NASA expected to be legally required to sterilize Mars samples at least to 2039 after allowing time for legal processes to protect Earth's environment, and to develop the ESF recommended new technology better than HEPA or ULPA filters to filter out 0.05 micron nanobacteria - proposal to return unsterilized samples to a safe high orbit above GEO to search for life by remote telerobotic study - and immediately return sterilized sub samples

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Please note this preprint is not yet peer reviewed and is currently in the process of development with frequent updates.

(previous DOI: 10.31219/osf.io/s8gcn for earliest versions)

Short abstract (central points, 2,000 characters)

- **Astrobiologists say Mars may still have surface microhabitats for life**. Early Mars had seas, and microbes could persist adapted to current extreme surface conditions.

- **Probability of returning extant life could be significant** in microhabitats or as viable spores in dust storms

- **Current technology can't meet ESF requirement** for extraterrestrial life air filters (0.05 microns)

- **Legal process might require prohibitory version of the precautionary principle** if it considers large scale effects such as from mirror life, and if legal decision is to rely on “Sagan’s criterion” that “we cannot take even a small risk with a billion lives” - a quote from “Cosmic Connection”

- **Legal process likely starts in 2022** when NASA hope to submit their draft Environment Impact Statement.

- **Minimum 17 years to prepare** as NASA needs legal clarity to start the build, while legal process takes at least six years, build at least 9 years and then 2 years to train technicians because of many lapses in protocols for the Apollo mission

- **The current paper reasons that NASA is expected to be legally required to sterilize Mars samples** to protect Earth's biosphere to 2039+ based on the shortest possible

timescale until the facility might be ready to receive samples.

- **Recommendation to return unsterilized samples to a safe orbit above GEO**, examine with remote controlled life detection instruments and return sterilized sub-samples to Earth immediately

- **Several recommendations to increase the chance of returning viable Martian spores** including dust sample and sample of the brines found by Curiosity by adding capabilities to take these samples to ESF fetch rover

- **New swansong Gaia hypothesis that life on Mars may remove CO_2 sufficiently to keep Mars just barely habitable** for billions of years but not uninhabitable as CO_2 emissions from volcanoes fluctuate – this would increase the chance of finding present day life on Mars

Graphical abstract

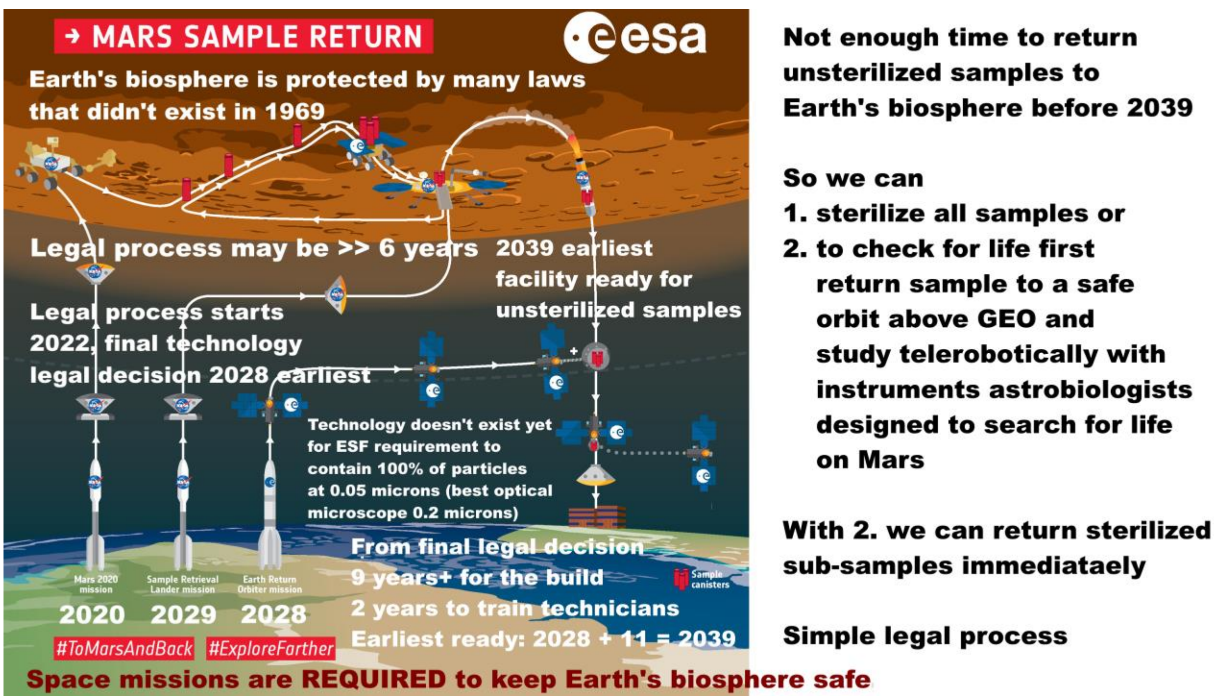


Figure 1: Text added to ESA graphic (Oldenburg, 2019) showing current proposed timeline (NASA, 2022mpfs) and time until the facility is ready to receive sample

Text on graphic:

Earth's biosphere is protected by many laws that didn't exist in 2039.

Legal process may be >> 6 years
Legal process beings 2022, final technology decision 2028 earliest

Technology doesn't exist yet for ESF requirement to contain 100% of particles at 0.05 microns (best optical microscope 0.2 microns)

From final legal decision, 9+ years to build
2 years to train technicians
Earliest ready: 2028 + 11 = 2039

Space missions are REQUIRED to keep Earth safe

Not enough time to return unsterilized samples to Earth's biosphere before 2039

So, we can
1. sterilize all samples or
2. test for life first - return samples to a safe orbit above GEO and examine them with remotely controlled life detection instruments designed by astrobiologists

With 2., we can return sterilized sub-samples immediately
Simple legal process

Abstract (long version, 5000 characters)

NASA plans to return samples from Mars by 2031. They believe there is no surface life in Jezero crater, but also agree Earth has to be protected, on the remote chance there is native Martian life in these samples. Martian life might survive as a swansong biosphere from a more habitable early Mars, perhaps in microhabitats such as are commonly found in Mars analogue deserts on Earth.

The Apollo sample return procedures had no legal process, and were first made public on the day of launch. During internal review, the experts Vishniac and Sencer told NASA its procedures would not protect Earth, but NASA overruled them on the grounds that their recommendations would postpone the launch date.

This would not be permitted today. We have many laws to protect Earth’s biosphere that didn’t exist in 1969.

The European Space Foundation study from 2012 says "release of a single unsterilized particle larger than 0.05 μm is not acceptable under any circumstances". This is to contain starvation-stressed nanobacteria which can pass through 0.1 μm nanopores. This technology doesn't exist yet, even as experimental filters in laboratories. This is far beyond the requirements for HEPA and ULPA filters.

The sample return facility is estimated to cost ~\$500 million. Before starting a build costing over \$250 million, NASA has to commit to Congress that the cost and schedule is adequate. They can't know this until the end of the 8+ years legal process, which can change build requirements. Adding 11+ years to complete the build, we find an earliest date of 2039 to return unsterilized samples, with further delays likely.

However, samples can be sterilized at a level sufficient for planetary protection while preserving evidence for past life, geological dating and structure, and ability to detect present day life. This becomes an unrestricted sample return; a relatively simple process under the Outer Space Treaty.

Perseverance's primary objective is past life, rather than present day life.

We recommend that the ESA fetch rover adds a dust sample, originally planned for Perseverance. This could be used to search for dust-storm resistant Martian propagules which could be transported in Martian dust storms.

We also recommend that the ESA fetch rover is modified to dig an extra sample of dirt to return the brine layers Curiosity discovered centimeters below the surface of sand dunes. This could help resolve the puzzling Viking lander results. Did Viking detect complex chemistry or native life in the 1970s?

If there is a significant chance of viable present-day life in these samples, we suggest returning them to a satellite in a stable inclined orbit above GEO in Earth's Laplace plane or "ring plane". This orbit has many advantages for protection of Earth, the Moon, and other satellites. Sterilized subsamples can be returned to Earth immediately for geological studies, and preliminary astrobiological work.

Should signs of life be found, either in the sterilized samples or in orbit, scientists can send instruments to the orbiting satellite, previously designed to search for life in situ on Mars. These can be teleoperated with close to zero latency. Decisions about what to do next depend on what they discover.

This article examines specific worst-case scenarios for Martian life, such as a blue-green algae with everything flipped as in a mirror: DNA spirals counterclockwise, and amino acids, carbohydrates, and sugars, are all in their mirror forms. Ordinary terrestrial life can't use these mirror organics.

Synthetic biologists have started a step-by-step process to flip a terrestrial cell to mirror life. They warn of a risk that this mirror life could gradually transform parts of terrestrial ecosystems to indigestible mirror organics, giving it a competitive advantage.

Synthetic mirror life will need containment to a higher level of safety than conventional biosafety laboratories. To keep Earth safe, mirror cells will depend on chemicals only available in the laboratory.

To achieve a similar level of safety for Martian mirror life, we might have to leave it in orbit.

In some of our scenarios, Martian life is harmless or even beneficial. However, in worst case scenarios Martian life can never mix safely with ours. Quarantine might also be insufficient to protect our biosphere from microbes in the microbiomes of astronauts returning from Mars.

Our future possibilities, and opportunities, depend on what form Martian life takes. Answering this seems a top priority for space colonization enthusiasts, and astrobiologists alike.

Perseverance seems unlikely to resolve this. However, future astronauts in orbit around Mars may be able to give answers quickly with rapid surveys of proposed potential habitats, controlling surface robots with low latency telepresence.

This article concludes that the complex laws already in place to protect Earth's biosphere are both understandable and necessary.

Public comment on NASA's draft environmental impact statement

The author submitted this comment to NASA as a public comment, on their draft environmental impact statement which summarizes some of the most salient points of this paper for the planetary protection review, see:

. Comment Submitted by Robert Walker

Are you aware of the ESF Mars Sample Return study (Ammann et al, 2012:14ff)? It said ***"The release of a single unsterilized particle larger than 0.05 µm is not acceptable under any circumstances"***. This is to contain starvation limited ultramicrobacteria which pass through 0.1 micron filters (Miteva et al, 2005). Any Martian microbes may be starvation limited.

This 100% containment at 0.05 microns is well beyond capabilities of BSL4 facilities. Even ULPA level 17 filters only contain 99.999995 percent of particles tested only to 0.12 microns (BS, 2009:4).

It IS possible to filter 0.05 micron particles from water, under high pressure. One study used carbon nanotubes loaded with silver. It eliminated polioviruses at 0.03 microns in diameter (Kim et al, 2016) (Singh et al, 2020:6.3).

However, this technology doesn't seem to exist for aerosol filters.

An experimental 6-layer charged nanofiber filter intended for coronaviruses filters out 88% of ambient aerosol particles at 0.05 microns (Leung et al, 2020).

Even this would not achieve 100% containment.

The ESF also set a minimum one in a million probability of release of a single unsterilized particle at 0.01 microns. This is to contain gene transfer agents which transfer novel capabilities overnight in sea water to unrelated species of archaea (Maxmen, 2010)..

The ESF said both requirements need periodic review. This might reduce those figures further.

Benner et al suggest structures in the Martian meteorite ALH84001 might be fossils of earlier life without ribosomes or proteins; simpler “RNA world” cells (Benner et al, 2010: 37).

Panel 4 for the 1999 “Size limits” workshop concluded that such a primitive free living lifeform could be as small as 0.014 microns in diameter and 0.12 microns in length, if there is an efficient mechanism for packing its RNA. They measured one of the smallest structures at approximately this size (Board et al, 1999: 117).

A review board would also consider research since 2012 into small synthetic minimal cells (Lachance, 2019) and protocells (Joyce et al, 2018).

Biologists have also seriously considered a shadow biosphere of nanobes (Cleland, 2019, pp 213 - 214) which could co-exist with modern life. None were found, but this shows such a possibility is biologically credible.

Smaller cells have a larger surface area to volume ratio, and so take up nutrients more efficiently, an advantage in an environment with low nutrient concentrations. Small cells also avoid protozoan grazing (Ghuneim et al, 2018).

If Mars has early life nanobes, even with less sophisticated biology, these nanobes might be able to occupy a shadow biosphere on Earth.

An example worst case would be Martian mirror life. With the right enzymes (isomerases), this might be able to convert normal organics in an ecosystem into mirror organics only available to mirror life, or the very rare terrestrial microbes able to make use of mirror organics.

Uhran et al estimate a minimum of 6-7 years to complete the legal process, from filing the environmental impact statement. This may be significantly extended if challenged in court. International bodies like the WHO and FAO likely get involved and international treaties triggered (Uhran et al, 2019).

NASA is required to provide preliminary design and engineering details for the Sample Return Facility before they start a build, and with a life-cycle cost over $250 million must also commit to Congress on cost and schedule (NASA, Science Engineering Handbook: section 3.5).

However, the legal process may change requirements, so should be completed before we launch the Earth return orbiter, Earth Entry Vehicle, and Mars Ascent Vehicle, or start to build the receiving facility.

Urhan et al estimate 9 years to build or repurpose the facility and 2 years to train scientists because of many lapses in Apollo sample handling. If the build starts in 2028, the earliest the facility could be ready is 2039.

The legal process might also conclude that the required technology doesn't exist yet.

I propose two possible solutions in my article.

1. sterilize samples during the return journey, perhaps with nanoscale X-ray emitters. Present day life in the sample would be recognizable after sterilization OR

2. return unsterilized samples to a safe orbit where astrobiologists study them remotely using miniature instruments designed for life detection on Mars. Return sterilized sub-samples to Earth immediately;

As a safe orbit, this paper recommends the Laplace plane above GEO where ring particles would orbit if we had a ring system.

A return to the ISS doesn’t break the chain of contact with Mars.

The Moon needs to be kept free of contamination for future astronauts and tourists (COSPAR, 2011)

The preprint examines ways to increase chances of viable spores, such as dust samples (Jakovsky et al, 2021)

This may make, 2 preferrable.

Details and cites: see preprint https://osf.io/rk2gd (attached).

Introduction

This introduction highlights some of the paper's most central points, with an overview of the basic arguments and links to sections that expand on them further.

Section titles are written like a mini-abstract, so you can get a fast overview of this introduction, by just reading the section headings, then drill down into any section of interest for more details. You can get an overview of the entire paper in the same way, skip through, just reading section headings then drill down into any section you want to expand on.

NASA agrees we need to protect Earth's biosphere from Mars samples though they believe the surface of Mars is too inhospitable for life today

When the public ask NASA, *"Is there any life on Mars "*, the answer given by NASA's associate administrator Thomas Zurbuchen is typical (NASA, 2021wnpr).

> *That's a question I ask myself, is anything alive there, and frankly at the surface where we're going right now with Perseverance* ***we do not believe there's anything alive right there****, because of the radiation that's there, it's chilling cold and there's really no water there.*

However, we need to ask a different question

> *"Do we have to protect Earth from the possibility of life in the samples returned by Perseverance?*"

NASA agrees, the answer is *"Yes"* (NASA, 2020nebmsr) (Foust, 2020).

Judging from their NEPA announcement, (NASA, 2022nepa), NASA seem to be of the impression that the consensus amongst scientists is that the Martian surface is too inhospitable for life.

> ***"The general scientific consensus is that the Martian surface is too inhospitable for life to survive there today. It is a freezing landscape with no liquid water that is continually bombarded with harsh radiation."***

However it is hard to find even one astrobiologists who would agree with NASA in their confidence that the Martian surface is too inhospitable for microbial life, and some astrobiologists say there is a significant possibility of present day life even in Jezero crater

However in the case of Mars, it is hard to find even one astrobiologist who goes as far as NASA's statement. Some think it has a high chance to be inhospitable but not certainty and many think Mars may have small niches suitable for life, similar to niches found in the soil or rocks of our driest coldest deserts which often have small communities of microbes, even if they are only habitable at microbial scales. Many also think it could have extant Martian life. A few think there is a possibility that Viking discovered life in the 1970s. This quote is from a paper about planetary protection in the forwards direction by Rummel and Conley, both former planetary protection officers for NASA (Rummel et al , 2014)

> ***"Claims that reducing planetary protection requirements wouldn't be harmful, because Earth life can't grow on Mars, may be reassuring as opinion, but the facts are that we keep discovering life growing in extreme conditions on Earth that resemble conditions on Mars. We also keep discovering conditions on Mars that are more similar—though perhaps only at microbial scales—to inhabited environments on Earth, which is where the concept of Special Regions initially came from."***

In the 2020 conference **"*Mars extant life: what's next?*"** (Carrier et al, 2020) a significant fraction of the participants thought that there is a possibility Mars has extant life.

> ***"Primary conclusions are as follows: A significant subset of conference attendees concluded that there is a realistic possibility that Mars hosts indigenous microbial life. A powerful theme that permeated the conference is that the key to the search for martian extant life lies in identifying and exploring refugia ("oases"), where conditions are either permanently or episodically significantly more hospitable than average. Based on our existing knowledge of Mars, conference participants highlighted four potential martian refugium (not listed in priority order): Caves, Deep Subsurface, Ices, and Salts."***

For more example quotes from the literature, see section:

- Views of astrobiologists on the possibility of present-day life on or near the surface (below)

NASA's attempts to protect Earth in 1969 for Apollo 11 were judged to be inadequate by representatives of the National Academy of Sciences and Public Health Service – but NASA overruled these objections, saying that they didn't have time to make the required changes

In 1969 NASA did make an effort to protect Earth from the possibility of life in the lunar samples, however, sadly, even at the time their plans were not considered adequate. The astronauts opened the door of the Apollo 11 capsule after splashdown in the open sea, letting out air that was exposed to lunar dust from the landing module. There would be dust also in the astronauts clothes. The astronauts donned biological isolation garments and exited into a life-raft bobbing in a heavy sea, and then swabbed the isolation garments with a bleach solution. They weighted the swabs and dropped them into the sea. They then disinfected the raft with an iodine solution (Meltzer, 2012:404) and sank the raft (Meltzer, 2012:205). This wouldn't be enough to protect the sea from microbial spores even with the scientific understanding of the 1960s. The view of Vishniac of the National Academy of Sciences is summarized by Meltzer as : (Meltzer, 2012:203).

> *Opening and venting the spacecraft to Earth's atmosphere after splashdown would, in his view, make the rest of Apollo's elaborate quarantine program pointless.*

The chairman of the Interagency Committee, David Sencer, from Public Health Service said these plans violated the concept of biological containment (Meltzer, 2012:203).

However, NASA set up the internal Interagency Committee with a requirement that all parties had to agree on any change to its plans. This consensus had to include NASA itself (Meltzer, 2012:129). This gave NASA the authority to block any objections. It used this power in 1969 to block requests for more stringent precautions on the basis that there wasn't enough time left before the launch of Apollo 11 to add the precautions required by interagency experts. For more on this, see:

- Apollo procedures didn't protect Earth even according to the Interagency Committee on Back Contamination (ICBC) that advised NASA

[links are to later sections in the current paper]

This time NASA won't be able to overrule objections by other agencies because of the NEPA legislation introduced in 1970

However, this time it will be different. NASA won't be able to overrule objections on the basis of a fast approaching launch date for the Mars Ascent Vehicle, the Earth Entry Vehicle etc or later because of the fast approaching date for the sample return itself. We have many laws in place now that didn't exist for Apollo starting with NEPA, which was signed into law on 1st January 1970 (EPA, n.d.), the year after Apollo 11. This mandates an Environmental Impact Statement (EIS); the starting point for the modern legal process in the USA.

After the EIS, many other agencies and experts will get involved, in an extensive legal process with open public debate and peer review. If this turns up flaws as serious as the ones independent experts found in 1969, NASA's plans will fail legal review and need revision.

The current paper finds many examples of potential objections that may be raised during this legal process, once scientists in other disciplines get involved.

With a minimum of 6 years for the legal process, 9 years for the build, and 2 years to train technicians, and objections that could change build requirements to the end of the legal process, NASA can't guarantee to be ready to receive unsterilized samples before 2039

Even with no objections, we find that NASA's proposed timeline seems unachievable, because they won't know until the end of the legal process that their initial design can pass without any objections. In summary:

- **NASA agrees that they have to protect Earth from unsterilized Mars samples.** They passed Key Decision Point A for the sample return mission in December 2020 - this is for the overall concept, including orbiter, ascent vehicle, Earth entry vehicle and fetch rover. It does not include a detailed specification for the Mars Receiving Facility building (Foust, 2020) (NASA, 2021nmttm) (Gramling et al., 2021)

- **One estimated minimum timeline for building a facility is 11 years** (Uhran et al, 2019) which would seem to give enough time to complete the facility by 2033 if they start work in 2022, if it weren't for the legal process

- **Estimated minimum timeline of the legal process of 6-7 years** not including the 2 years to develop a consensus legal position (Uhran et al, 2019). See:

  Minimum timeline: 2 years to develop consensus legal position, 6-7 years to file Environmental Impact Statement, 11 years to build sample return facility

- **Legal process starts in 2022 at earliest,** with the Environmental Impact Statement which will be submitted by NASA in 2022 at the earliest (NASA, 2022nic). It's not clear yet if NASA have reached the level of clarity about the sample facility requirements to count as a "consensus legal position" on the matter.

New to this study:

- **During the legal process, NASA's recommendations are likely to meet many objections.** For example, all proposals for Mars Receiving Facilities we looked at for the current paper use HEPA or ULPA filters, as is normal for a biosafety laboratory, and the MSR safety fact sheet for the draft EIS just says (NASA, 2022smsr).: "*Such a Mars sample receiving facility would have design and sample handling requirements equivalent to those of biological safety laboratories used for research studies of infectious diseases*"

  However, such an approach is likely to be challenged, because the most recent sample return study, the one by the ESF in 2012 required standards far beyond a normal biological safety laboratory. See: By European Space Foundation study (2012), particles larger than 0.05 µm in diameter are not to be released under any circumstances

The current paper finds that the technology for such 100% effective nanopore filters doesn't yet exist. See: Example of best available nanofilter technology from 2020, not yet commercially available, filters out 88% of ambient aerosol particles at 0.05 microns - far short of the ESF requirement to filter out 100% at this size – though this standard can be met with nanoparticles in water under high pressure

- **The current paper also finds that epidemiologists are likely to raise many issues with quarantine of technicians in the case of accidental exposure of the staff handling the facility.** One of many issues is the question of how to deal with a lifelong symptomless superspeader similar to Typhoid Mary (Korr, 2020) . This could lead to a requirement to use telerobotics to handle the samples. See Complexities of quarantine for technicians accidentally exposed to sample materials

Consequences:

- **If NASA attempts to start a build in 2022, there may be a mismatch between the final legal requirements and the technology for their new facility (e.g. filters) and overall architecture (e.g. whether samples are handled by humans directly or through telerobotics).** They might have to start again with a new design and new build or radical rebuild at the end of the legal process.

- **NASA could receive new objections at any point through to near the end of the legal process** as their recommendations are considered by external experts and other agencies in the US. ESA who are an equal collaborator with NASA could also receive new objections in the EU, UK, and Canada at any time which would impact on their launches.

  Then the process is expected to involve other countries and international organizations that could also raise objections with NASA's or ESA's recommendations as the process continues. Then others could get involved with litigation as happens often with Environmental Impact Statements.

  6-7 years is optimistic for the legal process already, and objections or litigation could extend it considerably. See: Legal process likely to extend well beyond 6 years with involvement of CDC, DOA, NOAA, OSHA etc, legislation of EU and members of ESA, international treaties, and international organizations like the World Health Organization

- **If a rebuild is needed, it restarts the clock for the build which may not end until 2039 at the earliest**. If the requirements change towards the end of the legal process, this potentially adds the 11 years of the build to the end of the legal process which at 6-7 years from 2022 would end in 2028 at the earliest.

NASA can't know if their build can go ahead at all until towards the end of the legal process as the necessary technology to contain the samples might not yet exist – for instane if required to contain 0.05 micron nanobes.

If the technology already existed for 100% containment of any conceivable exobiology, it could be possible to "over engineer" and design in advance for a facility with reasonable assurance that it would be approved whatever the outcome of the legal process. However that is not the current situation.

- **Even new technology for filters to contain 0.05 micron particles may not be enough.** The current paper finds that the minimum size might be reduced on review as a result of considering new research on the potential for life not based on DNA or proteins. Since the technology doesn't exist yet, we can't guarantee that it will be possible to achieve reliable maintainable filters that achieve 100% containment at 0.05 microns in 6 years and if this reduces to 0.01 microns it's even harder to do.
  See: Scientific developments since 2012 that may be considered in a new review of ESF's 0.05 micron / 0.01 micron size limits – if the review considers life not based on DNA and proteins such as minimum size RNA world cells, this could potentially reduce the 0.05 microns to a requirement that release of a 0.01 micron particle is not acceptable under any circumstances

- **We may need similar levels of assurance to synthetic biology as a possible decision is that the "gold standard" of a one in a million chance of release from biosafety laboratories might not be enough**. See: Discussion of potential large scale effects from mirror life could lead to a call for near certainty of containment, as for some experiments in synthetic biology and following sections: ESF study discussion of precautionary principle said we need to minimize risk using best available technology because if we require no appreciable risk of harm the mission has to be cancelled – considerations of large scale effects could lead to calls to re-evaluate this conclusion and Clarifying this question of which version of the precautionary principle to use with Sagan's criterion that "we cannot take even a small risk with a billion lives"

- **a possible final decision from the legal process is that the technology doesn't yet exist to return an unsterilized sample to Earth safely at this time** – either because the updated filter technology doesn't yet exist even by the end of the legal process, or because the decision is made that until we know what's in the sample, we need a similar level of assurance for Martian life as for synthetic life, leading to the prohibitory version of the precautionary principle, which requires no appreciable risk of harm.

So we can't know until near the end of the legal process if the mission samples can return at all with current technology.

Impact on timeline, with this end-to-end requirement, is that the build only starts after the legal requirements are clear, because NASA aren't permitted to risk such high levels of public funds before they know what to build.

Meanwhile the ESA spacecraft also can't be launched until the end of the legal process unless they risk launching spacecraft that don't comply with the legal requirements because of the impossibility of modifying a spacecraft after it is launched:

- **2028 (earliest end for legal process) is after the proposed 2027 launch date for the ESA Earth Return Orbiter and Capture, Contain and Return system** (NASA, 2022mpfs) unless it takes the risk of launching spacecraft that don't comply with the technological requirements for sample return. It is also just one year before 2029, the proposed launch date for the ESA sample fetch rover and the NASA Mars Ascent Vehicle (NASA, 2022mpfs) .

  Also, even if the legal process proceeds very quickly with no major issues or objections, and ends by 2028, extra time should be allowed for any modifications it requires.

  If these spacecraft are launched before the legal process ends, the risk is that they may not be permitted to return to Earth, and the Earth return vehicle may need to be retrieved in orbit for further processing, which would add to the expense of the sample return. See Need for legal clarity before launch of ESA's Earth Return Orbiter, Earth Entry Vehicle, and NASA's Mars Ascent Vehicle

- **This also makes 2028 the earliest start date to build the sample receiving laboratory, as NASA is not permitted to risk this level of public funds for a new building, until they can provide a detailed cost and schedule with engineering details.**

  The estimated cost is over half a billion dollars (Andrews, 2020) (Mattingly, 2010:20). At this level of funding, NASA will need to commit to Congress that the cost and schedule is adequate (NASA, Science Engineering Handbook: section 3.5).

  However they can't do this until they know what to build and they won't know what to build until they know the legal requirements. See NASA procedural requirements for mission planners to develop a clear vision of problems, show it's feasible and cost-effective, develop technology with engineering details and show it will meet requirements before build starts – because of significant costs involved in modifying designs at later stages in the build

- **Even if NASA's original design eventually does pass legal review, they won't know this until 2028 at the earliest, so they can't start the build right away.** They can only start after either their recommendation is approved, or they are certain that it will be approved

- **2039 is the earliest date for a sample return**. This date is reached by adding the minimum of 9 years for the build and 2 years to train technicians to the earliest date of completion of the legal process of 2028.

- **Delays beyond 2039 are likely**.
  - With such a complex legal process and many possible objections, the legal process may take longer than 6 years, see: Legal process likely to extend well beyond 6 years with involvement of CDC, DOA , NOAA, OSHA etc, legislation of EU and members of ESA, international treaties, and international organizations like the World Health Organization

  - The build may well encounter unexpected issues, for instance in integration and maintenance of biofilters for extraterrestrial nanobacteria, and take more than 9 years to complete,

  - Training of technicians could potentially take longer than expected too, for instance if the 0.05 micron requirement is used, they are likely to be required to show that they can sterilize cabinets containing the samples and replace the novel nanoscale filters after damage without releasing any unsterilized nanoparticles of 0.05 microns or larger. This is likely to involve challenge studies using nanoscale aerosols. They might not pass these tests initially with unfamiliar technology. See: Challenges for maintenance for future 0.05 micron compliant nanoscale filters – need to be designed for sterilization before any potential extraterrestrial biology is known, and may be easily damaged and hard to replace without risking release of nanoparticles

Proposed solutions: to sterilize samples, or return to a safe orbit above GEO

Proposed solutions:

- **Sterilization is the simplest solution**. If present day life is unlikely in the samples and if the past life samples are seriously degraded already by exposure to surface cosmic radiation, we find the extra radiation to sterilize the samples is not likely to impact on geological studies, while any extant life, while not viable, would still be recognizable as such by astrobiologists. As far as extant life is concerned, the mission would then be a technology demonstration, preparing for a future mission that is more likely to return any viable Martian microbes.

  See Sterilized sample return as aspirational technology demonstration for a future astrobiology mission

- **Alternatively the samples can be returned for preliminary study in a location not connected to Earth's biosphere.** This solution is a way to avoid the need to sterilize native life in the sample,

  We can then sterilize sub samples which can be returned for immediate study in terrestrial laboratories, while the unsterilized materials are studied in a safe location off-planet until we know what is in them. Future decisions then are made based on what we find in the samples.

  See **Recommendation** to return a sample for teleoperated 'in situ' study above Geosynchronous Equatorial Orbit (GEO)

- **For maximum planetary protection, the current paper recommends a particular class of orbits in the Laplace "plane" - where Earth's ring system would be situated if it had one, and high above GEO orbit.** Any debris shed from such an orbit is constrained to a region that doesn't intersect with Earth or with Geostationary orbit. These Laplace plane orbits have been proposed for disposal of GEO satellites at the end of their life, and the same rationale makes it useful for our purposes, returned to a higher orbit than the proposed end of life orbits for GEO satellites See: An orbit within the Laplace plane above GEO contains debris in event of an off nominal explosion or other events

These decisions are best made before ESA launches their spacecraft, with potential for large cost reductions. For example,

- **If NASA and ESA decide to sterilize the samples during the return journey from Mars,** this capability has to be built into the Earth Entry Vehicle before launch.

- **If NASA and ESA decide to return the sample to a safe orbit** such as the proposed orbits in the Laplace plane above GEO, or just to return the samples for sterilizing first in a sterilizing Earth return module that orbits Earth, the aeroshell of the EEV is unnecessary extra weight which could be replaced by fuel to help it get into its final orbit on its own.

See Need for legal clarity before launch of ESA's Earth Return Orbiter, Earth Entry Vehicle, and NASA's Mars Ascent Vehicle.

These two proposed solutions, sterilization of all the samples, or return to above GEO introduce no possibility of risk to Earth's biosphere.

With these solutions, all the materials that reach Earth are sterilized. These solutions are as straightforward for planetary protection as the unrestricted sample returns from comets and asteroids which we have already done.

So there should be no significant delays for either of these solutions. See:

- Comet and asteroid sample returns are straightforward - but are unrestricted sample returns - sterilized during collection - or Earth has a similar natural influx

If the samples are returned to above GEO, this adds complexity to the mission compared to the simplest solution to just sterilize the sample during the return journey.

So, is there enough chance of returning Martian life, to make it worthwhile to keep samples unsterilized above GEO?

Mars has a higher potential for habitability today than the Moon as understood in 1969

If we take the example of the Moon, if our current legislation had been in place in 1969 or NASA had taken more care to protect Earth back then, it probably wouldn't have been worth returning unsterilized samples with the first samples returned from the Moon. The chance of life in the lunar samples or dust seemed extremely low even at the time.

In 1969 NASA could have just done a sterilized robotic sample return, or maybe several, to confirm that the Moon was as uninhabitable as it seemed from other observations; Once they knew the surface of the Moon was sterile, which in the case of the Moon we now know they would have discovered quickly, within a year or two, they could then drop all planetary protection protocols and send humans.

However, there are differences that make Mars a better candidate for native life today than the Moon seemed to be with the understanding of the 1960s.

In summary:

- **We have clear evidence today, that early Mars had conditions favourable for evolution of life, with lakes and even seas.**
    - In the 1960s we had no clear evidence for a past habitable Moon. There was weak evidence suggesting the Mares were ancient sea beds, but this evidence was not persuasive (and of course soon turned out to be false)

- **Curiosity has detected ultra cold salty brines on the surface of sand dunes just before dawn / after dusk and below the surface just after sun rises and just before the sun sets.**
    - There was no detection of liquid water on the Moon, just a hypothetical layer that could exist at a depth of tens of meters enriched with organics.

- **Mars has a sparse atmosphere humid enough for thin layers of frost to form at night in many regions.** Some terrestrial blue green algae and lichen have been able to grow in Mars simulation conditions using just the night time humidity in partial shade.
    - The Moon has no atmosphere, only an exosphere. Frost can't form there at night, and by 1969 it was already clear no life could grow on the surface of the Moon.

- **The Martian dust storms can transport spores from distant regions of Mars**.
    - There is no way for life to be transported from distant parts of the Moon.

- **A small minority of scientists believe that the Viking landers may have detected life in the 1970s. These observations are puzzling because of an apparent circadian rhythm with the radiolabeled emissions offset by 2 hours from the maximum temperature. These observations match biological rhythms and are hard to explain using chemistry.**
    - There were no puzzling observations from lunar experiments that anyone interpreted as possibly due to life.

For details:

- How we understood the Moon in 1969 compared to Mars today - Mars with a thin atmosphere and liquid water, is more favorable for life than the Moon was thought to be back then

Proposals to modify the ESF lander and sample selections to increase potential for returning viable present day or identifiable past life

If there is extant life on Mars, is there a chance we can detect it using this sample return mission, perhaps modified in some way?

The current paper suggests we may spot it in Martian dust. Martian propagules could be up to half a millimeter in diameter carried through the process of saltation - repeated bounces across the Martian sand-dunes similarly to motion of dust in desert sand dunes on Earth.

- Native Martian propagules of up to half a millimeter in diameter (including spore aggregates and hyphal fragments) could travel long distances with repeated bounces (saltation) - if they can withstand the impacts of the bounces

Martian propagules may have evolved coatings of hard chitin-like substances or agglutinated particles of the iron oxide dust, to protect from UV and collisions with the Martian surface during saltation. Chitin is a hard substance common in fungi and in the fungal component of lichens, and also in insect exoskeletons and jaws. See

- Martian life could also use iron oxides from the dust for protection from the impact stresses of the saltation bounces - or it might use chitin - a biomaterial which is extremely hard and also elastic and is found in terrestrial fungi and lichens

The current paper finds that if there are small regions within reach of the dust storms as productive of spores as even the coldest driest terrestrial deserts, Martian dust could potentially contain detectable amounts of viable spores. Since the dust storms are sometimes global, it's possible a dust sample could collect propagules that originated almost anywhere on Mars. On Earth, spores and fungal hyphal fragments from distant deserts can be detected thousands of miles away. The same process could potentially occur on Mars.

- Potential for spores and other propagules from nearby or distant regions of Mars similarly to transfer of spores from the Gobi desert to Japan

The original plans for the Perseverance rover included a dust sample but this capability was later removed. The current paper recommends that the ESA fetch rover takes an extra sample tube to collect dust. Or better, it could use a rotary air sampler to collect and compress a sample of air. This is of interest for human missions too, to have a sample of Martian dust to test with terrestrial spores to check the potential for terrestrial life to spread in Martian dust storms - for forward contamination risk evaluation. It is also useful to study chemical hazards in the dust that could impact on astronauts such as the chlorites, chlorates and perchlorates.

Such a sample also has some geological interest as a random sampling of wind-eroded rock fragments from distant parts of Mars.

See:

- **Recommendation:** Extra sample of air and airfall dust to search for Martian life, assess forward contamination issues for terrestrial microbes, dust dangers for astronauts, and to return a random sample of wind-eroded rock from distant parts of Mars
- **Proposal:** magnets could be used to enhance dust collection
- **Proposal:** to use the sample return capsule as a dust collector – keep it open to the atmosphere before adding the sample tubes

The plans for Perseverance also originally included an atmospheric sample, another capability that was dropped from the mission. Dust collection can be combined with an atmospheric sample which would be valuable for studying trace gases in the atmosphere. Since this is a capability dropped by Perseverance, it is in the scope of the mission. An atmospheric sample can’t be added to Perseverance now, but it can still be added to the ESA fetch rover or the Mars Ascent Vehicle.

Perserverance’s In Situ Resource Utilization experiment Moxie collects carbon dioxide in the air to split it into oxygen, which may be useful for fuel on Mars in the future. To collect the carbon dioxide it uses an atmospheric compressor.

Jakovsky et al propose sending a similar atmospheric compressor to Mars but this time use it to collect an atmospheric sample and a dust sample to return to Earth.

- **Proposal:** by Jakovsky et al from the 2020 NASA decadal survey to combine a dust sample with a compressed sample of the Martian atmosphere

We also recommend modifications to the ESA fetch rover to add an extra sample of dirt since this is of special interest to astrobiologists. Ideally this would include the ultra cold brine layers observed indirectly by Curiosity, which form in sand dunes at night - which might perhaps shed light on the puzzling Viking observations.

These brines could potentially be habitable to native Martian life if it uses a biofilm to retain the liquid through to the warmer daytime temperatures, to modify habitability of the layers at a microscale. They could also be habitable to Martian life if it can tolerate lower temperatures than terrestrial life.

- **Recommendation:** modify ESA's sample fetch rover to grab a sample of the near surface temporary brine layers from sand dunes - perhaps Perseverance may be able to do this too with its regolith bit

These recommendations are all in the spirit of the mission as extra sample returns and are different from the “mission creep” of adding new instruments for other purposes.

The current paper also has a recommendation to increase the possibility for finding recognizable traces of early life. This doesn’t require any new instruments but is just a new way of using the instruments Perseverance already has, specifically the Marscopter, if it remains operational.

Any ancient organics in surface layers are likely to be seriously degraded by cosmic radiation to the point where traces of life would be hard to recognize. The current paper suggests searching for young craters near to the Perseverance rover in Jezero crater.

We find that there is a near certainty of young craters within travel distance of Perseverance less than 50,000 years old that have excavated the subsurface to a depth of several meters. This could let us return organics exposed to no more than a few tens of thousands of years of surface levels of cosmic radiation. This would increase the possibility of finding past life. Also it’s possible that such layers would be more habitable to present day life.

- **Recommendation:** use of Marscopter and Perseverance to help identify young craters with sharp rims to help sample subsurface organics excavated by meteorites

Some Mars colonization enthusiasts argue that no planetary protection is needed, however their arguments aren't accepted by NASA and wouldn't be persuasive for the general public, other agencies or justices

Some Mars colonization enthusiasts, notably Robert Zubrin, president of the Mars Society, have argued that Earth doesn't need to be protected from Mars samples using arguments that many colonization enthusiasts find persuasive (Zubrin, 2000).

However planetary protection experts say this reasoning is not valid (Rummel et al., 2000). Their arguments would carry weight, indeed NASA have already agreed that they need to protect Earth (Foust, 2020) (NASA, 2021nmttm) (Gramling et al., 2021).

This reasoning could not be used to bypass the legal process. For reactions of several planetary protection experts, see:

- Zubrin's arguments in: "Contamination from Mars: No Threat" - not likely to be decisive in legal process - response of planetary protection experts in "No Threat? No Way"

Zubrin's two main points are that he says (Zubrin, 2000)

- any Martian life in the samples has already reached Earth on Martian meteorites,
- Martian life is adapted to Mars and so can't harm humans.

To answer Zubrin's two main points,

- **Martian surface salts or dust can't get to Earth after any meteorite impact, indeed, we have no samples of them here on Earth. So life from those habitats at least can't get to Earth.**

  The meteorites we do have come from at least 3 meters below the surface. The rocks there are ultracold apart from any localized geothermal heating. They also come from high altitudes in the Southern Uplands where the atmospheric pressure is also much lower (the low pressure makes it easier for impactors to knock material into orbit). These are much less favourable locations for present day Martian life than the surface dust and salts in Jezero crater. See

- Could Martian life have got to Earth on meteorites? Our Martian meteorites come from at least 3 m below the surface in high altitude regions of Mars

On Zubrin's second point,

- **Microbes don't have to be adapted to us to harm us.** Indeed there are many microbes that harm humans that haven't adapted to us.

  They harm through accidental toxins, as for botulism and tetanus, or they are opportunistic pathogens as for legionnaires disease which is a pathogen of biofilms that uses the same methods to attack human lungs and sometimes leads to death.

  For another example, opportunistic fungal infections can be deadly to immunocompromised individuals. Potentially, we could all be immunocompromised to an alien biology that no terrestrial immune system has ever encountered. For these and many more examples, see

- Could present day Martian life harm terrestrial organisms?

He has several other arguments. For an outline of his main arguments and responses to them, see again:

- Zubrin's arguments in: "Contamination from Mars: No Threat" - not likely to be decisive in legal process - response of planetary protection experts in "No Threat? No Way"

Cockell's example scenario of a present day habitable but lifeless Mars, which perhaps had life in the past

Charles Cockell and others have argued that, amongst other scenarios, it's possible life may have evolved on Mars in the past but no longer be present (Cockell, 2014). In this scenario, present day Mars may well have uninhabited habitats even if there is no longer life there (Deighton, 2016)

> ***Most microbes can grow in different types of extremes and the extremes that we are looking at, things like radiation, perchlorate salts and also sulphate salts (found on Mars), they will grow in that. It's just a question of trying to determine what the limits are and that's the work we're doing at the moment. Anywhere where we've gone to the deep subsurface (on earth) today, where there is liquid water, there is a high chance that environments are habitable,***
>
> ***Simply because Mars is a planet of volcanic rock, and when volcanic rock weathers that provides an environment for microbes to grow and reproduce, I think we can already say there is a high chance there are habitable environments.***

So he thinks there may well be habitable environments today. The big question is whether life ever evolved on Mars, and whether past life has survived to the present day on Mars.

Early Mars was very hospitable to life with its seas and lakes. However it's been barely habitable most of the time for billions of years. Could life have continued for so long, or would a swansong biosphere (O'Malley-James, 2014) have only lasted a few hundred million years before life was extinct on Mars?

Cockell has suggested (amongst other possible scenarios) that if early Martian life went extinct, Mars could now have uninhabited habitats, i.e. which life could colonize but with nothing left by way of early Martian life to colonize them (Cockell, 2014).

Proposed solution of a self sustaining barely habitable Swansong Gaia which might explain current conditions on Mars, and increase potential for past life to continue to the present

The current paper proposes a new solution relevant to this debate, a self perpetuating “Swansong Gaia”, that creates conditions for life to continue at low levels for billions of years. The proposal is that without the presence of life, Mars would be far more habitable, but that life itself keeps the surface barely habitable, by sequestering more carbon whenever the atmosphere gets thick enough to permit liquid water. I.e. it’s a novel form of “Gaia” – not an anti-Gaia as that would make the planet uninhabitable, but a Swansong Gaia which maintains a planet perpetually in a Swansong state of minimal habitability.

With this proposal, Mars might even be so close to uninhabitable ***precisely because*** of the presence of life in trace amounts.

Whenever the atmosphere starts to thicken, life grows and draws down carbon, and soon makes the planet less habitable again. So long as the volcanoes continue to produce more than the minimum amount of CO_2 required for habitability, this could keep the planet barely habitable for billions of years.

Mars would likely still have uninhabited habitats, especially ones that are newly formed. But as in Mars analogue terrestrial deserts, life would colonize many of them slowly over thousands of years. With this proposal Mars may have a mix of some inhabited and some uninhabited habitats, as regions on Mars gradually swing between habitable and uninhabitable depending on local and global conditions.

The current paper suggests that an atmosphere so close to the triple point of water at the low temperature and low pressure limit for survival for life might even be a weak biosignature for a Mars-like exoplanet. Although no individual exoplanet could be shown to contain life in this way, statistically we could detect a high probability of life outside of our solar system if many more than expected Mars-like exoplanets are found to have atmospheres close to the triple point of water. The idea would be that without life many of these planets would have much thicker atmospheres, and the difference from predictions due to this Swansong Gaia effect could be noticed statistically.

- Suggestion of a self perpetuating “Swansong Gaia” maintaining conditions slightly above minimal habitability for billions of years - as a way for early life to continue through to present day Mars

Scenario based approach to explore mixing of biospheres based on different forms of biochemistry

The current paper uses a scenario based approach, and examines many scenarios for the potential impacts of Martian life on Earth’s biosphere, if it exists. If there are Martian microbes with a different biochemistry from terrestrial life, we have to consider the possibility of Earth developing a mixed biosphere with both biochemistries for life co-existing, Martian and terrestrial life. How such a biosphere develops will depend on the scenario.

Martian life would most likely not be originally adapted to most terrestrial habitats, but all it needs to establish a foothold on Earth is to find a niche somewhere. Martian microbes with short generation times could adapt and evolve and eventually may play an equal role in many biospheres with terrestrial microbes.

Would our ecosystems function in the same way if e.g. the plant soil microbiome and the human body microbiome eventually has equal amounts of familiar biology and a new non familiar exobiology?

There are many scenarios to consider for a mixed biosphere with microbial life using unfamiliar exobiology we could return from Mars. One possibility is an accidental similarity of Martian biochemicals to terrestrial amino acids.

Martian life could have an unfamiliar biochemistry that has some biochemicals that have an accidental similarity to amino acids used by terrestrial life. The current paper looks at the example of BMAA, which is produced by cyanobacteria. It is sometimes misincorporated in place of the amino acid l-serine, a substitution which has been implicated as a possible cause of ALS, or Lou Gherig's disease. In the case of a mixed biosphere where half the microbes use a biochemistry with a different vocabulary of amino acids, there may be potential for many such accidental neurotoxins.

See:

- Accidental similarity of amino acids forming neurotoxins such as BMAA

Another possibility is that in a mixed biosphere with two types of biochemistry, Martian life could produce accidental exotoxins, protoxins or allergens, again just due to our biology responding to unfamiliar chemicals from a different exobiology. See:

- Exotoxins, protoxins, allergens and opportunistic infection

Not all the scenarios considered are harmful to Earth. Using the archaea as an analogy, the current paper finds that if we accidentally introduce a new domain of Martian life to Earth's biosphere, or maybe even a new exobiology, it could also be harmless or even beneficial:

- Could Martian microbes be harmless to terrestrial organisms?
- Enhanced Gaia - could Martian life be beneficial to Earth's biosphere?

However, some of the worst case scenarios studied here such as the scenario of a Mars with mirror life could mean that there is no way for astronauts to return from Mars to Earth that keeps our biosphere safe. Even quarantine of astronauts might not be able to keep mirror life out of our biosphere. See:

- For some scenarios, quarantine would also be insufficient to protect Earth from return of astronauts, such as if Mars has mirror life

There are many other locations in our solar system where we can attempt settlement and colonization. The Moon is a likely starting point, whatever our future decisions for other planets, asteroids, icy dwarfs, comets and moons.

How to complete astrobiological knowledge gaps rapidly with future telerobotic study from Mars orbit

Everyone, including scientists and colonization enthusiasts, should want to know which scenario we face in our solar system. This needs an early answer. We need to know whether there is life on Mars and if so, what are the likely outcomes of the clash of biospheres of Mars and Earth if we let the two forms of life mix.

Perseverance's results are bound to be preliminary since Perseverance is

- not optimized to search for present day life
- hasn't visited a location with a high likelihood of finding life,
- is also not sterilized sufficiently to visit such a habitat if it detects one

So, Perseverance can't resolve this, except in the negative if it finds mirror life or some other form of life that can never be returned to Earth.

However, this is something we can resolve quickly in the future if we prioritize a rapid astrobiological survey of Mars, first with robotic explorers remotely controlled from Earth and then controlled from orbit by astronauts controlling robots on the surface similarly to avatars in computer games, with low latency telepresence.

- Resolving these issues with a rapid astrobiological survey, with astronauts teleoperating rovers from orbit around Mars

With this knowledge we can make wise decisions about the future for science, for commercial exploitation of Mars, and for space settlement.

Perseverance's mission within the wider context of an ambitious vigorous program of exploration and potentially settlement in our solar system

The current paper also examines the Perseverance sample return mission within the wider context of the exploration of Mars and the potential for future settlements in our solar system. The natural starting point is the Moon, in an ambitious vigorous approach to human exploration of our solar system, and an early rapid exploration of Mars via telerobotics from orbit would fit naturally into that vision.

- Planetary protection as an essential part of an ambitious, vigorous approach to human exploration - starting with exploration and settlement experiments on the Moon and preceding sections

The objective of the current paper is to help anticipate these potential scientific and legal problems early on, rather than 4-5 years from now. This will help NASA and ESA reduce the cost of the missions and achieve a better mission design.

I have written this paper with a general scientifically literate reader in mind. This is because of the multidisciplinary nature of planetary protection, and its wide ranging relevance, for instance to mission planners, engineers, legal experts, ethicists, and decision makers, as well as the general public.

The research for this paper turned up many surprises through connecting together widely separated disciplines such as synthetic biology, epidemiology, orbital dynamics, the engineering of filters, etc. These are likely to be considered in the legal process but haven't had much attention yet in the planetary protection literature.

The bulk of what is new in the paper is a result of this interdisciplinary approach.

- Highlights of what's new in this article
- Outline - and what's new to the planetary protection literature in this article

Modern legal processes didn't exist at the time of Apollo - no legal precedent for a modern restricted sample return

On September 18, 2020, (NASA, 2021prmtl), NASA's Perseverance rover arrived at Mars, to collect and cache rock and soil samples. ESA have started preparations for a mission to return them to Earth (Foust, 2021) (NASA, 2020sonr)

The current plan is for the rover to launch to collect the samples in 2029, and load them into the Mars Ascent Vehicle, which will send them to orbit around Mars where they are then captured by the ESA Earth Return orbiter and returned to Earth by aerocapture in our atmosphere in 2033. The Earth Return Orbiter launches in 2027 (NASA, 2022mpfs)..

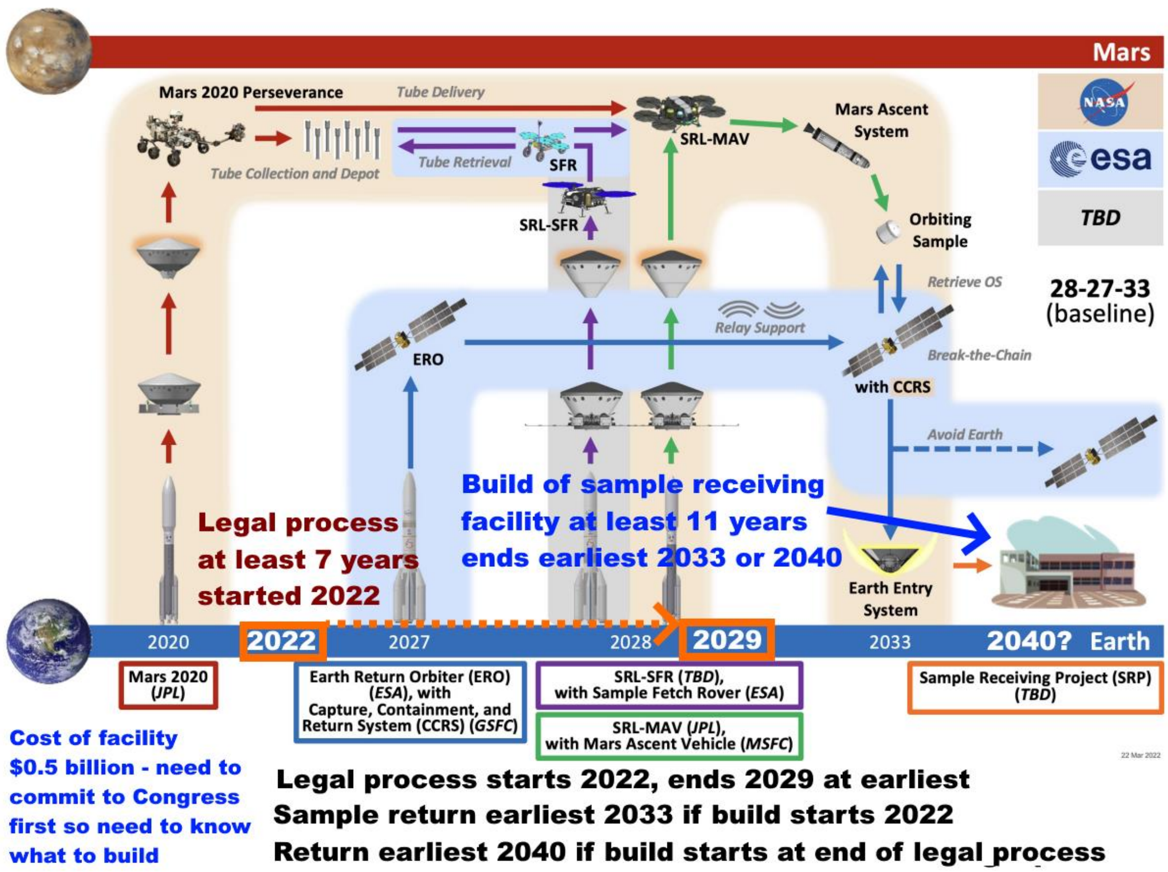


Figure 1: Text added to NASA / ESA graphic with current proposed timeline (NASA, 2022mpfs) and time until the facility is ready to receive samples

See also the current paper's Graphical abstract

The legal process to protect Earth's environment from any Martian microbes in the samples starts with the EIS submission in 2022 at the earliest (NASA, 2022nic) and will last at least 6-7 years and the build for the sample receiving facility lasts at least 9 years with an additional 2 years to train technicians and be ready to receive the samples

From this we can work out the sample return earliest dates:

- 2033 if build of sample return facility also starts in 2022 and has no delays
- 2039 if build of sample return facility starts at the end of the legal process and the legal process has no delays and completes in the fastest possible time, and the same for the build, no construction delays and build finishes as fast as possible.

The legal process to protect Earth's environment from any Martian microbes in the samples starts in 2022 at the earliest (NASA, 2022nic). So, the legal requirements on a facility to receive the samples are not yet known. Nor has work started on the build for this facility (Uhran et al, 2019).

At the time of the Apollo missions, the only legal protection of Earth was a clause in the Outer Space treaty, in a clause of article IX requiring States Party to the Treaty to: (Ireland, 1967)

> *"pursue studies of outer space, including the moon and other celestial bodies, and conduct exploration of them so as to avoid ... **adverse changes in the environment of the Earth resulting from the introduction of extraterrestrial matter and, where necessary, shall adopt appropriate measures for this purpose."***

NEPA didn't exist and there was no Environmental Impact Statement back then. NEPA was signed into law on 1st January 1970 (EPA, n.d.), the year after Apollo 11 and Apollo 12, both of which were launched in 1969.

There are now numerous laws to protect the environment of Earth which didn't exist at the time of Apollo (Race, 1996).

The Apollo 11 guidelines to protect Earth from back contamination were published on the day of launch, 16th July 1967, with no opportunity for public discussion. It also had no peer review and there were no legal challenges.

Many of the legal issues are the same for a sample return and for human astronauts. For instance, unless samples are handled telerobotically, NASA will have to develop guidelines to quarantine technicians who might get contaminated. This could happen as a result of breaches in sample handling, such as human error, filter malfunction or tears in the gloves.

In 1969, NASA was never granted legislative authority to promulgate quarantine regulations or enforce them. Issues could be raised about NASA's authority to deprive US citizens of liberty in a quarantine facility, so the legal status of guidelines on the 1969 quarantine issued by NASA is unclear (Meltzer, 2012:452). The quarantine itself was carried out under the legal authority of the Surgeon General (Mangus et al, 2004:32). These guidelines were rescinded in 1991 and a similar process would not be permitted today (Meltzer, 2012:452).

The upshot of this is that there is no prior legal review available for quarantine measures to protect Earth from an unsterilized sample return. This is legally new territory and as we'll see it leads to many complexities that haven't been considered in the planetary protection literature to date. See Complexities of quarantine for technicians accidentally exposed to sample materials

Much has changed since then in our understanding of extremophiles and limits of size of microorganisms. Sample return studies in 2009 (National Research Council. 2009) and 2012 (Ammann et al, 2012) each placed more stringent handling requirements on any Mars sample return, requiring containment of smaller and smaller particles.

1969 Apollo procedures didn't protect Earth even according to the Interagency Committee on Back Contamination (ICBC) that advised NASA – can we learn from their mistakes?

The Apollo missions attempted to protect Earth's environment from extraterrestrial contamination but as it turned out, they didn't do much by way of actual protection even according to the science of their day. It's useful to see why it was that they didn't do a better job of protecting Earth, as maybe we can learn from their mistakes.

If there had been life on the Moon it would have likely got into the lunar dust. This covered the surfaces of the lunar module, and floated in the air and got into the astronauts noses and sinuses. Neil Armstrong and Buzz Aldrin remember it like this (Hansen, 2012:531-2):

> Neil Armstrong:***"We were aware of a new scent that clearly came from all the lunar material that had accumulated on and in our clothes. I remember commenting that we had the smell of wet ashes."***
>
> *Buzz Aldrin:* ***"There was a hint of something, like it was going to catch fire"***

Other lunar astronauts described the smell of the dust as like the smell of burnt gunpowder. The reason for this is not known yet. One possibility is that volatiles in the lunar dust actually did combust, very slowly in the oxygen of the capsule atmosphere (NASA, 2016tmsom).

Although the dust eventually settled in the lunar module, the dust floated off the surface again as they entered zero g, and got into the air and into the command module. This is Pete Conrad's account for Apollo 12 (Wagner, 2006):

> ***"However, something we found out later and not until we got back to the ship, was that the fine dust was on the suits and on almost all of the equipment that was contained inside the bags. The dust is so fine and in zero g it tended to float off***

the equipment and it must have permeated the whole command module. It floated out of those bags; it floated out of the contingency sample bag.

There were three significant breaches of the chain of containment as the astronauts returned to Earth:

- The first breach of the chain of containment happened during the descent of the capsule when it vented some of the air inside the capsule.
- The second breach was when the crew opened the capsule door after splashdown (Uhran et al, 2019).
- The third breach was when the astronauts in the life-raft (now wearing the biological isolation garments thrown in to them by the frogmen) swabbed themselves with bleach quickly (Compton, 1989) (Meltzer, 2012:213), then they weighted the cloths and threw them into the ocean. Finally, they disinfected the raft with iodine solution (Meltzer, 2012:404) and sunk the raft in the sea (Meltzer, 2012:205)..

As Buzz Aldrin describes it (Aldrin et al, 2015):

"One of the frogmen helped us to stumble into the raft and another was right there with us from then on. Waves started rolling and splashing us, causing the hatch to slam into the head of one of the frogmen. He weaved for a moment as we all moved to catch him, but he recovered quickly and motioned us back down. Another handed us scrubbing cloths and detergent with which we had to thoroughly douse ourselves twice - once with one cleaning substance, the second with another, all to counteract any contamination we might have brought from the moon. The cloths we had used to scrub ourselves were tied to weights and dropped into the ocean."

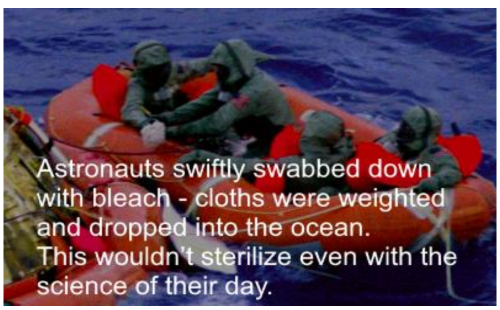


Figure 2: Astronauts swiftly swabbed down with bleach - then the cloths were weighted and dropped into the ocean. Background image: Apollo 11 crew await pickup by a helicopter from the USS Hornet (NASA, 2013ach).

In the dinghy are the Apollo 11 astronauts Neil Armstrong, Michael Collins, and Buzz Aldrin, and Navy Frogman Clancy Hatleberg

This wouldn't have sterilized the astronauts' outer garments of life, or the raft, even with the science of their day. To take an example, it takes twenty minutes contact with 10% bleach to sterilize Bacillus anthracis (the agent of anthrax, and a highly resistant spore). 10% of anthrax spores remain viable after ten minutes contact with bleach (Heninger et al, 2009).

Carl Sagan put it like this (Sagan, 1973:114):

> ***"The one clear lesson that emerged from our experience in attempting to isolate Apollo-returned lunar samples is that mission controllers are unwilling to risk the certain discomfort of an astronaut – never mind his death – against the remote possibility of a global pandemic. When Apollo 11, the first successful manned lunar lander, returned to Earth – it was a spaceworthy, but not a very seaworthy, vessel – the agreed-upon quarantine protocol was immediately breached. It was judged better to open the Apollo 11 hatch to the air of the Pacific Ocean and, for all we then knew, expose the Earth to lunar pathogens, than to risk three seasick astronauts. So little concern was paid to quarantine that the aircraft-carrier crane scheduled to lift the command module unopened out of the Pacific was discovered at the last moment to be unsafe. Exit from Apollo 11 was required in the open sea."***

NASA did try to get some oversight to identify potential problems as they set up a multi-agency "Interagency Committee on Back Contamination (ICBC)" to advise on procedures involving NASA, Public Health Service, Department of Agriculture, Department of the Interior, National Academy of Sciences, and NASA itself.

However in the end this agency turned out not to have much power to change anything. The issue was that NASA wished to retain the ability to override any objections. After months of negotiation, the regulatory agencies agreed (Meltzer, 2012:129)

> ***"not to take any actions that might have an effect on the lunar program (such as refusing to let astronauts back in the country) without the 'unanimous recommendation of the agencies represented on the [Interagency] Committee [on Back Contamination].'***

As a result, consensus was needed from all the agencies involved for any modification of NASA's plans. Since the agencies included NASA, it meant that NASA could override any objections. So the committee was just advisory with no actual power to change the procedures.

Wolf Vishniac from the National Academy of Sciences had serious objections to NASA's recommendation: to open the capsule door in the open sea (Meltzer, 2012:203). His view is summarized by Meltzer as :

> ***Opening and venting the spacecraft to Earth's atmosphere after splashdown would, in his view, make the rest of Apollo's elaborate quarantine program pointless.***

David Sencer, from Public Health Service, who was the Chairman from the committee, also had serious objections. His view is summarized by Meltzer as :

> ***... NASA's plan to open the CM after splashdown, allowing its crew to egress while the module was still bobbing in the ocean, violated the concept of biological containment. NASA had not responded adequately to ICBC recommendations and did not apparently recognize the necessity of protecting Earth's environment against any possibility of extraterrestrial contamination.***

But NASA's view prevailed. NASA did consider removing the capsule from the sea in a crane but were concerned that if they tried to lift it onto an aircraft carrier, the carrier might run down the capsule with the astronauts on board, or that the capsule might bump against the side of the ship while it was lifted out of the sea (it wasn't just Carl Sagan's seasick astronauts they were concerned about) (Meltzer, 2012:203).

They did have a functioning crane, which was used on the day of the Apollo splashdown. After the astronauts left the Apollo capsule, and just before the ship set sail, the capsule was recovered from the ocean with a crane (NASA, 2019ya). However, Deke Slayton, director of flight operations at the Manned Spacecraft Center in Houston, Texas, and a former Mercury astronaut, was concerned that the spacecraft was too heavy to lift with the crew inside (Carter, 2001).

These issues could have been fixed. NASA's problem was that with a fast approaching deadline, they felt they were out of time to test and fix these potential issues with lifting astronauts out of the sea with a crane.

Vishniac wrote (Carter, 2001):

> ***The Apollo Program is moving at a pace which we [the ICBC] can not stop. It is equally clear that this irresistible progress is being used to brush aside the inconvenient restraints which the Interagency Committee has considered to be an essential part of the Quarantine Program.***

The situation will be different today. In the legal process these lessons from the Apollo missions are likely to be used as examples of how not to do it.

This is before the National Environmental Policy Act which was signed into law on 1st January 1970 (EPA, n.d.), so there was no requirement for an Environmental Impact Statement (EIS) for Apollo. The EIS is the start of the modern legal process in the USA

With everything under legal scrutiny with open public debate, NASA won't be permitted to use a fast approaching deadline to bypass objections today.

We cover the legal process later under:

- Minimum timeline: 2 years to develop consensus legal position, 6-7 years to file Environmental Impact Statement, 11 years to build sample return facility

- Legal process likely to extend well beyond 6 years with involvement of CDC, DOA, NOAA, OSHA etc, legislation of EU and members of ESA, international treaties, and international organizations like the World Health Organization

There are many other issues with quarantine that were never considered for the Apollo mission, because it never had a proper review. The current paper raises the issue of a life-long symptomless superspreader similar to Typhoid Mary (Korr, 2020) which doesn't seem to have been covered before in the planetary protection literature but seems likely to be one of the first issues epidemiologists would raise with the plans when it gets their attention, e.g. when the WHO get involved.

Although the Mars sample return mission is only returning samples, not humans, similar quarantine issues arise because humans can get exposed to the sample materials either when the samples are retrieved, or even before then, if the sample container is breached on re-entry, or during lapses and malfunctions in sample handling procedures after the samples are delivered to the sample receiving facility.

Quarantine issues are sure to come up in the legal process and we discuss them later under: Complexities of quarantine for technicians accidentally exposed to sample materials

Comet and asteroid sample returns are legally straightforward - either sterilized during collection - or Earth has a similar natural influx

Several comet and asteroid samples have been returned without much legal complexity, under the auspices of COSPAR and the Outer Space Treaty. However these were unrestricted returns, either because the sample was considered to be sterilized already; or because the preponderance of scientific evidence indicated that Earth has a natural influx equivalent to the sample; or for both reasons. Here are examples of both types of mission:

- Stardust: samples were sterilized by spike heating on collection (JPL, 2003).
- Hayabusa 1 & 2: the first surface sample was naturally sterilized by cosmic radiation, and the second sample from an artificially induced impact crater was similar to material transferred to Earth through natural processes, and so needed no special treatment (Kminek et al, 1999) (Yano et al, n.d.).

Richard Greenberg originally proposed the natural influx criterion as his "Natural Contamination Standard" (Greenberg et. al, 2001).

Controversial 2019 report by Stern et al. recommended classifying parts of Mars similar to the Apollo 11 lunar requirements - no sterilization in the forward direction (Category II) – but Earth’s biosphere still protected in the backwards direction (restricted Category V)

From time to time there is controversy about whether we could classify parts of Mars in the forwards direction as like the Moon with only a remote chance of contaminating Mars with terrestrial life.

However, almost all agree that we need to protect Earth’s biosphere in the backwards direction from Mars back to Earth.

Stern et al’s report in 2019 was an example. They recommend that some regions of Mars are designated as safe for human landings, similarly to the Moon at the time of the Apollo 11 landing in the 1960s.

However, in the backwards direction, they recommended keeping the current classification for sample returns as a Category V, “Restricted Earth Return” (Zurbuchen, 2019).

From COSPAR (COSPAR, 2011):

> ***Category V missions** comprise all Earth-return missions. The concern for these missions is the protection of the terrestrial system, the Earth and the Moon. (The Moon must be protected from back contamination to retain freedom from planetary protection requirements on Earth-Moon travel.)*
>
> *… in a subcategory defined as “restricted Earth return,” the highest degree of concern is expressed by the absolute prohibition of destructive impact upon return, the need for containment throughout the return phase of all returned hardware which directly contacted the target body or unsterilized material from the body, and the need for containment of any unsterilized sample collected and returned to Earth.*
>
> *Post-mission, there is a need to conduct timely analyses of any unsterilized sample collected and returned to Earth, under strict containment, and using the most sensitive techniques. If any sign of the existence of a non terrestrial replicating entity is found, the returned sample must remain contained unless treated by an effective sterilizing procedure. …*

If these recommendations are adopted, precautions still have to be taken to protect Earth from a sample return.

At present the whole of Mars is categorized as Category IV in the forward direction (with subclassifications a, b, and c) which require various levels of sterilization to protect any potential habitats from contamination by terrestrial microbes on our landers (COSPAR, 2011).

> ***Category IV missions*** *comprise certain types of missions (mostly probe and lander) to a target body of chemical evolution and/or origin of life interest and for which scientific opinion provides a significant chance of contamination which could compromise future investigations.*
>
> *Requirements imposed include rather detailed documentation (more involved than Category III), including a bioassay to enumerate the bioburden, a probability of contamination analysis, an inventory of the bulk constituent organics and an increased number of implementing procedures. The implementing procedures required may include trajectory biasing, cleanrooms, bioburden reduction, possible partial sterilization of the direct contact hardware and a bioshield for that hardware. Generally, the requirements and compliance are similar to Viking, with the exception of complete lander/probe sterilization.*

A Category IV classification makes a human landing impossible. Humans can never be sterilized of microbes to the levels required, because of the diversity of microbial life that accompanies us. As of 2019, more than 150,000 strains have been detected in the human microbiome, in nearly 5,000 distinct species level genome bins [species essentially] (Du Toit, 2019). Other microbes also inhabit human occupied spaceships.

Stern et al. recommended that parts of Mars are reclassified as “Category II” similarly to the Moon, so that humans can land there without taking special measures to protect potential native Martian life. From COSPAR (COSPAR, 2011):

> ***Category II missions*** *comprise all types of missions to those target bodies where there is significant interest relative to the process of chemical evolution and the origin of life, but where there is only a remote chance that contamination carried by a spacecraft could compromise future investigations.*
>
> *The requirements are for simple documentation only. Preparation of a short planetary protection plan is required for these flight projects primarily to outline intended or potential impact targets, brief Pre-and Post-launch analyses detailing impact strategies, and a Post-encounter and End-of-Mission Report which will provide the location of impact if such an event occurs.*

Stern et al cited a recommendation from 2014 (Rummel et al , 2014) in support for their suggestion to map out regions of the Mars surface as Category II instead of Category IV (Zurbuchen, 2019) (NAS, 2020:26) writing:

> *NASA should reconsider how much of the Martian surface and subsurface could be Category II versus IV by revisiting assumptions and performing new analysis of*

transport, survival and amplification in order to reassess the risk of survival and propagation of terrestrial biota on Mars. ... Rummel et al. (2014) have shown that many areas of the surface are not locations of PP [planetary protection] concern

2020 Review committee modified recommendations of 2019 report, saying our knowledge is not yet sufficient to classify parts of Mars as suitable for an unsterilized Category II mission in the forward direction – agrees on need to protect Earth in backwards direction

The 2020 committee to review the Stern et al report modified some of the recommendations. They agreed on the backward contamination classification but in the forwards direction they say that a planetary protection category is given to a mission rather than a location, (NAS, 2020:27)

> *However, the PPIRB report perpetuates confusion about planetary protection terminology. The report suggests that much of the Moon can be re-defined as Category I, while areas of Mars may be reassigned to Category II (see Appendix E). However, in planetary protection policy, missions are categorized and not planetary bodies and their surfaces. NASA OPP typically reviews a mission's objectives and provides the mission with a categorization letter, and the assigned category determines the planetary protection requirements for the mission.*
>
> *As scientific understanding progresses, certain missions to the Moon, Mars, ocean worlds, and small bodies could receive a categorization that imposes fewer planetary protection requirements.*

Later the committee say that the current state of research is not yet sufficient to determine regions of Mars that could be targets of Category II missions (NAS, 2020:27)

> *The current state of research does not yet appear to be adequate to determine whether there are regions on Mars where human explorers or commercial missions might land with minimal planetary protection implications.*
>
> *… Through information obtained from past and future Mars missions and complementary research, some regions may have sufficiently low risk of forward or back contamination so that a lander would only be required to follow the current Category II requirements.*

Similar situation in 2014 / 2015: 2014 report said maps can identify areas of Mars of planetary protection concern in the forwards direction then 2015 review modified those recommendations, saying maps can't yet be used – due to knowledge gaps on survival of terrestrial life in dust storms and potential for life to survive in microhabitats hard to detect from orbit

It was a very similar situation in 2014/15. Stern in 2019 cited the 2014 report (Rummel et al , 2014) to support their recommendation to use maps to classify parts of Mars as category II.

But even as that 2014 report by Rummel et al was in publication, NASA and ESA commissioned a review which overturned many of its findings, similarly to the 2020 review of Stern et al.

In particular, the 2015 review overturned the suggestion from the 2014 review that areas not of Planetary Protection concern can be delineated using maps, saying a map of RSLs with buffer zones can only represent our incomplete state of knowledge at a particular time (Board, 2015 : Ch 5, p 28)

> *… As RSL studies are a very active field of Mars research, it is expected that the number of fully and partially confirmed RSL will increase from now to the near future, just as it has increased from their first. Hence, the map displayed in Figure 47 represents only a snapshot in time and will probably be outdated soon.*
>
> *While it is helpful to provide a general overview of regions that may be favorable for the formation of RSL, it is of limited use in the identification of Uncertain or Special Regions. The same applies to other maps that also may be updated soon.*
>
> *…*
>
> *Another potential source of misinterpretation related to the use of maps in Special Region studies is the issue of scale. … (see also the discussion in Chapter 2, "Detectability of Potential Small Scale Microbial Habitats")*
>
> *… Maps, which come necessarily at a fixed scale, can only provide information at that scale and are, therefore, generalizations.*
>
> *…*
>
> *Maps that illustrate the distribution of specific relevant landforms or other surface features can only represent the current (and incomplete) state of knowledge for a specific time—knowledge that will certainly be subject to change or be updated as new information is obtained.*

Chapter 2 which they refer to there mentions knowledge gaps for microhabitats and the possibility of biofilm to make microclimates for life not easy to detect from orbit, as well as the issue of translocation of terrestrial life to remote regions of Mars, in (Board, 2015 : Ch 2, p 12)

> *In particular, the issues of translocation of terrestrial contamination and the behavior of multispecies populations in extreme environments, produce uncertainty in the determination of Special Regions, because such regions might not be isolated from the rest of the planet (translocation), because microbial communities could occupy dispersed, small-scale habitats or might be able to alter local environmental parameters and syntrophic consortial interactions*
>
> *[**syntrophic interactions:** where microbes exchange metabolites in an overall combined metabolism that wouldn't be feasible for either species individually (Seiber at al, 2010)].*
>
> *These issues, together with the present lack of knowledge about the limits of life on Earth and the uncertainty of the relationship between the large-scale and micro-scale environments at any given place make the definition of Special Regions difficult.*
>
> *…*
>
> ***Detectability of Potential Small Scale Microbial Habitats***
>
> *There are many examples of small-scale and microscale environments on Earth … that can host microbial communities, including biofilms, which may only be a few cell layers thick. The biofilm mode of growth, as noted previously, can provide affordable conditions for microbial propagation despite adverse and extreme conditions in the surroundings. On Earth, the heterogeneity of microbial colonization in extreme environments has become more obvious in recent years.*
>
> *…*
>
> *To identify Special Regions across the full range of spatial scales relevant to microorganisms, a better understanding of the temperature and water activity of potential microenvironments on Mars is necessary.*
>
> *…*
>
> *Craters, and even microenvironments underneath and on the underside of rocks, could potentially provide favorable conditions for the establishment of life on Mars, potentially leading to the recognition of Special Regions where landscape-scale temperature and humidity conditions would not enable it.*

The 2015 review also identified a knowledge gap about whether viable microorganisms can be transported in Martian dust storms, especially in cell clusters or aggregates (Board, 2015 :12)

A potential problem with designating Special Regions on Mars is that viable microorganisms that survive the trip to Mars could be transported into a distant Special Region by atmospheric processes, landslides, avalanches (although this risk is considered minimal), meteorite impact ejecta, and lander impact ejecta. In addition to dilution effects, the flux of ultraviolet radiation within the martian atmosphere would be deleterious to most airborne microbes and spores.

However, dust could attenuate this radiation and enhance microbial viability. In addition, for microbes growing not as single cells but as tetrades or larger cell chains, clusters, or aggregates, the inner cells are protected against ultraviolet radiation. Examples are methanogenic archaea like Methanosarcina, halophilic archaea like Halococcus, or cyanobacteria like Gloeocapsa. This is certainly something that could be studied and confirmed or rejected in terrestrial Mars simulation chambers where such transport processes for microbes (e.g., by dust storms) are investigated. The SR-SAG2 report does not adequately discuss the transport of material in the martian atmosphere.

See also *(Race et al, 2015: 34)*.

Based on (Board, 2015) if we wish to make a Category II classification of a mission to a specific location on Mars we need to first fill in our knowledge gaps on transfer of terrestrial life in the dust storms, scrutinize any proposed landing site carefully for RSLs and fill in knowledge gaps on the potential for microhabitats on Mars not easily detected from orbit. They make several recommendations in the appendix A including (Board, 2015: App. A, p. 46)

Undertake in situ mapping of the microheterogeneity of biologically important environmental parameters in the landing ellipse of a future space mission dedicated to astrobiology.

Based on that knowledge we can then decide whether such microhabitats could occur in the proposed landing site and whether introduced terrestrial life could contaminate distant microhabitats elsewhere on Mars.

See: Scenario of localized forward contamination on Mars depends on whether terrestrial life can be transported in dust storms

All agree Mars sample returns need to be treated as restricted Earth return with potential for adverse changes to the environment of Earth

Whether in the future, parts of Mars are classified as Category II or remain Category IV in the forward direction makes no difference to the legal process for sample return, since Stern et al accept the need to protect Earth from returned samples from anywhere on Mars, including from the regions they recommend to classify as Category II in the forwards direction.

This is similar to the approach used for Apollo 11 when there was still thought to be a small chance of native lunar microbes that could have adverse effects on the environment of Earth, but there was already known to be no chance of terrestrial life spreading on the Moon.

With the Moon we soon proved that Earth is safe from samples returned from the Moon. But suppose we find samples returned from Mars are unsafe? What then happens to the classification long term?

Example future scenarios where we protect Earth's biosphere from backwards contamination indefinitely - but with no or minimal risk of forward contamination of Mars – i.e. similar to Apollo 11 era classification of the Moon but indefinitely

We saw that Stern et al's recommendation to classify parts of Mars as safe from terrestrial life in the forward direction is not likely to be adopted at present. See:

- 2020 Review committee modified the recommendations of the 2019 report, saying our knowledge is not yet sufficient to classify parts of Mars as suitable for an unsterilized Category II mission in the forward direction – agrees on need to protect Earth in backwards direction (above)

However, Stern et al's recommendation does remains a potential future scenario, that some day we will be able to classify most or all missions to parts or all of Mars as category II, in the forwards direction (COSPAR, 2011):

> ***"… where there is significant interest relative to the process of chemical evolution and the origin of life, but where there is only a remote chance that contamination carried by a spacecraft could compromise future investigations."***

If we are able to classify parts of Mars in this way, we might still need to be careful with samples returned to Earth as this classification is consistent with a "restricted Earth return" Category V sample return that may contain native life (COSPAR, 2011).

> ***"restricted Earth return," the highest degree of concern is expressed by the absolute prohibition of destructive impact upon return, the need for containment throughout the return phase of all returned hardware which directly contacted the target body or unsterilized material from the body, and the need for containment of any unsterilized sample collected and returned to Earth.***

Indeed this was the precise situation for the Moon in 1969. Originally in the early 1960s there was concern about forward contamination too (Sagan, 1961) and the first unmanned landings on the Moon were sterilized. But by 1969, the risk of forward contamination was thought negligible.

However, we will see that in an alternative history where the Moon is slightly more habitable, we need to use the Apollo 11 era classification indefinitely. We will see that this is also a possible future scenario for Mars.

So why was it necessary to protect Earth in 1969 when we already knew there was no need for sterilization in the forward direction?

Carl Sagan's hypothesis of a subsurface habitable layer on the Moon at a depth of tens of meters – which could risk backwards contamination of Earth – and originally there was thought to be a low risk of forwards contamination

A region can have habitats for native life, and yet have only a remote chance of terrestrial contamination compromising future investigations.

That was the exact scenario in 1969. It was still possible there could be viable life on the surface that could be returned to Earth even though it would be hard for the astronauts to contaminate those habitats in the forwards direction.

The reason this is possible is that before Apollo, Carl Sagan calculated a small chance of a habitable layer of organics at a depth of tens of meters below the lunar surface. The structure of the Moon's near subsurface was unknown at the time, and not much was known about how the Moon formed or its past history.

So now imagine an alternative history where the Moon had a more habitable past. In this scenario, the Moon has deep layers with organics from its earlier seas or lakes. It also has organics brought to the Moon on comets after it formed, or incorporated when the Moon formed, just as for early Earth.

Based on a scenario like this, Carl Sagan calculated that a layer at depth of tens of meters would be protected from cosmic radiation and coincidentally might also be warm enough for liquid water (Sagan, 1961).

Also, at the time of Apollo, scientists still didn't know much about surface processes on the Moon. For instance, it remained a possibility that there were some volcanic processes. Not enough was known about surface processes on the Moon to rule out the possibility of spores from this deep subsurface layer sometimes reaching the surface and surviving for a while, perhaps in the shadow of a rock or just below the surface dust.

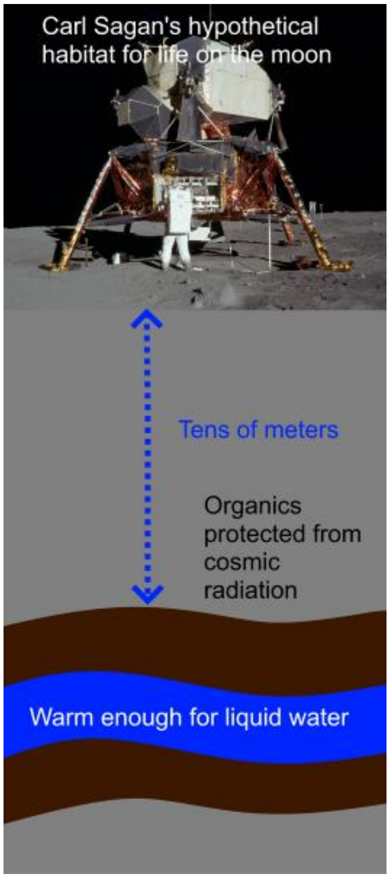


Figure 3: Sagan's hypothetical liquid water layer on the Moon (Sagan, 1961), photograph is of Buzz Aldrin and the Eagle lunar landing module, 1969 (NASA, 1995).

In this scenario, Carl Sagan's deep layer is safe from forward contamination by the astronauts, and yet, it is a possible source of spores that could contaminate the astronauts or their samples on the Moon, which can then be returned to Earth.

At first scientists thought it was possible that deep subsurface habitats could be contaminated in the forward direction and the early missions were sterilized out of concern of forward contamination of potential habitats below the surface on the Moon (Sagan, 1961). Since microbes survived even inside electronic components such as capacitors and resistors, JPL sterilized Rangers III, IV and V with dry heat sterilization followed by external sterilization with ethylene oxide just before launch (Phillips, 1974:26).

Decision to stop sterilizing missions to the Moon in 1963 because any forward contamination was expected to be localized – even if there were habitats below the surface

On Sept 9, 1963 with publication of NASA Management Manual NMI-4-4-1 they relaxed this requirement saying (Phillips, 1974:30)

> ***"The NASA policy is based on acceptance of the scientific opinion that lunar surface conditions would mitigate against reproduction of known terrestrial microorganisms and that, if subsurface penetration of viable organisms were to be caused by spacecraft impact, proliferation would remain highly localized."***

So, by the time of Apollo, forward contamination of the lunar surface was thought to have only a remote chance of compromising future investigations elsewhere on the lunar surface. Some local contamination might be possible but there were no processes that could move it around to other parts of the Moon.

This is not mentioned in the manual, however, even Carl Sagan's deep warm habitable layer, if it existed, would have been hard to contaminate from the surface in the conditions as they were known by then.

So, in this way, a region can have habitats for native life, and even be vulnerable to terrestrial contamination locally on the surface, yet with no way for the contamination to spread, it can have only a remote chance of terrestrial contamination compromising future investigations. So long as any local contamination is documented it would be classified as class II (COSPAR, 2011):

> ***"… where there is significant interest relative to the process of chemical evolution and the origin of life, but where there is only a remote chance that contamination carried by a spacecraft could compromise future investigations."***

Although Sagan's model is no longer supported in its original form, some scientists think there may be isolated patches of ice well below the surface supported by volatiles vented from deep below the surface of the Moon, and even a potential deep biosphere today, similarly to Sagan's suggestion, see

- Suggestion by Crotts of a subsurface ice layer on the Moon deep enough for liquid water and by Loeb of a subsurface biosphere on the Moon (below)

It's not a likely scenario in our solar system. But in an alternative history, suppose the Transient Lunar Phenomena, transient brightening that could be escapes of volatiles - actually carry spores from the deep subsurface to the surface. Suppose that this is mirror life or in some way with potential to ham Earth's biosphere. Then in that alternative history, we need to protect

Earth indefinitely from these spores but the risk of forward contamination would be effectively zero as the spores are transported in one direction only from the subsurface to the surface.

Scenario of localized forward contamination on Mars depends on whether terrestrial life can be transported in dust storms

Whether a similar scenario is possible for Mars depends on whether there is life on Mars, of course, and then on whether terrestrial microbes can be transported to other parts of Mars in the Martian dust storms.

If dust transport of terrestrial life is possible on Mars, it will be hard to impossible to prevent forwards contamination of the rest of the Martian surface once any part is contaminated

However, if dust transport is impossible, terrestrial life might still spread over the surface but this would be a slow process, likely taking millennia. Depending how isolated the habitats are, it might even be next to impossible for life to spread beyond a very localized more habitable region such as a forward contaminated site of Recurrent Slope Lineae.

A worst case in the forward direction would be a crash of a spaceship containing astronauts. When space shuttle Columbia crashed, it resulted in debris spread over a distance of over 400 kilometers and an area of several thousand square kilometers on Earth (CAIB, 2003:306-7)

If the dust storms can't transport terrestrial life, even after such a crash, contamination would still remain localized to a few thousand square kilometers of the Martian surface. Most of Mars would remain uncontaminated. The crash would have no serious effect on future scientific experiments, as scientists would know where the contamination is and would study the rest of the surface of Mars. There would be many similar regions that aren’t contaminated and very unlikely that such a crash would impact on a habitat with some unique form of life that can’t be found elsewhere on Mars.

The issue of dust transport of terrestrial organisms is a knowledge gap and needs to be investigated carefully. The UV may not be a limiting factor. Billi et al found that a dried biofilm of chroococcidiopsis mixed with regolith only 0.015 to 0.03 millimeters thick (15 to 30 microns) could survive 469 days of Mars surface UV attenuated by a 0.1% neutral density filter to conditions of partial shade on Mars (Billi et al, 2019b).

They calculated that this dose is equivalent to 8 hours of full sunlight on Mars. Even in full sunlight, that would give the biofilm enough time to get transported 100 km at 5 km/s (Billi et al, 2019a). The UV is also greatly reduced in the Martian dust storms.

The reactive perchlorates that pervade the Martian dust may make dust transport of terrestrial life less likely, as they can sterilize the dust from terrestrial microbes and spores. However many microbes are able to live in perchlorate brines and some can even use the perchlorates in their metabolism. The dust perchlorates are mainly an issue if they are activated to chlorates and chlorites by UV. For a discussion which also looks at hypothetical more resilient Martian life, see discussion in:

- Could Martian life be transported in dust storms or dust devils, and if so, could any of it still be viable when it reaches Perseverance?

This question is not likely to be resolved until we can return samples of dust from Mars and study the effect of UV and the dust storms on the dust. See

- **Recommendation:** Extra sample of air and airfall dust to search for Martian life, assess forward contamination issues for terrestrial microbes, dust dangers for astronauts, and to return a random sample of wind-eroded rock from distant parts of Mars

It doesn't seem to be established yet that forward contamination will be localized on Mars to the area explored by a spacecraft or human mission or crash site. However, if after detailed scientific study we find it is localized, this leads to future scenarios where forwards contamination is of concern mainly local to the landing sites, exploration regions and crash debris fields, at least over timescales of decades. There might even be no way for terrestrial life to get from one Martian habitat to another except through humans moving it.

Even local colonization by terrestrial life might also take a long time if habitats are also colonized only on timescales of millennia as for some habitats in cold dry deserts.

In this scenario, it might be possible to protect sites on Mars of special scientific interest from contamination with terrestrial life almost indefinitely, e.g. a selection of the RSLs, even in a region close to a human base.

Scenario of localized forward contamination by terrestrial life, but with Martian life still able to spread in Martian dust storms using spores adapted to Mars and more resilient than terrestrial spores

Native Martian life might be adapted to spread in Martian dust storms in ways that terrestrial life is not. See:

- Native Martian propagules of up to half a millimeter in diameter (including spore aggregates and hyphal fragments) could travel long distances with repeated bounces (saltation) - if they can withstand the impacts of the bounces

Also

- Martian life could also use iron oxides from the dust for protection from the impact stresses of the saltation bounces - or it might use chitin - a biomaterial which is extremely hard and also elastic and is found in terrestrial fungi and lichens

So there are scenarios where Martian life in dust from other parts of Mars could potentially be a backwards concern, and yet with no forwards contamination concern for windblown terrestrial microbes, see:

- Could Martian life be transported in dust storms or dust devils, and if so, could any of it still be viable when it reaches Perseverance? below.

Scenario of no possibility of forward contamination because Martian life occurs in extreme habitats inaccessible to terrestrial life

Another way backward contamination could be a concern, when forward contamination is not, is if Martian life occupies a microhabitat on Mars that is not habitable for terrestrial life. Mars could have polyextremophiles with capabilities that extend beyond those of terrestrial life.

In this scenario more capable Martian life might survive in extreme conditions on Mars beyond the limits of terrestrial life as well as terrestrial habitats, while terrestrial life might only be able to survive on Earth and not on Mars.

For examples of how this could be possible see

- How Martian life could make perchlorate brines habitable when they only have enough water activity at -70 °C – biofilms retaining water at higher temperatures - chaotropic agents permitting normal life processes at lower temperatures – and novel biochemistry for ultra low temperatures

All possibilities remain open: no need for sterilization to protect Mars, while Earth needs to be protected indefinitely – or no protection either way - or protection indefinitely both ways - or need to sterilize spacecraft to protect Mars indefinitely with no need to protect Earth or astronauts from returned materials

In summary, some of the ways Mars could resemble Sagan's ideas for the Moon in 1969, with the need to use a protection classification similar to Apollo 11 indefinitely include these example three safe forward contamination scenarios:

- **Scenario 1: Native habitats for Martian life can't be colonized with terrestrial life** (e.g. too cold for terrestrial life), but they are habitable to Martian life extremophiles more capable than terrestrial life, OR

- **Scenario 2: Contamination with terrestrial life spreads only with great difficulty over thousands of years from one part to another of the Martian surface**. In this scenario, **forward c**ontamination is possible, slowly, and there are habitats that both Martian and terrestrial life can colonize, but there is only a remote chance of it compromising near future investigations as the habitats will be colonized only slowly (depending on views about whether it matters to compromise investigations millennia in the future), OR

- **Scenario 3: We find that terrestrial life can't be transported in the Martian dust storms but Martian life can be transported in the dust** so the spores can reach our spacecraft even if they don't land near a habitat on Mars.

In these scenarios, the level of protection needed for Earth depends on the form of life we find on Mars if any.

For an example of a scenario where Mars astronauts can visit Mars with no risk of forward contamination but never return to Earth, we can combine any of those three safe forward contamination scenarios with a discovery of Martian mirror life extremophiles capable of surviving on Mars, and also on Earth. The microbes brought by the astronauts to Mars would then be either unable to spread on Mars or limited in how far they can travel, while the mirror life on Mars could return to Earth on any return trip in their spaceships and it may be impossible to prevent the two biospheres mixing after such a return trip. See:

[Similar considerations apply to astronauts returning from Mars - in some scenarios such as mirror Martian life, astronaut quarantine would be insufficient to protect Earth's biosphere]

Scenarios here range from no possibility of astronauts ever returning, to scenarios with a need for quarantine whenever astronauts return to Earth, and to scenarios where astronauts can travel both ways with no restrictions, as for the Moon, because though there is life on Mars it's known to be harmless for Earth, and Earth life is known to be harmless for Mars.

However, we don't know that any of those three safe forward contamination scenarios apply at present. With our current limited understanding of Mars, it remains a possible future scenario that we have to protect Mars indefinitely from terrestrial life

One way this could happen is if:

1. Native habitats can be colonized with some terrestrial species AND

2. At least some of those terrestrial species can also spread easily in dust storms

In that case once the relevant terrestrial life gets established on Mars, it will eventually get to all the native habitats on Mars within reach of the dust storms. This depends also on how far the terrestrial life can spread in each storm and on how far apart the oases are of habitable regions for terrestrial life on Mars.

If it can spread hundreds, or thousands of kilometers in a dust storm as for dust blown off terrestrial deserts, and if there are habitats such as RSLs widespread over Mars, then, since the dust-storms are global there will be almost nowhere on the surface that won't eventually be contaminated. For a discussion in the opposite direction of distant Martian microbes spread thousands of kilometers, see:

- Potential for spores and other propagules transferred from distant regions of Mars similarly to transfer of spores from the Gobi desert to Japan

One possibility here is that terrestrial and Martian life both have the capability to spread throughout the planet in dust storms. In this scenario, we might show that the two biospheres can mix safely (both ways, or in one direction), but if not, planetary protection would be needed both ways indefinitely.

It's also possible that unlike the Moon we find vulnerable habitats on Mars that terrestrial life could contaminate, but with no risk of that life harming Earth – an example here could be some early form of life of limited capabilities that astrobiologists assess is of no risk to Earth as it can't compete with terrestrial life, but that is vulnerable to terrestrial life which is easily spread in the dust storms.

In this case we need to sterilize spacecraft to explore Mars indefinitely – unless we decide it is okay to contaminate Mars with terrestrial life, perhaps protecting early Martian life or recovering it and saving it in orbital habitats simulating the Martian environment.

Finally we have scenarios as for the Moon where there is no life on Mars and no possibility of terrestrial life surviving on Mars, and a similar situation, scenarios where life on Mars and on Earth can co-exist and the biospheres mix harmlessly or even beneficially to both planets.

If the two biospheres can mix harmlessly, we might still want to keep them separate for some period of time, long enough to study the differences and how Mars' current biosphere operates, and to see how much the two forms of life have diverged if the life on Mars is closely related through panspermia.

That is a future scenario, but there are many other scenarios, and until we know more, we need to continue to be careful of forward contamination too. Until we know more we have to be specially careful about forward contamination via windblown dust, or via contamination of local microhabitats that may be hard to detect from orbit, as we saw in:

- ;

So, we shouldn't assume in advance that once we find out more, planetary protection for Mars will develop in the same as for the Moon and that we soon find that astronauts can travel both ways with no problems. That is only one of many possible future scenarios.

The reason we need to do planetary protection is because we don't know the answer to these questions yet.

Another possibility we need to look at is the situation where Mars has only uninhabited habitats, but ones that terrestrial life could colonize and spread through rapidly.

Scenario of no present day life on Mars could give unique opportunity to study uninhabited habitats on another terrestrial planet, and microbes accidentally introduced to an uninhabited planet in the wrong sequence could make Mars less habitable for colonists – need to allow time for study first

The scenario where Mars has uninhabited habitats might seem at first to be a scenario with no risk of forward contamination harming future scientific experiments. However, in that scenario, Mars gives us a unique opportunity to study uninhabited habitats on another terrestrial planet and find out about complex chemistry and test theories about what came before life, such as proto life or naked RNA etc.

- Scenario of a pre-biotic uncontaminated Mars of great scientific value - microhabitats with autopoetic cells, Ostwald crystals breaking the mirror symmetry of organics, or naked genes, adsorbed on mineral particles with impenetrable membrane caps, but not yet quite life

So these scenarios also may need some care in the forwards direction.

Also early unplanned future contamination with whatever gets to Mars on the first spacecraft could interfere with plans for step by step terraforming of Mars if that is what colonists wish to do.

The strong Gaia hypothesis might seem to suggest that any life on Mars would automatically make it more habitable for us. However that is not necessarily true.

Life on Mars might not automatically make it more habitable, indeed, it could make it colder and drier by taking CO_2 out of the atmosphere, which might perhaps have already happened if there is life there, as we'll see in:

- Suggestion of a self perpetuating "Swansong Gaia" maintaining conditions slightly above minimal habitability for billions of years - as a way for early life to continue through to present day Mars

One terraforming suggestion is to warm Mars up with methanogens. But methanotrophs would have the opposite effect, of removing native abiotic methane, while photosynthetic life would rapidly remove any CO_2 liberated into a warming planet.

Life introduced to Mars could also make it more habitable for some forms of life, but not for us, e.g. a methane rich atmosphere would lead to a warmer Mars but one that is less habitable for humans than an atmosphere that has oxygen in it.

Finally, depending on the microbes that seed Mars, it is also possible for them to stimulate reactions deep below the surface to calcify subsurface water, cement it, preventing methane reaching the surface as well as making it unavailable for use by colonists. See:

- Self limiting consortiums of methanogens, methanotrophs, and Fe(III)-reducing bacteria converting underground aquifers to calcite, and so maintaining a subsurface barely habitable Swansong Gaia hydrology

Then there is the possibility of rapid evolution of microbes with new harmful capabilities. Martian environment would be novel for terrestrial extremophiles. Life from Earth seeded on Mars accidentally could evolve new capabilities. For instance, it might rapidly evolve the ability to use chaotropic agents to survive at lower temperatures than usually encountered on Earth – so that it could survive in freezers, or it might evolve greater resistance to UV, or to ionizing radiation, in extreme conditions of higher levels of UV, far colder temperatures, and far more ionizing radiation than it encounters on Earth.

In those scenarios it may still be possible to colonize Mars, but the sequence of microbes introduced to Mars may be important, to make it optimal for human colonization and to eliminate risks such as calcifying aquifers in an early stage in the process.

The difference from human settlements on the Moon is that a Mars with uninhabited habitats MIGHT have the potential for terrestrial life to transform it or to evolve and change on Mars and the uninhabited habitats may also be of interest for prebiotic chemistry that would be destroyed by terrestrial life. There is no such risk for the Moon.

How we understood the Moon in 1969 compared to Mars today - Mars with a thin atmosphere and liquid water, is more favorable for life than the Moon was thought to be back then

The Moon was soon shown not to be a contamination risk for Earth. The surface layers of the Moon are now known to consist of fine grained regolith reworked by meteorite impacts and to be on average 10 meters deep (though the depth is highly variable) and this layer is ultra dry with no possibility of life in it: (Hiesinger et al, 2006). The samples returned by Apollo contained only trace amounts of organics, most of which are probably due to contamination (Elsila et al, 2016).

The Moon is now classified as Category II in the forwards direction and unrestricted Category V in the backwards direction meaning astronauts just need to document contamination in the forwards direction.

Since the time of Apollo, we have detected organics and water at the poles. However, the moon remains classified as Category II because the conditions are too cold for life at the poles.

However there are differences that favour life on Mars more than they did for life on the Moon in the 1960s or even today with our more complex understanding of the Moon.

In summary:

- **We have clear evidence today, that early Mars had conditions favourable for evolution of life, with lakes and even seas.**
    - In the 1960s we had no clear evidence for a past habitable Moon. There was weak evidence suggesting the Mares were ancient sea beds, but this evidence was not persuasive (and of course soon turned out to be false)

- **Curiosity has detected ultra cold salty brines on the surface of sand dunes just before dawn / after dusk and below the surface just after sun rises and just before the sun sets.**
    - There was no detection of liquid water on the Moon, just a hypothetical layer that could exist at a depth of tens of meters enriched with organics.

- **Mars has a sparse atmosphere humid enough for thin layers of frost to form at night in many regions.** Some terrestrial blue green algae and lichen have been able to grow in Mars simulation conditions using just the night time humidity in partial shade.
    - The Moon has no atmosphere, only an exosphere. Frost can't form there at night, and by 1969 it was already clear no life could grow on the surface of

the Moon.

- **The Martian dust storms can transport spores from distant regions of Mars**.
    - There is no way for life to be transported from distant parts of the Moon.

- **A small minority of scientists believe that the Viking landers may have detected life in the 1970s. These observations are puzzling because of an apparent circadian rhythm with the radiolabeled emissions offset by 2 hours from the maximum temperature are hard to explain using chemistry.**
    - There were no puzzling observations from lunar experiments that anyone interpreted as possibly due to life.

So, to fill that out in more detail, this time the other way round, the Moon cases first:

In the 1960s it seemed possible that the Moon was more habitable in the past, and some still believed that the lunar Mares could be the beds of ancient seas (Gilvarry, 1964a) (Gilvarry, 1964b). **However there was nothing by way of clear proof and we soon found out that the Mares were basalt plains flooded by ancient lava flows.**

With Mars, however, we now have clear proof it had past conditions favourable for life, with water flowing at times, forming lakes, and temporary rivers and a recurrent ocean covering most of the northern hemisphere from time to time in early Mars. This ocean may have been covered with a thick layer of ice most of the time, but it would still be habitable in hydrothermal vents beneath the ice (Vago et al, 2017). Mars' ocean may also have been mostly liquid at times. That includes at least two tsunamis, likely the result of impacts (Rodriguez et al, 2019) which are challenges to explain but suggest at least a temporary largely liquid ocean (Turbet et al, 2019), as recently as 3.4 billion years ago (Rodriguez et al, 2019). We also have clear evidence of deltas flowing into the ancient seas and evidence that strongly suggests the lake in Gale Crater was open water not covered by a layer of ic and most recently evidence from the Chinese Zharong rover of substantial amounts of surface water in Utopia Planitia as recently as 700 million years ago.. See: Evidence of temporarily more habitable Mars backs up the modelling including evidence from the Zharong rover of substantial amounts of water in Utopia Planitia about 700 million years ago – would life survive in a planet with these frequent changes of habitability or does it go extinct easily, and if so does it re-evolve?

-

Another difference is there was no detection of liquid water in any form on the Moon in 1969 (or since then). In the vacuum conditions even cold salty brines are impossible. But the thin atmosphere of Mars is below the triple point of at the lowest points meaning water can be briefly stable for instance in the Hellas basin (Schulze-Makuch et al, 2010b).

- **We already have good evidence for salty water on present day Mars, from Curiosity in Gale crater.** Salty water is stable at higher temperatures and these brines are closer to the equator and a drier location than Jezero crater. These are thought to be calcium perchlorate brines which stay liquid at extremely low temperatures. However, though too cold for terrestrial life they may still be habitable to more cold tolerant unfamiliar life, or biofilms may be able to modify the habitability of the brines.

See: Detection by Curiosity rover of liquid water as perchlorate brines in Gale crater sand dunes and similar conditions are predicted in Jezero crater dunes

Surface conditions on the Moon were already known to rule out any possibility of reproduction of life on the surface exposed to the vacuum conditions (Sagan, 1961:25). We knew of the near vacuum surface conditions already from radio observations (Stern, 1999),

- **Surface conditions on Mars however to our best knowledge don't yet rule out the possibility of reproduction of life adapted to extreme conditions in microbial niches.**

  See: Surface conditions of ionizing radiation, UV radiation, cold and chemical conditions don't rule out the presence of life

  We have also managed to find photosynthetic polyextremophiles on Earth able to grow in our best attempts at Mars surface simulation conditions using only the night time humidity in partial shade. Nothing resembling this is possible on the Moon.
  See: Experiments with black yeasts, fungi and lichens in Mars simulation conditions suggest life could use the night time humidity directly without liquid water

In 1969, nobody thought they had detected life on the Moon. They did look for life in the samples returned from the Moon, trying to culture it in petri dishes of agar, and there was nothing resembling life in them (NASA, 2019nsfl)

Video: NASA Searches for Life from the Moon in Recently Rediscovered Historic Footage

- **A small minority of scientists including Levin and Miller believe that there is a possibility that the Viking landers detected life on Mars in the 1970s**, with the labelled release experiment. This view was strengthened by the discovery in the old data of what look like circadian rhythms offset by two hours from the temperature fluctuations (Levin et al, 2016) (Miller et al, 2002).

  See: Could Perseverance's samples from Jezero crater in the equatorial regions of Mars contain viable or well preserved present day life?

Also: Puzzles from the Viking landers – why some think Viking detected life already in the 1970s – evolved gases in the labelled release experiment offset from temperature fluctuations by as much as two hours, more typical of a circadian rhythm than a chemical reaction

The Moon doesn't have any processes with the potential to transport viable spores from distant locations.

- **Mars is more physically connected through atmospheric processes than the Moon and especially through the dust storms**. It's not yet known to be impossible that viable spores from more distant parts of Mars might get to the rover carried in the global dust storms.

  See: Potential for spores and other propagules from nearby or distant regions of Mars

Views of astrobiologists on the possibility of present-day life on or near the surface of Mars

Many astrobiologists have expressed a view that present day Mars may well be habitable to terrestrial life in part. This need not mean that there is life there, it could have uninhabited habitats i.e. which life could colonize but with nothing left by way of early Martian life to colonize them (Cockell, 2014).

However many astrobiologists also think that present day Martian life is possible or even likely, , at least in the form of microbes. A few believe Martian life may have been detected already by the Viking landers in the 1970s.

Rummel and Conley, both former planetary protection officers for NASA, put it like this: (Rummel et al , 2014)

> ***"Claims that reducing planetary protection requirements wouldn't be harmful, because Earth life can't grow on Mars, may be reassuring as opinion, but the facts are that we keep discovering life growing in extreme conditions on Earth that resemble conditions on Mars. We also keep discovering conditions on Mars that are more similar—though perhaps only at microbial scales—to inhabited environments on Earth, which is where the concept of Special Regions initially came from."***

Davila et al. (Davila et al, 2010).

> ***"We argue that the strategy for Mars exploration should center on the search for extant life. By extant life, we mean life that is active today or was active during the recent geological past and is now dormant. As we discuss below, the immediate strategy for Mars exploration cannot focus only on past life based on the result of the Viking***

missions, particularly given that recent analyses call for a re-evaluation of some of these results. It also cannot be based on the astsumption that the surface of Mars is uniformly prohibitive for extant life, since research contributed in the past 30 years in extreme environments on Earth has shown that life is possible under extremes of cold and dryness."

Westall (Westall , 2013:192)

"This presupposes that the ephemeral surface habitats could be colonized by viable life forms, that is, that a subsurface reservoir exists in which microbes could continue to metabolize and that, as noted above, the viable microbes could be transported into the short-lived habitat

.... Although there are a large number of constraints on the continued survival of life in the subsurface of Mars, the astonishing biomass in the subsurface of Earth suggests that this scenario as a real possibility."

Morozova (Morozova et al, 2006)

"The observation of high survival rates of methanogens under simulated Martian conditions supports the possibility that microorganisms similar to the isolates from Siberian permafrost could also exist in the Martian permafrost"

Crisler et al (Crisler et al, 2012)

Our results indicate that terrestrial microbes might survive under the high-salt, low-temperature, anaerobic conditions on Mars and present significant potential for forward contamination. Stringent planetary protection requirements are needed for future life-detection missions to Mars

Renno (Renno, 2014):

"This is a small amount of liquid water. But for a bacteria, that would be a huge swimming pool - a little droplet of water is a huge amount of water for a bacteria. So, a small amount of water is enough for you to be able to create conditions for Mars to be habitable today'. And we believe this is possible in the shallow subsurface, and even the surface of the Mars polar region for a few hours per day during the spring."

Stamenković (Wall, 2018)

There is still so much about the Martian habitability that we do not understand, and it's long overdue to send another mission that tackles the question of subsurface water and potential extant life on Mars, and looks for these signals

De Vera et al (de Vera et al, 2014)

> ***"This work strongly supports the interconnected notions***
> ***(i) that terrestrial life most likely can adapt physiologically to live on Mars (hence justifying stringent measures to prevent human activities from contaminating / infecting Mars with terrestrial organisms);***
> ***(ii) that in searching for extant life on Mars we should focus on "protected putative habitats"; and***
> ***(iii) that early-originating (Noachian period) indigenous Martian life might still survive in such micro-niches despite Mars' cooling and drying during the last 4 billion years"***

Cockell (Deighton, 2016)

> ***Most microbes can grow in different types of extremes and the extremes that we are looking at, things like radiation, perchlorate salts and also sulphate salts (found on Mars), they will grow in that. It's just a question of trying to determine what the limits are and that's the work we're doing at the moment. Anywhere where we've gone to the deep subsurface (on earth) today, where there is liquid water, there is a high chance that environments are habitable,***
>
> ***Simply because Mars is a planet of volcanic rock, and when volcanic rock weathers that provides an environment for microbes to grow and reproduce, I think we can already say there is a high chance there are habitable environments.***

Cabrol (Cabrol, 2021)

> ***Arguably, dispersal does not imply seeding, but it provides the potential for it and, if life started on Mars, odds are that not only is it still there, but it is everywhere it can be where conditions allow dormancy or metabolic activity. Here, terrestrial analogues in extreme environments show that 'everywhere it can be' does not, however, mean easy to see. Hidden oases are often measured in centimetres to micrometres, their presence intimately linked to the subtle interplay and feedback mechanisms between living things and their environment.***

Bianciardi et al (Bianciardi et al, 2012)

> ***"These analyses support the interpretation that the Viking LR experiment did detect extant microbial life on Mars"***

Miller et al (Miller et al, 2002).

> ***"Did Viking Lander biology experiments detect life on Mars? ... Recent observations of circadian rhythmicity in microorganisms and entrainment of***

terrestrial circadian rhythms by low amplitude temperature cycles argue that a Martian circadian rhythm in the LR experiment may constitute a biosignature."

Levin et al (Levin et al, 2016)

> ***"It is concluded that extant life is a strong possibility, that abiotic interpretations of the LR data are not conclusive, and that, even setting our conclusion aside, biology should still be considered as an explanation for the LR experiment. Because of possible contamination of Mars by terrestrial microbes after Viking, we note that the LR data are the only data we will ever have on biologically pristine martian samples"***

In the 2020 conference Mars extant life: what's next? (Carrier et al, 2020) a significant fraction of the participants thought that there is a possibility Mars has extant life.

> ***Primary conclusions are as follows: A significant subset of conference attendees concluded that there is a realistic possibility that Mars hosts indigenous microbial life. A powerful theme that permeated the conference is that the key to the search for martian extant life lies in identifying and exploring refugia ("oases"), where conditions are either permanently or episodically significantly more hospitable than average. Based on our existing knowledge of Mars, conference participants highlighted four potential martian refugium (not listed in priority order): Caves, Deep Subsurface, Ices, and Salts.***

Suggested sources for native life in equatorial regions such as Jezero crater include local microhabitats such as salty brines, and spores in windblown dust – while the dust and salts are not likely to be transferred to Earth via asteroid impacts

There are several suggestions for microhabitats for native Martian life in the equatorial regions of Mars.

First, Curiosity has already found a cold brine layer in equatorial sand dunes (Martin-Torres et al, 2015) a few cm below the surface. Nilton Renno has suggested this could be habitable to a biofilm that can regulate its microhabitat, for instance, retain the water through to warmer conditions in daytime (Nilton Renno cited in Pires, 2015).

These brines could also be habitable for Martian life at lower temperatures than for terrestrial life. The abundant perchlorates on Mars can reduce minimum temperatures for metabolic reactions (Rummel et al , 2014:897) and Martian life may be capable of replicating at lower temperatures than Earth life (Schulze-Makuch et al, 2010a) (Houtkooper et al, 2006).

- Detection by Curiosity rover of liquid water as perchlorate brines in Gale crater sand dunes and similar conditions are predicted in Jezero crater dunes

The Viking results, still not fully explained (Quinn et al, 2013) (Levin et al, 2016), add to the possibilities for native Martian life in the sample. The emissions of the evolved gases labeled with carbon 14 were offset by as much as two hours relative to the temperature changes. Such a large offset resembles circadian rhythms, and is hard to explain by purely chemical processes (Miller et al, 2002).

- Puzzles from the Viking landers – why some think Viking detected life already in the 1970s – evolved gases in the labelled release experiment offset from temperature fluctuations by as much as two hours, more typical of a circadian rhythm than a chemical reaction

Some of the seasonal linear dark streaks, the Recurring Slope Lineae (RSL's) on steep hill slopes in the equatorial regions may provide microhabitats for Martian life.

There is evidence now that some may be predominantly caused by dry granular flows, however other evidence still suggests that some may be formed by a wet dominated mechanism or a mix of wet and dry mechanisms, and if so they remain potentially habitable (Stillman, E., 2018:81). See

- Could local RSL's be habitable and a source of wind dispersed microbial spores? Both dry and wet mechanisms leave unanswered questions - may be a combination of both or some wet and some dry

The dust storms block out UV (Smith, 2019). This could make any nearby habitats such as the RSL's a source of wind dispersed spores. Indeed, global dust storms (Shirley, 2015) could potentially transport spores to Perseverance's samples from almost anywhere on Mars. Martian life might be adapted to propagate via dust storms. See

- Could Martian life be transported in dust storms or dust devils, and if so, could any of it still be viable when it reaches Perseverance? (below)

These near surface layers of dust and salts are not likely to be transferred from Mars to Earth by asteroid impacts. All the Martian meteorites we have so far come from at least three meters below the surface after glancing blows into the high altitude southern uplands of Mars (Head et al, 2002).

So the "Natural Contamination Standard" (Greenberg et al, 2001) doesn't apply. We can't assume that Earth has already been exposed to any spores spread in Martian dust storms or any viable life in near surface brines. See:

- Could Martian life have got to Earth on meteorites? Our Martian meteorites come from at least 3 m below the surface in high altitude regions of Mars

First restricted (potentially life bearing) sample return since Apollo, but needs much stricter planetary protection than was realized for Apollo – especially after discovery of starvation mode nanobacteria that pass through 0.1 micron nanopores

This is the first restricted sample return since Apollo. Much has changed since then in our understanding of polyextremophiles, the limits of size of life, and our modern environmental protection laws. However, several studies have looked in detail into how such a mission can be carried out in a way that is safe for humans and the environment of Earth. It's likely that these studies become a basis for legal requirements on such a mission, as it goes through legal review.

The 2009 study by the National Research Council (NRC) set a limit of 0.25 microns diameter for released particles (National Research Council. 2009).

This was followed up by the 2012 study by the European Space Foundation (ESF) which reduced this to a limit of between 0.01 microns and 0.05 microns (Ammann et al, 2012:14ff).

The ESF study considered evidence of free living microbes cultivated after passing through 0.1 micron filters (Miteva et al, 2005). Such small sizes may be an adaptation to starvation survival stresses, which makes this similar to situations one might expect on Mars.

The ESF study found a similar theoretical minimum size for free living terrestrial life with their estimated minimal genome of 750 genes, concluding that such a theoretical microbe could have a width of less than 0.1 microns, and length greater than 0.2 microns (Ammann et al, 2012:15)

Example real life microbes match these figures. This is a SEM of a bacteria with width less than 0.1 microns and length about 0.2 microns:

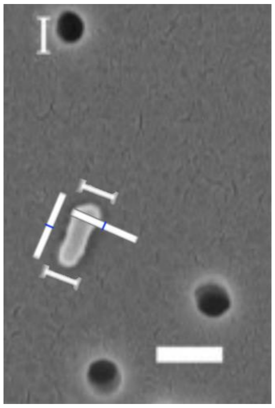


Figure 4: SEM of a bacterium that passed through a 100 nm filter (0.1 microns), large white bar is 200 nm in length (Liu et al, 2019).

The ESF study also considered Gene Transfer Agents (GTAs) with a minimum size of 0.03 microns, which transfer properties to unrelated microbes rapidly overnight in sea water (Maxmen, 2010).

By European Space Foundation study (2012), particles larger than 0.05 microns in diameter are not to be released under any circumstances

The European Space Foundation study summarizes their conclusions in this figure (Ammann et al, 2012:14ff). :

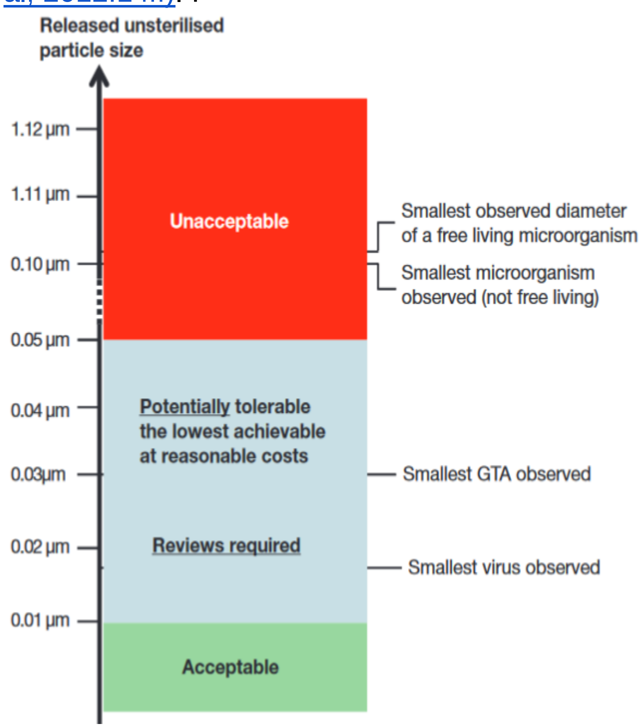


Figure 5: ESF summary of containment requirements

The report concluded that

> *"the release of a particle larger than 0.05 µm in diameter is not acceptable in any circumstances"* (Ammann et al, 2012:21).

The three proposed methods of containing samples in a Mars sample receiving facility, BSL-4 in a clean room, clean room in a BSL-4 and triple wall - with examples for each design

A Mars sample receiving facility has to function both ways, to protect the Martian sample against any contamination by Earth life, and to protect Earth's environment from release by any Martian life in the sample.

To protect the Martian sample requires a clean room, which is normally under positive pressure to help keep contamination out of it.

To protect Earths environment requires a BioSafety Laboratory, which is normally under negative pressure to help contain whatever is in it,

The three main designs of sample receiving facility consist of (Uhran et al, 2019):

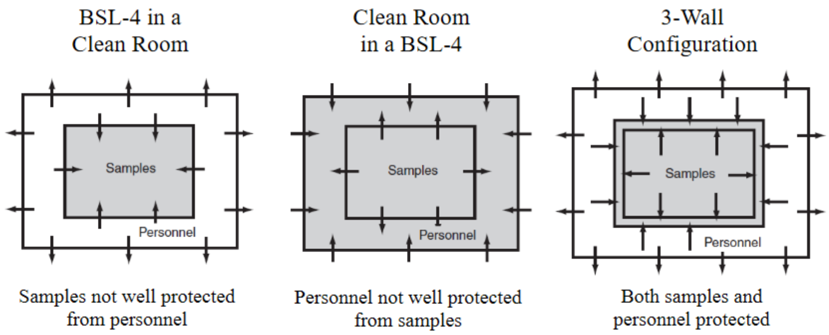
- a clean room inside a biosafety level 4 facility
  (personnel not well protected from samples)
- a biosafety level 4 facility inside a clean room
  (samples not well protected from personnel and terrestrial life),
- a biosafety level 4 facility surrounded by a vacuum barrier inside a clean room in a novel triple wall facility
  (protects both sample and personnel)
- 

Figure 6: the three ways of containing a returned sample.

(Figure 4 of (Carrier et al, 2019:8) adapted from Figure 1 of (Rummel et al, 2002))

This shows how the inner double wall of the triple wall system works:

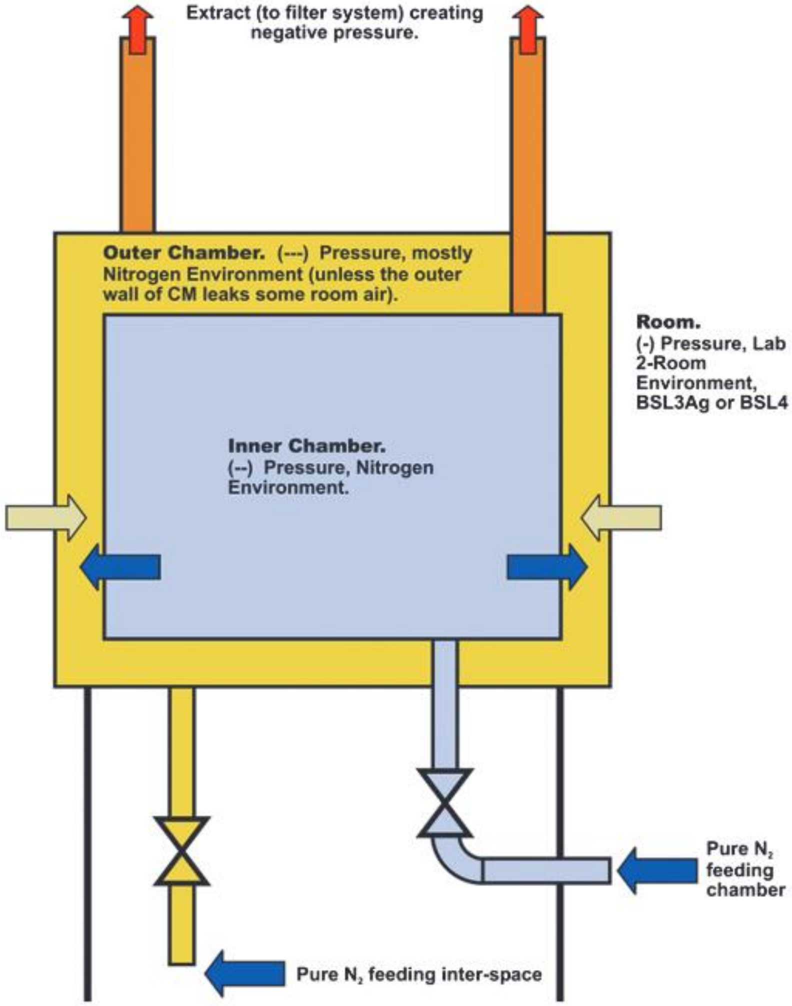


Figure 7: schematic illustration of double wall system to both isolate and contain the sample, Credit NASA / FLAD (Hsu, 2009)

As examples of these types of design:

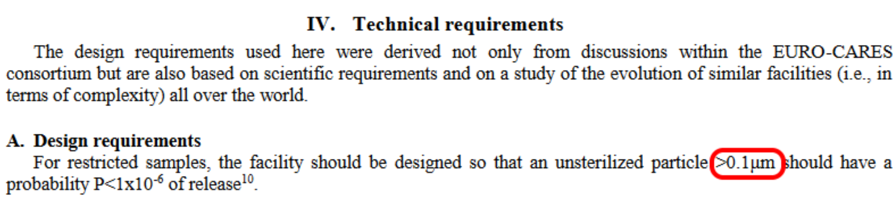
- EURO-CARES (Hutzler et al, 2017) consists of a clean room inside a BSL4 so the staff need extra protection from the samples, which they provide with personal protection equipment suits.
- Calaway et al proposed a lower cost mobile laboratory that could be attached to existing biosafety labs, and this is a BSL4 inside a clean room so there's more risk of contamination of the samples (Calaway et al, 2017).
- The FLAD team concept is an example 3-wall design which also included a proposed concept for novel double walled glove boxes with the pressure reduced inside the double wall so this protects both samples and personnel (Beaty et al, 2009:750).

There is also a breadboard design for a cabinet constructed by the University of Leicester. This is a smaller three wall design where the double wall encloses a smaller cabinet in a lab (Carrier et al, 2019:23). This is a “no glove” remote manipulation negative pressure system.

EURO-CARES sample return facility design filter requirements are out by an order of magnitude, due to unfortunate typo - ESF study’s probability of less than one in a million is for unsterilised particles of 0.01 microns (NOT 0.1 microns) – and ESF requires 100% containment for particles of 0.05 microns

The EURO-CARES design (Hutzler et al, 2017:5) cites the ESF study but summarizes it incorrectly as a requirement of a one in a million probability for release of an unsterilized particle > 0.1 microns.

This seems to be an unfortunate typo or a simple misreading. In this screenshot, the EURO-CARES cite 10 is to the ESF study.



IV. Technical requirements

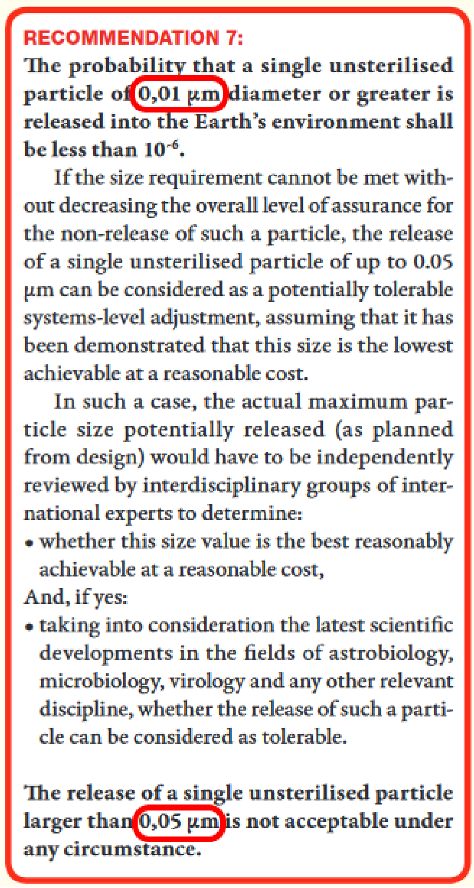
The design requirements used here were derived not only from discussions within the EURO-CARES consortium but are also based on scientific requirements and on a study of the evolution of similar facilities (i.e., in terms of complexity) all over the world.

A. Design requirements

For restricted samples, the facility should be designed so that an unsterilized particle >0.1µm should have a probability $P<1x10^{-6}$ of release[10].

Figure 8: screenshot from the EURO-CARES study

The ESF study does have a one in a million requirement, but this is for an unsterilized particle **≥ 0.01µm** or 10 nm (Ammann et al, 2012:48).



RECOMMENDATION 7:

The probability that a single unsterilised particle of 0,01 µm diameter or greater is released into the Earth's environment shall be less than 10^{-6}.

If the size requirement cannot be met without decreasing the overall level of assurance for the non-release of such a particle, the release of a single unsterilised particle of up to 0.05 µm can be considered as a potentially tolerable systems-level adjustment, assuming that it has been demonstrated that this size is the lowest achievable at a reasonable cost.

In such a case, the actual maximum particle size potentially released (as planned from design) would have to be independently reviewed by interdisciplinary groups of international experts to determine:

- whether this size value is the best reasonably achievable at a reasonable cost,

And, if yes:

- taking into consideration the latest scientific developments in the fields of astrobiology, microbiology, virology and any other relevant discipline, whether the release of such a particle can be considered as tolerable.

The release of a single unsterilised particle larger than 0,05 µm is not acceptable under any circumstance.

Figure 9: screenshot from the ESF report

"The probability that a single unsterilized particle of 0.01 micron diameter or greater is released into the Earth's environment shall be less than one in a million"

"Release of a single unsterilized particle at 0.05 microns is not acceptable under any circumstances"

Notice that the one in a million probability in the ESF study is cited incorrectly too, as it is used in an unusual way.

The ESF defines their one in a million as the probability of release of A SINGLE UNSTERILIZED PARTICLE of 0.01 microns. This means over the ENTIRE LIFETIME of the facility.

To achieve this level of assurance requires filters capable of removing far more than 99.9999% of the particles at this size, depending on the number of nanoscale particles expected to encounter the filters for the duration of the facility.

The EURO - CARES design would need to be modified to incorporate filters capable of orders of magnitude better containment of particle sizes than their design requirement of one in a million containment at 0.1 microns. The maintenance, operating procedures etc of the filters would also need to comply with this standard.

If this requirement is impossible the ESF study says that best available technology is permitted. However they say such a decision needs independent review by an interdisciplinary group of international experts.

The ESF study says release of a single particle larger than 0.05 microns in diameter is not permitted in any circumstances, not even a one in a million probability for a single particle at that size is adequate. This is beyond the capabilities of current filter technology as we'll see in:

- Example of best available nanofilter technology from 2020, not yet commercially available, filters out 88% of ambient aerosol particles at 0.05 microns - far short of the ESF requirement to filter out 100% at this size – though the ESF requirement at 0.05 microns can be met with nanoparticles in water under high pressure

The Mars Sample Return Facility designs that predate the 2012 ESF study naturally do not comply with the 0.05 microns standard (Beaty et al, 2009).

However, I also searched for published designs that postdate the ESF study. I have not yet found a design which the authors say complies with 100% containment at 0.05 microns, which as we will see seems currently to be beyond current technology.

For example, in the studies that postdate the ESF study,

- Calaway et al (Calaway et al, 2017) and Carrier et al (Carrier et al, 2019:23) don't cite the ESF study, and make no mention of the 0.05 micron requirement.
- The University of Leicester breadboard design is based around a commercially available class III isolator, which would not comply with that requirement as it uses HEPA filters. Their double wall enclosure operating at -250 Pa also uses an H14 HEPA filter (Carrier et al, 2019:23)

All these studies use HEPA filters, except the EURO-CARES design which uses the more stringent ULPA filters.

Even the EURO-CARES design's ULPA filters have not been tested to meet these Mars sample return ESF design standards and would need to be upgraded.

The ESF requirement would not be ignored in the legal process. We can't know in advance whether it would be adopted but it surely would be discussed and is a possible requirement as an end result of the legal process.

Indeed, if the legal process leads to an update of the ESF study, or a totally new study, the requirements can be expected to be at least as stringent as in 2012 as it would be based on a review of the same science, together with new discoveries made since then.

The starvation limited nanobacteria able to pass through 0.1 micron nanopores are established science as are the Gene Transfer Agents. New research may lead to smaller potential Martian microbes or gene transfer nanoparticles that need to be contained.

See:
Scientific developments since 2012 that may be considered in a new review of ESF's 0.05 micron / 0.01 micron size limits – if the review considers life not based on DNA and proteins such as minimum size RNA world cells, this could potentially reduce the 0.05 microns to a requirement that release of a 0.01 micron particle is not acceptable under any circumstances

HEPA and ULPA filters are not tested for such small particles as 0.05 microns and not required to contain them

The standards for biosafety level III cabinets, or biosafety level 4 facilities are based on HEPA filters, for instance, a biosafety level III cabinet has to be exhausted to the outside air through two HEPA filters (Richmond et al, 2000:37). These HEPA filters are required to trap 99.97% of particles of 0.3 microns in diameter and 99.99% of particles of greater or smaller size (WHO, 2003:35). These requirements don't set any minimum size above which escape of a single particle is unacceptable under any circumstances.

In the US, HEPA filters are tested down to 0.1- 0.2 microns (depending on the class of filter, some are tested only at 0.3 microns). In Europe they are tested at the most penetrating particle size which may vary depending on the filter. In both cases, the filters are tested according to probabilities (Zhou et al, 2007) (EMW n.d.).

ULPA level 17 filters are rated to filter out 99.999995 percent of particles (BS, 2009:8) in the range 0.12 microns to 0.25 microns (BS, 2009:4), according to BS EN 1822-1:2009, the British implementation of the European standard (BS, 2009).

This still doesn't comply with the ESF standard of no release of a 0.05 micron particle in any circumstances. They are not even tested over this size range.

The filters are tested with challenge aerosols such as dioctylphthal (DOP) generated on the intake side of the filter, and measured with a photometer on the discharge side (Richmond et al, 2000:33). These photometers have limited sensitivity to nanoaerosols below the 100 nm limit. In a study of a DOP aerosol using TSI model 8130 Automated Filter Tester in 2008 (table III of Eninger et al, 2008), although particles below 100 nm (0.1 microns) constituted 10% of the count of particles in the test aerosol, and 0.3% of the mass, they provided almost none of the light scatter in the testing photometer (less than 0.01%)

So, is it possible to filter out particles down to 50 nm (0.05 microns)? And if so, how can such a filter be tested?.

Example of best available nanofilter technology from 2020, not yet commercially available, filters out 88% of ambient aerosol particles at 0.05 microns - far short of the ESF requirement to filter out 100% at this size – though this standard can be met with nanoparticles in water under high pressure

Aerosols are more of a challenge than water contaminants.

It is possible to remove most or all nanoparticles from water with nanofilters under high pressure. A 2020 review of the literature found several studies that achieve a million fold reduction or more of small viruses in water. (Singh et al, 2020:6.3). Singh et al found one study using carbon nanotubes loaded with silver that achieved 100% removal of very small viruses such as the polio, noro and Coxsackie viruses (Kim et al, 2016) (Singh et al, 2020:6.3).. The poliovirus is only 0.03 microns in diameter (Hogle, 2002).

This fulfills the requirement to filter out 100% of particles at 0.05 microns in water. These tests don't tell us how well the filters would perform with the ESF's more stringent requirement to filter out almost all particles down to 0.01 microns from water.

However the sample handling facility would have to filter the nanoparticles from air, not water.For the state of the technology for aerosols, we can consider an experimental filter designed to contain SARS - Cov2, the virus that causes COVID19. This virus has a minimum diameter of 60 nm (0.06 microns) and could in principle be dispersed in an aerosol droplet not much larger than this (Leung et al, 2020). This is not far from the ESF requirement of 0.05 microns.

A single coronavirus is well below the limit for the current HEPA filters in respirators for intensive care, but the capability of HEPA filters to filter out most particles over 0.1 microns has been adequate for personal protection equipment for COVID19 (WHO, 2020tosi) (van Schaik, 2020).

COVID 19 personal protection equipment doesn't currently use filters with the capability to filter out 100% of SARS-Cov2 particles from the air. Nevertheless, a more stringent way of filtering out viruses might be of some interest for the COVID19 response, and also to filter out ambient nanoaerosols at less than 0.1 microns from traffic (Leung et al, 2020). With this motivation, Leung et al constructed a 6-layer charged nanofiber filter.

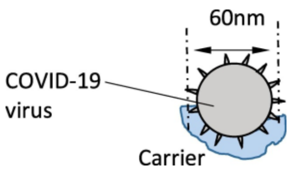


Figure 10: schematic illustration of coronavirus attached to a 60 nm (0.06 microns) diameter carrier water droplet which then becomes airborne (Schematic 1a, from (Leung et al, 2020).)

Leung et al found that their test filter was capable of filtering out 88% of ambient aerosol particles at 0.05 microns (50 nm) (Leung et al, 2020). This is a useful level of filtration for coronaviruses and for traffic fumes, but is still not not close to sufficient for the ESF study.

This suggests that best available technology is not yet able to comply with the ESF standard to contain 100% of particles at 0.05 microns and nearly 100% of particles at 0.01 microns (or this study would have used it). So, this requirement mandates development of a new design, which will then need to be developed, tested, manufactured and integrated into equipment such as suits and glove boxes for the facility.

In short, it is already technically possible, with an experimental filter, to filter out most particles at 0.05 microns.

However, ULPA filters can’t do this. Also, the standard tests for ULPA and HEPA filters can’t test a filter adequately with aerosols small enough to certify such a filter (previous section).

It doesn’t seem to be possible yet to filter out 100% of particles from the air at 0.05 microns with the best available filter technology

However if the particles are in water under high pressure, it is possible to filter out 100% of 0.05 micron particles using experimental nanofilters made of carbon nanofibers loaded with silver.

Challenges for maintenance for future 0.05 micron compliant nanoscale filters – need to be designed for sterilization before any potential extraterrestrial biology is known, and may be easily damaged and hard to replace without risking release of nanoparticles

In the future once these new 100% effective 0.05 micron compliant filters have been designed, developed, tested and proven to work, there will also be the need to show that they can be replaced and maintained, while still maintaining 100% containment at 0.05 microns. Biosafety level III cabinets need to be checked annually (Richmond et al, 2000:33) and equipment will sometimes need to be repaired.

HEPA filters often fail these annual tests and need replacement. When these filters are changed, the Biological Safety Cabinets (BSCs) must be decontaminated (WHO, 2003:35).

For Martian samples, decontaminating the filters or the cabinet before changing them is likely to be challenging, since properties of any viable life in an unsterilized Martian sample are not yet known. The method used for decontamination has to be capable of sterilizing not only known pathogens, not only all Earth life, but also capable of sterilizing any possible extraterrestrial extremophile with possibilities of increased resistance to the sterilizing agents compared to terrestrial life.

Meanwhile however the sample itself needs to be kept unsterilized while the cabinet housing it is sterilized to replace the filters. In addition the maintenance including replacing the filter must be carried out in such a way as to prevent the leak of a single particle larger than 0.05 microns.

The filters for smaller nanoparticles for water treatment are easily damaged, through chemical and biological deterioration by aging, scratches by particle like substances, or fouling of the membrane (Singh et al, 2020:8).

This suggests that once the technology is available for new filters to filter out 100% of particles from the air at 0.05 microns, these new aerosol nanofilters may have similar challenging maintenance requirements.

It seems that to comply with the ESF 0.05 micron standard will be a significant future scientific and technical challenge for filter technology, and filter maintenance and may involve major new learning curves for the technicians that run the facilities.

I have found no previous study of this issue of filter maintenance at 0.05 microns in the planetary protection literature.

Similar challenges with meeting this 0.05 micron standard could be expected for other aspects of maintenance, such as repairing the cabinet itself in the case of electrical fault. This also must be done in a way that doesn't permit a single 0.05 micron particle to escape.

However, before this work on the new filter technology, we also have to review the minimum size requirements. This is recommended in the ESF study.

ESF study's recommendation for regular review of the size limits

The ESF study said that future reductions in the size limit are possible. They expected later reductions to happen at a slower pace, but say the size limit will need to be reviewed in the future, adding (Ammann et al, 2012:21):

> *Based on our current knowledge and techniques (especially genomics), one can assume that if the expected minimum size for viruses, GTAs or free-living microorganisms decreases in the future, and this is indeed possible, it will be at a slower pace than over the past 15 years*
>
> *However, no one can disregard the possibility that future discoveries of new agents, entities and mechanisms may shatter our current understanding on minimum size for biological entities. As a consequence,* ***it is recommended that the size requirement as presented above is reviewed and reconsidered on a regular basis.***
> *[bolding as in original cited text]*

The minimum size was reduced from 0.25 microns to 0.05 microns / 0.01 microns in just three years from 2009 to 2012.

By 2020, eight years later, another review is certainly required.

Scientific developments since 2012 that may be considered in a new review of the ESF study's size limits – life with a simpler biochemistry such as minimum size RNA world cells without DNA or proteins could potentially lead to a requirement that release of even a particle of 0.014 microns is not acceptable under any circumstances

A size requirement review of new agents and mechanisms might consider

- extracellular vesicles. These have recently been discovered to provide another method for gene transfer at the 0.02 microns scale (Biller et al, 2017).
- microbes with less than the minimal genome of independent free living microbes, which depend on a host as symbionts, or epibionts (living on the surface of an organism)

(Ghuneim et al, 2018).

The symbionts can benefit the host, or be parasitic or have a more complex relationship (Pérez-Brocal, 2011).

Another matter a review panel might consider is whether to look at size limits for terrestrial life only or to examine size limits for potentially smaller extraterrestrial life.

The 2012 ESF study focused on limits of size for terrestrial life, and concluded that if Mars has life with a similar biology to ours it couldn't be much smaller than the starvation limited nanobacteria and still be able to contain the ribosomes. These are large molecules 0.02 microns in size, essential for translating mRNA to proteins and common to all terrestrial life (Ammann et al, 2012:14, 15).

However, a review board might look at the research since 2012 into synthetic minimal cells (Lachance, 2019) and protocells (Joyce et al, 2018) which might lead a review board to consider possibilities for a non proteinous lifeform which doesn't need the large ribosomes as it doesn't need to construct proteins.

A simpler form of life than DNA based life could use enzymes based on the much smaller ribozymes which are constructed from fragments of RNA with no use of amino acids, the usual building blocks of proteins.

Ribozymes are constructed from small fragments of RNA interlocked in a complex pattern, with no need for amino acids to catalyze replication, the RNA serves both as genetic material and for catalysis.

Figure 11: the key to the RNA world hypothesis - a ribozyme. This particular example is the "hammerhead ribozyme", made up of fragments of RNA, stitched together without any use of protein chains, to make the enzyme, and gives an idea of how they are constructed (Sehnal et al, 2018).

Joyce's enzyme is an example of a ribozyme that can catalyze replication (Joyce, 2007).

RNA world cells might have no need for proteins or the amino acids that proteins are made of. Their interior would consist largely of RNA strands and ribozymes. This might permit cells far simpler than the simplest modern DNA based life.

The structures in the Martian meteorite ALH84001

Steven Benner and Paul Davies are of the view that these structures just ***might*** be fossils of simpler RNA world cells from an earlier form of life without those large ribosomes and without the proteins (Benner et al, 2010: 37).

> *"Why should proteins be universally necessary components of life? Could it be that Martian life has no proteins?*
>
> *... Life forms in the putative RNA world (by definition) survived without encoded proteins and the ribosomes needed to assemble them. ... If those structures represent a trace of an ancient RNA world on Mars, they would not need to be large enough to accommodate ribosomes. The shapes in meteorite ALH84001 just might be fossil organisms from a Martian "RNA world".*

These structures are 20 to 100 nm in diameter (0.02 to 0.1 microns) (Treiman, n.d.),

Panel 4 for the 1999 workshop on "Size limits of very small microorganisms" considered whether non terrestrial life could be as small as these structures (Board et al, 1999: 107). They assumed:

- A single biopolymer system
- Semipermeable membranes (assumed constructed of fatty acids)
- No proteins, using randomly formed peptides and other biomolecules instead
- Each single strand RNA gene has about 1,500 bases
- five genes (ligase, replicase, monomer synthase, fatty acid synthase, and membrane synthase ribozymes)

They calculated that a minimal free living cell would need a 3,580-nm^3 volume in addition to the volume of the surrounding membrane. Some proteins and other biomolecules would likely be present as well bringing the volume up to 50,000 nm^3 (Board et al, 1999: 117)

The 1999 panel made a crude approximation for a measurement of one of the putative ultramicrobacteria for ALH84001. They found it had a length of 120 nm (0.12 microns) and diameter of 10 nm (0.01 microns). The diameter was the least reliable measurement so they assumed a 14 nm diameter (0.014 microns). Its internal diameter would then be 6 nm

(0.006 microns) and internal length 112 nm (0.112 microns) assuming a 4 nm (0.004 microns) thick phospholipid membrane layer

[Figure needs permission, redraw or new source]

> Figure 12: Panel 4 from the limitations of size workshop measured one of these putative ultramicrobacteria (Board et al, 1999: 117). They measured it from Figure 6B of (McKay et al, 1996:928) and found a dimension of 120 nm long and 10 nm in diameter. Ruler shows 20 nm divisions. The report doesn't say which is the one they measured.

Allowing for a 4 nm thickness membrane they found an internal volume of 3,170 nm^3. This is close to their estimate of 4 to 5 genes if closely packed.

They gave an example of such a closely packed organism, the RNA phage Q-beta, which contains three genes consisting of about 1,500 bases each (Board et al, 1999: 117).

Panel 4 for the 1999 study concluded that a primitive free living lifeform of these dimensions, 14 nm (0.014 micron) diameter and 120 nm length, is possible, if there is an efficient mechanism for packing its RNA.

This 1999 estimate for the size limits for non-proteinous Martian life wasn't considered in the 2012 ESF report, which only looked at the limits for terrestrial life from that study (Ammann et al, 2012:15)

However, perhaps there has been enough new research since then into ideas for early life using ribozymes instead of ribosomes to consider the possibility that a sample returned from Mars could contain RNA world cells?

There seems to be universal agreement that terrestrial life with its dual informational biopolymer system and the complex ribosomes and proteins didn't arise in one go from abiotic precursors.

Modern life is just too complex to arise in one go and there is a causality issue too.

DNA needs certain proteins to replicate, however, cells need DNA to make proteins So it's hard to see how either could come first (NASA, 2001):

> *Scientists studying the origins of DNA are confronted with a paradox. DNA needs certain proteins to replicate. But in order to make the correct proteins for this function, modern cells need to have DNA. Since DNA and the proteins are dependent on each other, it is hard to see how either of them could have come first.*

Some less complex intermediary must have developed before modern life. This need not reproduce exactly, and may be more tolerant of errors of transcription. Such less complex life might be able to fit into a smaller cell size than modern DNA based life, and be able to pass through a smaller nanopore.

The usual solution is that some less complex intermediary must have developed before modern life. This need not reproduce exactly, and may be more tolerant of errors of transcription. Such less complex life might be able to fit into a smaller cell size than modern DNA based life, and be able to pass through a smaller nanopore.

Could the postulated RNA world nanobacteria 0.014 microns in diameter spread through Earth's environment (or other simpler forms of life)? Answer seems yes, possibly, with similar advantages to the postulated nanobes of the shadow biosphere hypothesis

There has been enough interest in the possibility of nanobacteria with a simpler biochemistry to search for a terrestrial "shadow biosphere". Phillipa Unwin observed nanobes only 20 nm in diameter, far too small for familiar life, but she found some disputed evidence that they could contain DNA (Cleland, 2019, pp 213 - 214). The theory that these nanobes are life is controversial, and Earth has not been proven to have a shadow biosphere of DNA based life or RNA world life, or any other simpler forms of life.

Although we haven't found a shadow biosphere on Earth, this research suggests the possibility that Mars could still have nanobacteria with the same simpler form of life it may have had in its early oceans and lakes billions of years ago. This simpler life could co-exist with familiar life in a shadow biosphere on Mars, or it could be the only form of life on Mars but co-exist in a shadow biosphere when returned to Earth.

Such small cells would have some major evolutionary advantages on Mars. Smaller cells have a larger surface area to volume ratio, and so can take up nutrients more efficiently, which is an advantage in an environment with low nutrient concentrations. Small cells also avoid protozoan grazing because they are so small compared with protozoa (Ghuneim et al, 2018). The smallest nanobacteria cells typically occur in aquatic environments on Earth, but the Martian near subsurface brines would give them similar advantages.

So surface habitats on Mars might well favour nanobacteria, if they exist on Mars. Lifeforms of a simple enough biochemistry to fit into a nanobe might also be variable size, shrink to nanobacteria when starvation limited at low nutrient concentrations and then grow to larger sizes in nutrient rich environments when returned to Earth.

So, could such nanobes compete with terrestrial life?

They would have the same advantages on Earth as the 0.05 micron to 0.4 micron diameter nanobacteria we already have (Ghuneim et al, 2018), and even more so, better able to take up nutrients in microhabitats with low nutrient concentration, and ignored by large secondary consumers that preferentially prey on larger microbes. Also mirror life might convert normal organics in the habitat into mirror organics that can only be used by more mirror life, or by rare terrestrial microbes that can make use of mirror organics. This would make its habitat or microhabitat more favourable to its own biochemistry than to terrestrial life. This would give it an extra advantage, as we explore more in: Example of mirror life nanobacteria spreading through terrestrial ecosystems (below)

A review board would reconsider these ideas in light of the eight years research done since 2012. Perhaps this would lead them to re-examine the possibility of Martian nanobes smaller than 0.05 microns in diameter.

Based on these considerations it seems not impossible that they set the limit as small as the 0.014 microns (14 nm) diameter RNA world cells considered by the working group in 1999, for the size of particle that must not be released under any circumstances.

If they use the same factor of two safety factor as for ultramicrobacteria they might reduce the limit further to 0.005 microns or 5 nm.

Priority to decide on minimum size of released particle for filter requirements early in legal process and to outline future technology to achieve this standard

The filter design will depend on the minimum size and other aspects of the recommended requirements, so the first priority is to complete the review of the minimum size requirements. We need to know if the 0.05 micron / 0.01 micron standard is still considered sufficient after a review of relevant scientific discoveries in the 10 years from the 2012 ESF review through to 2022 or does it need to be increased, perhaps to 0.01 microns or even 0.005 microns?

Given the significant technological challenges involved already at 0.05 microns, it would seem to be a high priority to complete this review and to outline a technology research approach that has the potential to achieve the resulting standard before embarking on the legal process for a Mars sample return.

The filters themselves could be developed and tested in parallel with the legal process. and brought to technological readiness during the first few years of the legal process so that they are ready or almost ready when the build starts.

The issue here is that until the required technology is mapped out, there will be no information available about installation methods, maintenance requirements, failure modes and so on. To get this information seems likely to require practical experience of building at least breadboard versions of the filters, and the technology for testing them for compliance with the standard, and then testing methods for replacing damaged filters without releasing any nanoscale particles during the maintenance procedures. This information would likely be needed well before the end of the legal process.

See:

- Challenges for maintenance for future 0.05 micron compliant nanoscale filters – need to be designed for sterilization before any potential extraterrestrial biology is known, and may be easily damaged and hard to replace without risking release of nanoparticles.

NASA will also need to show this level of understanding of the technology they propose for the filters by phase A in the NASA project development process (NASA, n.d.SEH: section 3.4) which happens well before the NASA budget request for the build. See NASA procedural requirements for mission planners (below)

Discussion of potential large scale effects from mirror life could lead to a call for near certainty of containment, as for some experiments in synthetic biology

Some of the potential outcomes could in the worst case scenarios lead to large scale effects on the Earth's biosphere. See:

- Example of mirror life nanobacteria spreading through terrestrial ecosystems (below).

We don't know how terrestrial life developed homochirality (Blackmond, 2019) (Brazil, 2015), so with our current level of scientific understanding, it doesn't seem to be possible to assign a likelihood to whether or not Mars could have mirror life microbes. See:

- Potential for mirror life on Mars and survival advantages of mirror life competing with terrestrial life that can't metabolize mirror organics (below).

It is a similar situation for other worst case scenarios for extraterrestrial biology on Mars. Terrestrial biology may or may not be representative of evolution on other planets, making it hard for astrobiologists to give expert opinion on what we may find. When designing experiments to look for life in situ on Mars, astrobiologists are careful not to make assumptions that it will resemble terrestrial life.

Experiments in synthetic biology are conducted in ways that make it impossible for any synthetic life to escape the laboratory and reproduce in the wild. If similar requirements were adopted for a sample return, the one in a million “gold standard” for BSL-4 laboratories may be made more stringent and changed to a requirement for near certainty.

It is very hard to design for near certainty of containment. Synthetic life experiments can do this by designing the novel biology to be dependent on nutrients only available in the laboratory. This is not an option for a sample return with naturally evolved biochemistry. See:

- A requirement for similar levels of safety to those used for experiments with synthetic life would lead to the Prohibitory version of the Precautionary Principle and make unsterilized sample return impossible with current technology and current understanding of Mars, which could impact on the discussion by the ESF study of whether to use the prohibitory version of the legal precautionary principle

The 2012 ESF study in their discussion of precautionary principle said we need to minimize risk using best available technology because if we require no appreciable risk of harm the mission has to be cancelled – considerations of large scale effects could lead to a need to re-evaluate this conclusion

One way to resolve questions like this is with the precautionary principle, which is used for situations where there is insufficient information for decision makers to fully know all the possible consequences of their action

The European Space Foundation sample return study in 2012 considered four variations on the precautionary principle. One of these was the Prohibitory version (Ammann et al, 2012:25) (Stewart, 2002).

> ***Prohibitory Precautionary Principle**: Activities that present an uncertain potential for significant harm should be prohibited unless the proponent of the activity shows that they present no appreciable risk of harm*

However, the authors of the ESF report determined that it is impossible to use this version of the principle for this mission, on the basis that if it was adopted, the sample return mission couldn’t go ahead. (Ammann et al, 2012:25)..

It is not possible to demonstrate that the return of a Mars sample presents no appreciable risk of harm. Therefore, if applied, the Prohibitory Precautionary Principle approach would simply lead to the cancellation of the MSR mission..

Instead, they recommended the "best available technology" version. With this version, the level of protection depends on the technology available at the time of the mission, which is used to minimise the risk of harm.

***Best Available Technology Precautionary Principle**: Activities that present an uncertain potential for significant harm should be subject to best technology available requirements to minimise the risk of harm unless the proponent of the activity shows that they present no appreciable risk of harm.*

Stewart (the academic the ESF cited) said in his essay that stringent preventative environmental regulation is often justified and appropriate because of the very high regard society places on the environment (Stewart, 2002:15):

In critiquing strong versions of PP [Precautionary Principle], this essay does not argue that stringent preventive environmental regulation should never be adopted. … As society places a very high value on the environment and its protection, stringent preventive regulation of uncertain environmental risks is often justified and appropriate.

He ends the essay by suggesting the need to develop criteria for situations where the prohibitory version of the principle should be used.

The discussion of the potential for large scale effects could lead to re-evaluation of this decision by the ESF study board.

Based on the high value society places on the environment, some members of the general public, scientists and other agencies may consider the prohibitory version of the principle to be required in this situation. These could include synthetic biologists, or the WHO or CDC, or FAO (because of potential impact of mirror life on farming and wild caught food) or NOAA (for fisheries) and so on.

This is also a possible conclusion of any review board tasked with updating the recommendations from the 2012 study to take account of advances in understanding of science in the last decade. The 2012 study board were asked to recommend handling requirements for the sample return (Ammann et al, 2012:1).

"Recommend the level of assurance for the exclusion of an unintended release of a potential Mars life form into the Earth's biosphere for a Mars Sample Return mission"

A review board would likely have the same mandate. However, it ***is*** possible for such a board to respond saying they can't find any appropriate level of assurance for the mission to go ahead with current technology.

For a more detailed analysis, see: Which variation on the precautionary principle is appropriate for a Mars sample return? (below)

Clarifying this question of which version of the precautionary principle to use with Sagan's criterion that "we cannot take even a small risk with a billion lives"

The main difference between a sample return and experiments with known infectious diseases in biosafety laboratories is that we need to consider the potential for a small but appreciable risk of large scale changes in Earths' biosphere.

This current paper formulates a potential new criterion which could help clarify such decisions, which we can call "Sagan's criterion" based on a quote from Carl Sagan in his book "Cosmic Connection" (Sagan, 1973:130)

> *"The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives."*

This is made rigorous in the form of a challenge to mission planners:

> ***Sagan's criterion:*** *If it is impossible to show that there is no appreciable risk of significant harm to the lives or livelihoods of a billion people, the Prohibitory version of the Precautionary Principle must always be used.*

Then to resolve this issue, first we ask

- Should we adopt Sagan's criterion?

If the answer is "Yes", the next question is:

- Is there any *appreciable risk of significant harm to the lives or livelihoods of a billion people?*

If the answer is "No appreciable risk of this severe form of harm", the best available technology version of the precautionary principle can be considered and it may be appropriate to use the one in a million "gold standard" of a biosafety laboratory – though likely with better quality filters to filter down to 0.05 microns / 0.01 microns.

If the answer is “yes there is appreciable risk of this severe form of harm” precautions must be taken similar to synthetic biology to prevent any appreciable risk of harm.

For a more detailed analysis, see: Formulating Sagan’s statement that “we cannot take even a small risk with a billion lives” as a criterion for the prohibitory version of the precautionary principle (below)

In the case of the Mars sample return mission, whether NASA and ESA can meet this challenge might depend on the state of our scientific understanding of Mars and our technological capabilities by 2028 or whenever the legal process ends.

Urhan et al recommend an advanced planning and oversight agency set up two years before the start of the legal process - Rummel et al recommend it should include experts in legal, ethical and social issues – while the ESF recommends an international framework should be set up, open to representatives from all countries

With so much to be sorted out, Urhan et al recommended that an oversight agency should be set up long before the legal process starts. Uhran et al recommend this is done two years before filing the environmental impact statement to develop a consensus position on the margin of safety for sample containment (Uhran et al, 2019).

Since the aim is to develop a consensus position, this would need to be based on up to date information. So it would need to include the review of the size limits required in the ESF sample return study (Ammann et al, 2012:PG). In the current paper we suggest we also need to review filter technology and provide a preliminary study of the technological advances needed to achieve the specified size limits, since the technology doesn’t seem to exist yet.

Rummel et al advise that clear communication with the public is essential from an early stage, for success of the mission. They recommend that this should avoid a NASA centric focus and include links with other government agencies and international partners and external organizations (Rummel et al, 2002).

Rummel et al warn that the mission might attract viral sharing of misinformation, a concern that now seems similar to the “infodemic” for COVID19. Potentially the sample return mission, and the facility, could also attract intentionally disruptive events, by bioterrorists, or by members of the public opposed to sample return (Rummel et al, 2002). Perhaps this may need to be managed based on the emerging discipline of infodemiology (WHO, 2020wic).

Rummel et al say that the oversight committee would need to contain experts in legal, ethical and social issues in addition to the experts in astrobiology, space engineering and mission planning. It should conduct ethical and public reviews. Broad acceptance by the public is essential at an early stage for success of the mission (Rummel et al, 2002).

The ESF recommends that since negative consequences from an unintended release could be borne by countries not involved in the program, a framework should be set up at the international level open to representatives of all countries, with mechanisms and fora dedicated to ethical and social issues of the risks and benefits from a sample return (Ammann et al, 2012:59). This again would be best done before the start of the legal process to make sure everyone is on the same page before it starts.

NASA did set up a review board for sample return missions on August 14th 2020 (NASA, 2020nebmsr). However, it is not clear yet what its scope is. It is not clear whether it will consider these wide ranging issues, or include experts in legal, ethical and social issues, as recommended by Rummel et al.

NASA procedural requirements for mission planners to develop a clear vision of problems, show it's feasible and cost-effective, develop technology with engineering details and show it will meet requirements before build starts – because of significant costs involved in modifying designs at later stages in the build

NASA has a procedural requirement for mission planners to do an Earth Safety Analysis Plan to present to the Planetary Protection Officer (NASA, 2005npr: 2.7.4.1)

The Mars Receiving Facility is likely to be built in the US by NASA (Carrier et al, 2019). All NASA facility project managers are mandated by NPR 8820.2G to comply with NASA-required best practices.(NASA, 2014fpr:10)

In pre-phase A they need to develop a clear vision of the problems and how they can be solved (NASA, n.d.SEH:3.3):

It is important in Pre-Phase A to develop and mature a clear vision of what problems the proposed program will address, how it will address them, and how the solution will be feasible and cost-effective.

In phase A the technical risks are examined in more detail (NASA, n.d.SEH: section 3.4):

Technical risks are identified in more detail, and technology development needs become focused.
… Develop breadboards, engineering units or models identify and reduce high risk concepts

Then in phase B, the design is set out in more engineering detail and shown to comply with requirements. (NASA, n.d.SEH: section 3.5):

The project demonstrates that its planning, technical, cost, and schedule baselines developed during Formulation are complete and consistent; that the preliminary design complies with its requirements; that the project is sufficiently mature to begin Phase C; and that the cost and schedule are adequate to enable mission success with acceptable risk. For projects with a Life Cycle Cost (LCC) greater than $250 million, this commitment is made with the Congress and the U.S. Office of Management and Budget (OMB). This external commitment is the Agency Baseline Commitment (ABC). Systems engineers are involved in this phase to ensure the preliminary designs of the various systems will work together, are compatible, and are likely to meet the customer expectations and applicable requirements.

All these phases have to be completed before the start of the build. The issue here is that there are significant costs involved in modifying a design later on. It can cost 500 to 1000 times more to modify a design at a late stage in the process.

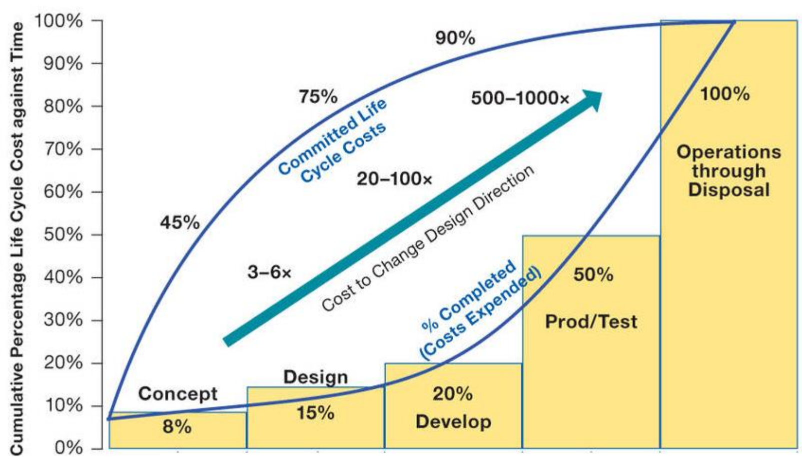


Figure 13: Life-Cycle cost impacts from early phase decision making
(from figure 2-5-1 of NASA, n.d.SEH: section 2.5)

The design requirements for the Mars Sample Receiving Facility will not be known until the legal process is complete.

Small discretionary funds are made available for concept studies before the budget request. However, the budget itself depends on the design requirements.

A study in 2010 estimated the cost of the facility as $471 million in 2015 US dollars, see Table 5-1 of (Mattingly, 2010:20). This is from before the ESF study, and so the cost is likely to be higher with the newer 0.05 micron requirements. Adjusted to 2021 dollars allowing for inflation, this is already over half a billion dollars, and informal cost estimates today are similar, $500 million per facility. NASA would need legal clarity before allocating such a budget.

It is also possible that ESA might build a second facility to contain the samples (Andrews, 2020) which could bring the total cost up to a billion dollars.

Examples of how sample return facility requirements might change during the legal process – more stringent filter requirements than for BSL-4 – quarantine to be replaced by telerobotics – and required safety levels far higher than the one in a million “gold standard” for a BSL-4 facility

NASA’s recommendation has to go through legal review and the requirements may well change as a result of legal considerations. Here are some examples of changes that may be required

- **NASA’s recommendation is likely to use HEPA or ULPA filters** (since nearly all published designs do).

  Legal review might lead to a requirement of 100% containment at 0.05 microns after consideration of the ESF sample return study or even a smaller 0.01 or 0.005 microns after the size limits review - and NASA may be legally required to fulfil those requirements.

  See: By European Space Foundation study (2012), particles larger than 0.05 µm in diameter are not to be released under any circumstances

  to Scientific developments since 2012 that may be considered in a new review of ESF’s 0.05 micron / 0.01 micron size limits – if the review considers life not based on DNA and proteins such as minimum size RNA world cells, this could potentially reduce the 0.05 microns to a requirement that release of a 0.01 micron particle is not acceptable under

any circumstances (above)

- **NASA's recommendation is likely to use quarantine of technicians to protect Earth's biosphere.**

  Legal review might find that quarantine is not sufficient (this has never been legally tested). If so, the facility might be required to be telerobotic.

  See Complexities of quarantine for technicians accidentally exposed to sample materials

- **NASA's recommendation is likely to be based on similar levels of security to a biosphere level 4 facility, with a one in a million chance of escape**

  We will see that some synthetic biologists consider the 1 in a million level of assurance for a biosafety level 4 laboratory to be insufficient for synthetic mirror biology - as there are occasional leaks from even high biosecurity laboratories.

  Instead they design synthetic life so it is dependent on chemicals only available in the laboratory. This solution is not available for extraterrestrial life. It is possible the outcome of the legal process is that no unsterilized sample return is possible until we know what is in the sample.

  See A requirement for similar levels of safety to those used for experiments with synthetic life would lead to the Prohibitory version of the Precautionary Principle and make unsterilized sample return impossible with current technology and current understanding of Mars

There is no precedent for these decisions. So the legal outcome is not known in advance.

Though preliminary studies for the facility can be done early on, it seems that the overall design (e.g. human operated or teleoperated) and engineering details (e.g. filter technology) may be modified during the legal process and can't be known in advance.

Minimum timeline: 2 years to develop consensus legal position, 6-7 years to file Environmental Impact Statement, 9 years to build sample return facility and 2 years to train scientists and technicians in its use

Race doesn't give a timeline to complete the legal process, although it is clear from her analysis that it would take many years (Race, 1996). Uhran et al however have mapped out a minimum timeline (Uhran et al, 2019). From table 2, their estimate is:

- Preparation including founding the oversight agency and developing consensus position on containment margin of safety (2 years)
- File environmental Impact statement, approximately 6-7 years, may be significantly longer if challenged in court.

Total: 8 to 9 years for legal process (including preparation)

- NASA plan to file the impact statement in 2022 (NASA, 2022nic)

So that makes

- 2028-9 the earliest date to complete the legal process, 6-7 years from 2022, though it may be considerably longer.

For the Sample Receiving Facility, they have to

- Build or repurpose containment facility - 9 years
- Train scientists and technicians, 2 years
  [this is because of the many lapses in sample handling for the Apollo facility]

Total: 11 years for the build

Note, the NRC in 2009 estimated a similar 10 to 16 years for the build, before the facility is ready for samples (National Research Council. 2009 : 59)

Going by the lowest of these estimates, if the build started in 2022, the facility could be ready to receive samples by 2033, as required for the planned mission. The legal process could be complete by 2028 if there were no delays.

However, as we saw in the previous section (NASA procedural requirements for mission planners), NASA need to have a clear account of what they will build, and how to service it, before they can go ahead with such an expensive build project. These will not be possible until the legal status is clear and the details of what is required are settled.

Need for legal clarity before build starts - NASA has reached keypoint A for the budget for entire program, but not for the facility – they can't know what they will be legally required to build for the facility – perhaps they can pass keypoint A without legal clarity – but keypoint B requires detailed engineering knowledge of what to build

NASA's planetary protection office has started to think in depth about the implementation of backwards planetary protection. They reached key decision point (KDP A) for the budget for the program in December 2020 (Foust, 2020) (NASA, 2021nmttm) (Gramling et al., 2021) However this is for the whole program including the orbiter, earth return vehicle, mars ascent vehicle, fetch rover etc. The sample receiving facility will need an architectural plan and dedicated budget at some point in the future.

It is hard to see how a build can go ahead when Mars sample return facility design requirements have not yet been legally approved. Perhaps they can pass keypoint A for the sample return facility without this legal clarity, but the more stringent keypoint B, which also has to be passed before the build starts, requires detailed engineering knowledge of what they are required to build.

They can't know this for as long as it remains possible the design will need to be modified during the legal process. The recommended design could be challenged at any point until near the end of the legal process which as we saw is not likely to be completed before 2028, six years after the EIS filing (NASA, 2022nic),.

Need for legal clarity before launch of ESA's Earth Return Orbiter, Earth Entry Vehicle, and NASA's Mars Ascent Vehicle

There would be similar issues for the launch of ESA's Earth Return Orbiter and NASA's.Mars Ascent Vehicle. Before these missions are finalized, ESA and NASA need to know the legal requirements for the Earth Return Orbiter, Earth Entry Vehicle (which will be launched in the same payload) and Mars Ascent Vehicle. There is no way to modify them post launch.

ESA and NASA also need to know what's required well before the launch dates, as unexpected legal requirements may impact on the design and construction of the Earth Return Orbiter (ERO) and Mars Ascent Vehicle (MAV).

To take an example, the ERO as currently conceived transfers the capsule to an Earth Entry Vehicle which then uses a direct flight back from Mars to Earth followed by aerocapture using an aeroshell to protect the contents (Huesing et al, 2019).

So – will the final legal decision approve use of an aeroshell?.

This has planetary protection implications and needs to be considered as part of the legal process.

The legal process will also need to look at how the mission is designed to break the chain of contact with Mars when the Earth Entry Vehicle is opened to retrieve the sample.

The legal requirements on the Earth Return Orbiter could change through to the end of the legal process.

This also may have implications on the capsule design and the design for the Mars Ascent Vehicle.

As well as the risk of delaying the launch, until the legal process is complete, ESA may need to consider to what extent they can risk public funds to design, build and test spacecraft that may need expensive redesign, modifications and retesting to comply with the final legal requirements.

Then – it may well become clear during the legal process that the build won't be completed in time.

- **If NASA decide to sterilize the samples during the return mission – they need to build in the capacity to do this before they launch the Earth Return Orbiter**. It can't be sterilized on a return journey from Mars unless this has already been planned for, and s, the aeroshell would be unnecessary weight as it would need to be captured in Earth orbit by some other spacecraft before it can be sterilized.

- **If NASA decide to return the samples to another location such as above GEO, again the aeroshell is unnecessary weight**, that could be used for extra fuel to help the satellite to reach its intended final orbit

Legal process likely to extend well beyond 6 years with involvement of CDC, DOA , NOAA, OSHA etc., legislation of EU and members of ESA, international treaties, and international organizations like the World Health Organization

There is potential for many delays in the legal process. Six years from filing the EIS is the bare minimum. The legal process starts with the EIS (EPA, n.d.). First, since there is a potential for damage to Earth's environment, various executive orders mandate NASA itself, as a federal agency, to consider such matters as:

- impact on the environment,
- impact on the oceans,
- impact on the great lakes,
- escape of invasive species,
- lab biosecurity against theft
  (NASA, 2012fdg)

After the environmental impact statement is filed, Uhran et al mention many other agencies likely to declare an interest such as the

- CDC (for potential impact on human health),
- Department of Agriculture (for potential impact on livestock and crops),
- NOAA (for potential impact on oceans and fisheries after a splashdown in the sea)

- Occupational Safety and Health Administration, to consider questions of quarantine if a scientist or technician gets contaminated by a sample (Uhran et al, 2019) (Meltzer, 2012:454)
- Department of Homeland Security,
- Federal Aviation Administration because the sample returns through the atmosphere
- Department of Transportation for bringing the sample to the receiving laboratory from where it touches down and to distribute to other laboratories
- Occupational Safety and Health Administration - for any rules about quarantine for technicians working at the facility
- U.S. Customs and Border Protection and the Coast Guard to bring back sample in case of an water landing or the Department of Defense if it lands on land, likely the Utah Test & Training Ranges
- Department of the Interior which is the steward for public land and wild animals which could be affected by release of Martian microbes
- Fish and Wildlife Service for the DoI who maintain an invasive species containment program and may see back contamination as a possible source of invasive species
- National Oceanic and Atmospheric Administration (NOAA)'s fishery program for sea landing in case it could affect marine life and NOAA fisheries
- Integrated Consortium of Laboratory Networks (ICLN) for laboratories that respond to disasters - a partnership of the Department of Agriculture, Department of Defense, Department of Energy, Department of Health and Human Services, Department of Homeland Security, Department of the Interior, Department of Justice, Department of State, and Environmental Protection Agency
- The state where the receiving laboratory is stationed may have regulations on invasive species, environmental impacts, disposal of waste, and possession of pathogens, similarly also for any states the sample may have to transit to from the landing site to the facility

The NEPA process doesn’t provide for judicial review directly, but decisions are often litigated. One common basis for judicial review is inadequate analysis in an EIS - that the agency either failed to consider some of the impacts or failed to fully consider the weight of the impacts they did review. Plaintiffs can’t claim damages, but the court can remand the case to the agency for further proceedings and may specify what those proceedings must include (Congressional Research Service, 2021).

Meanwhile, since this is a joint NASA / ESA mission, it involves ESA. Most of the ESA member states are in the EU (ESA, n.d.MS) so the EU will get involved.

This leads to a separate legal process in Europe, starting with the Directive 2001/42/EC (EU, 2001). I haven’t located any academic reviews for the European process, but as for the case in the USA, this would spin off other investigations which would involve the European Commission (Race, 1996)sion.

The UK, as a member of ESA but not in the EU, might also be involved in a separate process with its domestic laws. Canada also sits on the governing council of ESA, so perhaps may get involved. These countries are all members of ESA and also all potentially impacted by an adverse outcome, as are all countries on Earth.

The potential impacts on the environment of Earth, and on human health worldwide bring many international treaties into play (Uhran et al, 2019),

> ***In an address given to the Space Studies Board Task Group on Issues in Sample Return in 1996, attorney George Robinson presented a list of 19 treaties or international conventions and 10 domestic categories of law, including the rights of individual states and municipalities to quarantine, that may affect return missions***

Also several international organizations are likely to be involved such as the WHO (Uhran et al, 2019).

We will see below that the very worst case scenarios involve degradation of Earth's environment (such as by mirror life). It seems unlikely that these would be ignored. If the legal discussions expand to focus on these scenarios, this could involve many other organizations such as the Food and Agriculture Organization (UN, 1945) because of potential impact on agriculture and fisheries, and the World Health Organization because of effects on human health globally, in the USA, the Environmental Protection Agency partners with the United Nations Environment Programme (UNEP), and Arctic Council, so they'd likely get involved.

EPA, n.d., Partnering with International Organizations.

Indeed there would be few aspects of human life that would not be relevant in some way in discussions of the very worst case scenarios. As the legal process continues, surely there would be open public debate about these scenarios, and if the discussion expands in this way, potentially it might lead to much wider involvement in the international community. It would be necessary to convince the public, and interested experts in all these agencies that this is a safe mission and that all their concerns have been answered.

Race (Race, 1996) says that experts will have challenges deciding in advance whether the sample should be classified as potentially:

- an infectious agent
- an exotic species outside its normal range
- a truly novel organism (as for genetic engineering)
- a hazardous material

The choices here would change which laws and agencies would be involved.

Presidential directive NSC-25 requires a review of large scale effects which is done after the NEPA process is completed. (Race, 1996)

There are numerous treaties conventions and international agreements relating to environmental protection or health that could apply.

Including those to do with (Race, 1996)

- protection of living resources of the sea
- air pollution (long range pollution that crosses country boundaries)
- world health, etc

Individual groups in other countries could invoke domestic laws such as laws on accidents at sea or on land if they argue back contamination of Earth can cause measurable damage. (Race, 1996)

Race says scientists are likely to focus on (Race, 1996)

- technical details
- mission requirements
- engineering details
- costs of the space operations and hardware

General public are likely to focus on

- risks and accidents
- whether NASA and other institutions can be trusted to do the mission
- worst case scenarios
- whether the methods of handing the sample, quarantine and containment of any Martian life are adequate

Six to seven years seems a bare minimum to complete all this. Any addition to the legal process would push the sample return date further back than 2039.

The legal process and public debate for NASA's mission as precedent for China's mission to return a sample too – perhaps as soon as 2030 – with sterilization a likely solution for a country that wants to be first to return a sample

China currently plans to launch a mission possibly as soon as 2028, to return a sample by 2030. It would consist of two rockets, one with a lander and ascent vehicle, and the other with an orbiter and reentry capsule to return the sample to Earth, using two Long March rockets (Jones, 2021)

China had one of the most rigorous of all responses to the COVID pandemic. Professor Bruce Aylward, leader of the joint team that studied their response (McNeil, 2020) put it like this in the press briefing about their findings (United Nations, 2020)

> ***They [the Chinese] approached a brand new virus [that] has never been seen before that was escalating and quite frightening in January … and they have taken very basic public health tools … and applied these with a rigor and an innovation of approach on a scale that we've never seen in history***

If China considers the Mars sample return to be potentially hazardous it is likely to be especially careful just as it has been especially careful with COVID.

The debate that is sure to happen with the NASA mission will help bring widespread awareness of the issues of a sample return and the need to be careful.

China's mission is far simpler than the NASA one and similar to the proposal for NASA by the astrobiologist Chris McKay for a mission that does no more than land, gather a scoop of dirt and immediately return, see Sample return as a valuable technology demo for astrobiology – and proposals to keep the first sample returns simple, a scoop of dirt or skimming the atmosphere to return micron sized dust samples

China's first mission may have a higher chance of returning present day life than the NASA mission as currently envisioned - because they plan to scoop up some dirt which could have viable spores from dust storms, or the life that Viking detected (if it did find life).

Perhaps China may be able to accelerate their legal process or bypass elements of it though they would still have the international treaties and responses of international organizations and other countries to deal with.

However, once this topic enters public debate widely, the public can be expected to raise many issues as NASA has already seen with the comments so far on their draft environmental impact statement (NASA, 2022msrc).

The general public in Chinese likely raise similar issues, which would get the attention of leaders in China, given their recent experience of COVID and the high level of importance they assign to matters of public health.

If this turns into a space race with NASA competing with China, NASA can't accelerate the legal process to "win the race" with an unsterilized return before 2030.

However, NASA can accelerate its timeline if they do a sterilized return or a return to a safe orbit and sterilized subsamples, as that has almost no legal process.

Another way that NASA could "win" the race to return a sample of Mars would be to do a separate low cost sample return such as SCIM skimming the Mars atmosphere to return micron sized "Mars rocks" from dust storms, or Chris McKay's "grab a sample of dirt and return". NASA could have done either of those a decade ago or more.

It would likely be hard for NASA to find the budget for an extra sample return mission in competition with existing programs, but if Congress authorized the expenditure, they could do such a mission very quickly, and with their previous experience and expertise, surely faster than China. See:

- Sample return as a valuable technology demo for astrobiology – and proposals to keep the first sample returns simple, a scoop of dirt or skimming the atmosphere to return micron sized dust samples **Error! Bookmark not defined.**

A fast sterilized sample return, or return to a safe orbit, might lead to China doing the same.

As a response to public concerns, China could use either of the solutions suggested here:

- to sterilize the sample during the return mission.
- to return it to a remotely operated satellite in a safe orbit, and sterilize some of the dirt to return to Earth for immediate study while the rest is tested for signs of life in orbit.

These wouldn't significantly impact on the prestige value of returning the first samples from Mars and they are well within China's capabilities.

For details see

- Sterilized sample return as aspirational technology demonstration for a future astrobiology mission
- **Recommendation** to return a sample for teleoperated 'in situ' study above Geosynchronous Equatorial Orbit (GEO)

If they do this, it could then become the norm for samples returned from another planet – that when you don't know if there is life in them or what form of biochemistry or exobiology might be involved, you return the samples to a safe orbit for preliminary study first, or sterilize them.

Both missions are likely to be of most interest as a technology demo to show we can return a sample from Mars, at a later stage, once we know how to select the samples intelligently. But it's not impossible either mission might return viable present day life.

Sterilization or return to a safe orbit is the simplest solution both from a practical point of view and legally.

However, we need to look in more detail into the challenges involved in an unsterilized return, since that is NASA's current proposal.

Public health challenges responding to release of an extraterrestrial pathogen of unfamiliar biology

Pugel at al analysed the public health challenges involved in responding to release of an unknown extraterrestrial pathogen, for instance after an accident during the sample return that breaches the sample return container (Pugel et al, 2020). This happened with the Genesis mission. The parachute failed to deploy and the capsule broke open on impact with the desert. This was an unrestricted sample return, so there were no human health issues.

To avoid a similar mishap the Earth Entry Vehicle for the Mars samples would be designed to re-enter without a parachute *"It just comes in, and, wham, it hits the ground,"* (Andrews, 2020). However there still could potentially be a design issue or oversight or another unanticipated off nominal event.

Pugel at al. found that there would be a need for public health training before the return of the sample to be ready to respond promptly in a novel situation. If there were a release from containment, our health responders would be faced with a situation outside of the range of usual hazards, such as a natural outbreak, intentional attack or laboratory accident.

As an example, an extraterrestrial organism might not be classifiable using the conventional taxonomy of microorganisms. Hospital preparedness scenarios depend on laboratory testing and medical management. However, existing methods for rapid development of diagnostic tests for terrestrial pathogens might not be suitable for an extraterrestrial pathogen based on an unfamiliar biology (Pugel et al, 2020).

As we'll see, that could include mirror life, or life with novel nucleotides and amino acids or non proteinous life.

If biosafety level 4 procedures are required, medical staff would need to sterilize equipment with autoclaves with a bioframe (hermetically sealed outer container) that heats any vented air to sterilize it. Appropriate procedures would need to be developed to sterilize surfaces and dispose of contaminated personal protection equipment and other materials.

Public communication during such an event would be of crucial importance including rapid communication with health workers of new findings about the pathogen, and health responders and the general public would need open and clear communication (Pugel et al, 2020).

They don't consider the possibility where Biosafety level4 procedures are insufficiently rigorous to contain extraterrestrial life.

Failure modes for sample containment

We will see later that it is possible the legal requirement for containment for a sample return could be much better than the "gold standard" for a BSL-4 facility of one in a million. Depending on the level of precautions taken, it could be a requirement to take no appreciable risks. See:

- Formulating Sagan's statement that "we cannot take even a small risk with a billion lives" as a criterion for the prohibitory version of the precautionary principle

If that is the decision, even highly unlikely failure modes might need to be looked at. So this list includes some scenarios that are low likelihood.

1. Design flaw in the facility - e.g. Martian life smaller than the minimum size of particle the filters are designed for
2. Faulty equipment - such as the gloves which developed holes during the Apollo sample return
3. Human error - the most usual cause of breach of containment for biosafety laboratories
4. Humans taking shortcuts in safety protocols to speed up the research.
5. Mistaken decision that it is safe to separate out the sample with undetected novel Martian life still in it (e.g. not based on DNA or RNA and not using proteins)
6. Faulty design leading to release during replacement of filters, laboratory gloves and other equipment
7. Human error during maintenance of the equipment
8. External accident such as a plane crashes into the facility
9. Intentional harm e.g. a pilot flies a plane into the facility - or the pilot is deluded
10. Domestic terrorism or war that involves the facility intentionally or unintentionally
11. Overlooked design flaw in the sample return mission leading to a chain of contact between Martian surface and the exterior of sample return container
12. Undetected chain of contact set up, e.g. undetected micrometeorite breach of the container
13. Capsule breaks during landing (as happened with the Genesis mission) - perhaps due to design flaw in the capsule
14. Targeting error and capsule lands on something that explodes or in a war zone, or in the deep sea and is damaged or hard to retrieve
15. Targeting error and a human retrieves tbe capsule, and opens it out of curiosity, not knowing what it is.

Then in all these scenarios

1. Directly releases viable Martian life into the environment or
2. Human is contaminated and is kept in isolation - but then needs urgent medical attention

Then these scenarios need to be evaluated with the assumption that there actually is life in the sample that is later proven to be a an unacceptable risk to ever contact Earth's biosphere, such as some scenarios involving mirror life.

For instance, the facility needs to be designed to deal with a possible decision that the sample can never be safely removed unsterilized. It is not sufficient to have a design lifetime of a few years - it needs to be possible to keep it safe indefinitely, in case that is what needs to be done.

Proposal: in some of these failure modes, one solution might be a built in method to sterilize the sample if containment failure is imminent, for instance equipment failure and no safe way to repair it without risking a breach of containment. But others would happen too quickly for some such system to activate as sterilization takes a while and isn't instant.

If human quarantine is part of the containment strategy, we will find in the next section that one possible scenario is that like Typhoid Mary, it might never be safe for the human to leave quarantine. See:

- How do you quarantine a technician who could be a life-long symptomless super-spreader of an unknown Martian pathogen?

Complexities of quarantine for technicians accidentally exposed to sample materials

A Mars sample return facility will need quarantine procedures if technicians can be exposed to the samples or indeed if the general public can be exposed after some off nominal event with the sample return container. However, this has major legal, ethical and scientific ramifications.

During the Apollo sample returns, there were several times that technicians were accidentally exposed to the samples and had to isolate (Mangus et al, 2004:51). For instance, two technicians had to go into isolation after a leak was found in a sample handling glove for Apollo 11 (Meltzer, 2012:485), and then 11 technicians had to go into isolation in 1969 when a small cut was found in one of the gloves during preliminary examination of one of the samples returned by Apollo 12 (Meltzer, 2012:241).

As we saw above, the Apollo quarantine duration and protocols were never subject to legal review or public scrutiny (Meltzer, 2012:452). There was considerable discussion internally, but not externally. They were decided in consultations between NASA and the newly formed Interagency Committee on Back Contamination and in these discussions NASA had the final decisions (Meltzer, 2012:129). The quarantine was carried out under the legal authority of the Surgeon General (Mangus et al, 2004:32) since NASA doesn't have the authority to impose a quarantine, and a similar process would not be permitted today (Meltzer, 2012:452).

The Occupational Safety and Health Administration in the USA is sure to declare an interest for questions of quarantine. Then the WHO is likely to declare an interest at an international level

(Uhran et al, 2019). If unsterilized material from the sample is to be delivered to Europe, the European Commission would be involved, considering the implications of health and safety at work under Article 153 of the Treaty on the Functioning of the European Union (EC, n.d.).

A legal review of the Apollo guidelines would have been likely to raise many issues. There is no legal precedent to draw on here.

This is a graphical summary of some of the quarantine issues that may be raised, which we will go into in more detail in the following sections:

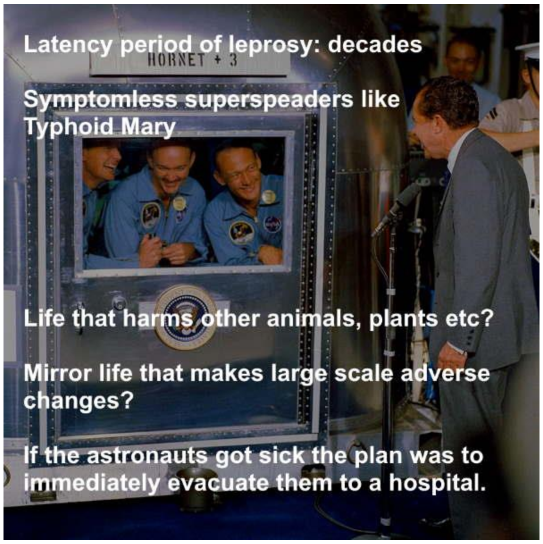


Figure 14: quarantine issues

- Latency period of leprosy: decades
- Symptomless superspreaders like Typhoid Mary
- Life that harms other animals, plants etc?
- Mirror life that makes large scale adverse changes?
- If the astronauts got sick the plan was to immediately evacuate them to a hospital.

Background image: President Richard M. Nixon welcomes the Apollo 11 astronauts aboard the U.S.S. Hornet,July 24, 1969 (NASA, 1969)

Vexing issue of authorizations to remove technicians from quarantine to treat life threatening medical incidents in hospital

First, there is the vexing issue of serious medical incidents, which has been discussed previously in planetary protection articles.

Suppose that one of the Apollo astronauts became seriously ill and needed urgent treatment that wasn't available within the quarantine facility. In this situation, NASA's stated plan was to immediately take them out of quarantine and to a hospital, as an authorized breach of quarantine (Meltzer, 2012:472).

The same ethical dilemma presents itself for a sample handling technician as for an Apollo astronaut. Suppose that there was some sudden medical condition such as a heart attack, unrelated to sample handling, that required urgent expert attention in a hospital. It would be hard to legally or ethically justify keeping the technician isolated unless there was clear proof that removing them from quarantine constituted an overriding significant danger to others.

The ethical conundrum here is that an unknown and probably low probability of severe risk to Earth's environment or to other humans or organisms is difficult to balance against the immediate certainty that without treatment an individual may die. The legal issue is one of human rights; could a technician legally be kept in quarantine in this situation, when it is known that removing them could save their life?

Even if a technician's condition is known to be caused by the sample, the situation is ethically problematic. The Apollo era guideline in this case is at least understandable, that any clear risk to the individual astronaut's life and health always has priority over planetary protection considerations.

This seems likely to be the policy for a Martian sample return handling incident too, that technicians will be immediately removed from quarantine if their life or health is seriously at risk, whether or not this is believed to be due to materials from the sample. It would be ethically difficult to argue for any other policy. However if this is the policy, it significantly reduces the capability of the quarantine procedures to protect Earth.

Example of a technician in quarantine with acute respiratory distress and symptoms similar to Legionnaires' disease – a disease of biofilms and amoebae that adventitiously infects humans – and sometimes mentioned in planetary protection discussions

Let's look at the example of a technician who suddenly suffers from acute respiratory distress in quarantine. The first question would be, is this terrestrial or Martian in origin?

It's not likely that a sample would contain an exact analogue of the microbes that cause Legionnaires' disease, as they need an aquatic environment to survive, can't survive drying and don't form spores.

Legionella pneumophila is aerobic and requires 6.0 to 6.7 mg of oxygen per liter and can reproduce down to 2.2 mg per liter (Wadowsky, 1985). However we shouldn't exclude the possibility of aerobes on Mars. See

- Some Martian brines could be oxygen rich permitting aerobes or even primitive sponges or other forms of multicellularity - Stamenković's oxygen-rich briny seeps model (below)

Although we can't expect an exact analogue of Legionnaires' disease, the way it invades the lungs could still be relevant for a possible Martian pathogen, especially if Mars has aerobic amoebae or other protists or biofilms.

Legionnaires' disease is caused by various species of Legionella, usually L. pneumophila, an intracellular disease of protists (single cell eukaryotes, with nucleus and organelles, but not part of a fungus, animal or plant).

This microbe is not adapted to humans but can sometimes cause a deadly opportunistic infection of our lungs. When white blood cells (phagocytes) engulf this bacterium, it is able to avoid digestion, using the same methods it uses to avoid digestion by protists in a biofilm and is then able to replicate inside the white blood cell (Best et al, 2018) (WHO, 2007). See *(Alberts et al 2002)*:

> *Legionella pneumophila is normally a parasite of freshwater amoebae, which take it up by phagocytosis. When droplets of water containing L. pneumophila or infected amoebae are inhaled into the lung, the bacteria can invade and live inside alveolar macrophages, which, to the bacteria, must seem just like large amoebae.*

Legionella bacteria can also live in the intercellular space of biofilms (Abdel-Nour et al 2013).

The concept of a Martian analogue of Legionnaires' disease is used as an example in planetary protection discussions to show that a Martian microbe would not need to be adapted to infect multicellular animals to be a pathogen of humans (Warmflash, 2007)

> *In essence, all that a potentially infectious human pathogen needs to emerge and persist is to grow and live naturally under conditions that are similar to those that it might later encounter in a human host. On Mars, these conditions might be met in a particular niche within the extracellular environment of a biofilm, or within the intracellular environment of another single-celled Martian organism.*
>
> *...*
>
> *To be sure, the genetic similarity between humans and protozoa is much greater than could be expected between humans and the Martian host of a Martian microbe.*
>
> *However, the L. pneumophila example does bring into question the rationale of the need for host-pathogen coevolution. Even in the context of a planetary bio-sphere that is limited to single-celled life, and even where there is unlikely to have been a co-evolution between agent and host organism, the possibility of infectious agents, even an invasive type, cannot be ruled out.*

For more about how an alien pathogen could evade our immune systems, with a discussion of Legionella and many other examples, see the section Could present day Martian life harm terrestrial organisms? (below).

So, what happens if a technician gets sick with acute respiratory distress, and there is no other known cause? Even for a suspected Martian pathogen, it would be hard to justify leaving a technician to die in the quarantine facility, when it's known that intensive care with ventilators and oxygen equipment could save their life.

Indeed, even if the symptom was not only suspected but known for sure to be caused by a Martian pathogen, it would likely still be unknown whether it is contagious and harmful to others, or how contagious it is. This would lead to some difficult decisions.

Indeed, even if it was a known Martian pathogen, and considered to be potentially contagious, if a technician's life was at stake, they might well still be removed from the quarantine taking as much care as possible to avoid infecting others. Anything else would be hard to justify ethically.

However if this is what happens, quarantine gives little by way of real protection of other humans even for a clear case of a Martian pathogen causing life-threatening symptoms in a quarantined technician. Meanwhile, this breach of quarantine would give potential routes for the pathogen to spread to other terrestrial microbiomes and also expose Earth's biosphere to any other Martian microbes that got into the technician's microbiome.

These considerations suggest some quarantine issues may require extensive discussion and study as it goes through the legal process. This could lead to further delays.

Arbitrariness of technician's quarantine period for an unknown pathogen – Carl Sagan gives the example of leprosy which can take 20 years or more to show symptoms

Apollo's three week quarantine period was arbitrary. Carl Sagan observed that we do not know the necessary quarantine period for a sample return. He gave the example of leprosy (Sagan, 1973:130)

> *There is also the vexing question of the latency period. If we expose terrestrial organisms to* Martian *pathogens, how long must we wait before we can be convinced that the pathogen-host relationship is understood? For example, the latency period for leprosy is more than a decade.*

We now know that leprosy can take 20 years or more to show symptoms (WHO, 2019).

Also, prion diseases can have very long incubation periods of anything from 13 years for a study of vCJD in France (ECDC, n.d.), to over 50 years for Kuru in South Fore where the native population have a mutation that gives them protection against it (Collinge et al, 2006).

It would be hard to justify any particular quarantine period for a technician who has been exposed to a Martian sample. For a known and well characterized Martian pathogen, quarantine periods might work, depending on the disease. However, for an unknown pathogen, in a situation where we don't know if they were exposed to anything, never mind what the potential pathogens are from Mars, there is no way to calculate a suitable quarantine period.

However there is another even more recalcitrant issue that needs to be looked at.

How do you quarantine a technician who could be a life-long symptomless super-spreader of an unknown Martian pathogen?

I can't find any previous discussions of asymptomatic carriers in the planetary protection literature. However, infectious disease specialists such as the WHO experts are certain to raise this issue once they become involved in the legal review process. Typhoid Mary (Korr, 2020) is a famous example of an asymptomatic carrier of a disease. She was healthy, symptomless, but transmitted typhoid to the people she cooked meals for. She was a symptomless carrier, or she could be called a life-long "symptomless super-spreader". She had to be isolated to protect others.

Typhoid Mary had no endpoint to her isolation either, no isolation period. She continued as a symptomless carrier capable of spreading typhoid throughout her life. Her isolation only ended with death. How would you quarantine a potential "Typhoid Mary" symptomless super-spreader of an unknown alien pathogen?

So, could there be symptomless spreaders of a Martian pathogen?

Let's look at Legionnaires' disease again which as we saw is used as an analogue of a Martian pathogen in planetary protection discussions.

In many individuals Legionnaires disease is asymptomatic, or at least, subclinical (Boshuizen et al, 2001). By analogy, a technician whose lungs are infected by a Martian microbial disease of biofilms might be asymptomatic, yet on release from quarantine they might then spread it to others who are worse affected by this microbe.

An analogous Martian disease may be naturally resistant to antibiotics because of the difference in biochemistry, and if so, mortality may be high. In the original outbreak of Legionnaires' disease in 1976, 29 people died out of 182 individuals who developed the disease as far as an acute respiratory illness (Winn, 1988:61).

So, Legionnaires' disease has symptomless carriers, yet at the same time it has a deadly effect on some individuals.

An exact analogue of an outbreak of Legionnaires' disease would be comparatively easy to control, as it doesn't spread easily from person to person and instead spreads to humans from other sources in the environment, via a route that is reasonably easy to guard against.

However its Martian analogue could still spread in the same way as Legionnaires' disease, from the technician to biofilms in the environment first, then later from biofilms back to humans again. It might not be as easy to prevent the transmission from the biofilms back to humans as for Legionnaire's disease depending on the details.

We can also ask, could such a disease spread directly from human to human through respiratory droplets like another microbial respiratory disease, Hib? Legionnaires' disease is also transmitted through respiratory droplets occasionally, though in that case it is rare. But could other pathogens spread more easily - including alien life that is just ignored by our immune system? The answer would seem to be that yes it can. This is discussed later in this article under: Could a Martian originated pathogen be airborne or otherwise spread human to human?

For another example, Serratia liquefaciens is one of the best candidates to survive in the low atmospheric pressures on Mars (Schuerger et al, 2013) (Fajardo-Cavazos et al., 2018). It is an opportunistic pathogen of humans which is occasionally fatal (Mahlen, 2011). A Martian analogue could survive in a human carrier without causing symptoms yet add to the opportunistic pathogens of humans in our biosphere.

Martian microbes could participate harmlessly or even beneficially in the human microbiome but harm other terrestrial organisms when the technician exits quarantine - example of wilting Zinnia on the ISS

Martian microbes could also be harmless or even beneficial to humans but potentially harmful to other organisms or to ecosystems. They could join the diverse microbial communities of bacteria, archaea and fungi inhabiting the sweat glands, hair follicles, dermal layers etc of our skin (Byrd et al, 2018), in our mouths (Deo et al, 2019), sinuses (Sivasubramaniam et al, 2018), and respiratory tracts (Kumpitsch et al, 2019). They could make use of many sources of food from our bodies in the form of dead skin cells, hair, and secretions such as sweat, sebum, saliva and mucus.

An example of this happened on the ISS, which because of its natural isolation from the terrestrial biosphere makes such issues particularly easy to study (Avila-Herrera et al, 2020). In 2018 the Zinnia Hybrida plants in a plant growth experiment on the ISS wilted. Two of the plants that displayed stress died and two survived (NASA, 2016hmossf).

Figure 15: Mold growing on a Zinnia plant in the ISS. The mold fusarium oxysporum is thought to have got to the ISS in the microbiome of an astronaut (Urbaniak et al, 2018). Two of the four infected plants died (NASA, 2016hmossf). It would be impossible to keep a pathogen of terrestrial plants out of the terrestrial biosphere with quarantine of technicians or astronauts.

The cause was a fungal pathogen Fusarium oxysporum probably brought to the ISS in an astronaut's microbiome (Urbaniak et al, 2018). The same fungus was cultured from an isolate from the astronaut's dining table (Urbaniakt al, 2019). The composition of the populations of microbes colonizing surfaces in the ISS seems to vary depending on the composition of the microbiome of crew members (Avila-Herrera et al, 2020).

All the astronauts have two weeks preflight "health stabilization" in quarantine. This is intended to reduce the risk of preventable infectious diseases of humans. It is not designed to keep out a fungus that can infect plants (NASA, n.d.hsp). Although fusarium oxysporum can be an opportunistic human pathogen, on this occasion it was only harmful to the plants.

As another example, microalgae produce accidental hepatotoxins that can damage livers of cattle and dogs that eat algal mats, a common occurrence in the Great Lakes (Hoff et al, 2007). Such toxins wouldn't harm humans, since we don't eat the algal mats, but are harmful to other creatures.

Although these microalgae are not a natural part of the human microbiome it would be hard to guarantee that the technicians in quarantine are not carrying any microalgae on their skin. This does sometimes happen. Leptolyngbya ramose is the first representative of a cyanobacteria to be isolated in a clinical specimen from the human microbiome (Bilen et al, 2019).

In a similar process, a microbe that is harmless as part of a human microbiome could be a pathogen of other organisms to Earth or generate accidental toxins that harm other organisms in our ecosystems.

For a more detailed discussion of these and many other examples, see again the section Could present day Martian life harm terrestrial organisms? (below).

What if mirror life becomes part of the technician's microbiome?

Let's suppose that the microalgae of the last section is mirror life. Or, suppose nanobacteria mirror life was to become part of the human microbiome, perhaps on our skin or in our respiratory tracts. This mirror life could be harmless or benign for humans, and co-exist with the numerous other species in the human microbiome, yet might cause major issues once released to the external world. There might be no way to "break the chain of containment" to let the technician leave isolation without spreading this mirror nanobacteria to the environment of Earth, with consequences that may be hard to predict.

On average we shed one layer of our skin from the surface each day in the form of corneocytes, replaced by new keratinocytes at the base of the layer (Wickett et al, 2006:S101). The average turnover time for an individual cell is about 28 days (Abdo et al, 2020) though with a lot of individual variation depending on the location (e.g. the forearm sheds faster than the thigh), age (young people shed skin faster than older people), and individual variation (Roberts et al, 1980) (Grove et al, 1983)..

In one study, children in a classroom released approximately 14 million bacteria and 14 million fungal spores per child per hour, for a total of 22 mg per child per hour (Hospodsky et al, 2015).

If mirror life did become part of a human microbiome, able to grow on our skin, potentially large quantities of mirror life could be shed into the environment, thousands or millions of spores a day.

Once the technician leaves quarantine, they would shed mirror life into the environment. It could then spread through any terrestrial microhabitats that it encounters.

If we found we need to eliminate an alien biochemistry from the human microbiome, to keep Earth safe, there is no guarantee that we would succeed, even once we understand it. In the worst case there might be no way to break the chain of containment to let the technician safely leave the facility.

So, if a technician has been infected with a strain of microbe that we know would harm the terrestrial biosphere and there is no way to eradicate it - they might have to stay in isolation for the rest of their life like Typhoid Mary (Korr, 2020).

So, could the samples contain mirror life and could such life be harmful to other creatures or the environment of Earth? We look at this in the next section.

Potential for mirror life on Mars and survival advantages of mirror life competing with terrestrial life that can't metabolize mirror organics

It's not known how terrestrial homochirality evolved, with many proposed mechanisms (Blackmond, 2019). Some experts such as Blackmond and Vlieg have expressed the view that it is just the *"luck of the draw"* and that we could find another planet out there with mirror life (Brazil, 2015). So we have to consider the possibility that technicians could be contaminated by mirror bacteria.

Mirror bacteria are likely to have a survival advantage on Earth. Most terrestrial life would be unable to metabolize most mirror organics such as starches, proteins, and fats (Dinan et al, 2007) (Bohannon, 2010).

Some species of terrestrial microbes might develop the ability to metabolize mirror organics. Our biosphere already has a few species of microbes that can express the isomerases and racemases needed to flip organics into their mirror molecules, to metabolize mirror organics (Pikuta et al, 2006) (Pikuta et al, 2010) (Pikuta et al, 2016).

However, most terrestrial microbes would not be able to do anything with mirror organics. Meanwhile, Martian life could already have the equivalent enzymes to metabolize normal organics. This has to be a possibility, given that some terrestrial microbes can already metabolize mirror organics.

One way this could happen is if Mars already has a biosphere where mirror and non mirror life co-exist. They might for instance have evolved separately in different habitats on early Mars and then two forms of life encounter each other later. Each form of life might then evolve the enzymes to metabolize organics from the other form of life. The result could be that mirror life from Mars is already able to metabolize non mirror starches, proteins and fats, giving it an initial competitive advantage over terrestrial life that has never been exposed to mirror organics.

Mirror Martian life might also need these enzymes to metabolize organics from the infall of meteorites, as these have both normal and mirror forms of carbohydrates, amino acids and other organics.

Most organics on Mars may well come mainly from the infall of meteorites, comets and interplanetary dust (Frantseva et al, 2018) rather than from life processes even if there is life there. If there was no degradation of the organics, Mars should have 60 ppm of organics deposited into the regolith, averaged over its entire surface to a depth of a hundred meters (Goetz et al, 2016:247).

This would lead to a strong selection advantage for life able to make maximal use of both isomers of sugars and amino acids in meteoritic material.

The outcomes for terrestrial ecosystems from release of such a lifeform could be serious, as mirror life gradually converts terrestrial organics to indigestible mirror organics through one ecosystem after another. This is covered later in this article:

- Example of a mirror life analogue of chroococcidiopsis, a photosynthetic nitrogen fixing polyextremophile

Martian life might also be of mixed chirality itself, with organics of both chiralities in a single cell. This is a proposal for early life, that it might use something like Joyce's enzyme, a ribozyme that can catalyze replication of its mirror image, possibly permitting "ambidextrous" life (Joyce, 2007).

Ambidextrous life might also transform half the organics in an ecosystem to mirror organics. Similarly to mirror life, this could reduce the habitability of ecosystems for terrestrial life, and give a selection advantage to the ambidextrous life.

Then if either the mirror life or ambidextrous life has a simpler biochemistry it could be able to shrink to nanobacteria as small as the hypothetical RNA world nanobacteria. For instance it might be mirror RNA world cells, and this would give it all the same selection advantages as the nanobes of the shadow biosphere hypothesis.

- Could the postulated RNA world nanobacteria 0.014 microns in diameter spread through Earth's environment (or other simpler forms of life)? Answer seems yes, possibly, with similar advantages to the postulated nanobes of the shadow biosphere hypothesis

If there is mirror life or ambidextrous life in the sample, it could find a niche as a minor component in the human microbiome of technicians, almost undetectable and harmless to humans. Nothing would happen until the technician exits quarantine and the mirror life starts to spread through the terrestrial biosphere.

It is hard to see how this risk could be prevented by legal work on the duration of quarantine or conditions for quarantine of technicians.

Similar considerations apply to astronauts returning from Mars - in some scenarios such as mirror Martian life, astronaut quarantine would be insufficient to protect Earth's biosphere

These considerations apply equally to the return of astronauts from Mars. Once a species of mirror bacteria or nanobacteria becomes part of an astronaut's microbiome, even if it is harmless to the astronauts, it might well be impossible to sterilize the astronauts from mirror life. It would then no longer be possible to safely return those astronauts to Earth without risking introduced mirror life.

All the other considerations here for technicians in a sample return facility, such as symptomless super spreaders, and no guaranteed quarantine period also apply equally to humans returned from Mars. In short there seems to be no way to use quarantine to protect Earth from extraterrestrial life that may infect human astronauts or technicians, unless the capabilities of that life are known.

For known life there may be ways to protect Earth during the return of an astronaut, depending on the lifeform. However, before we can design suitable precautions such as a quarantine period, we need to know what we need to protect against.
Our conclusion is that we can't design a quarantine method that is guaranteed to protect Earth from all conceivable forms of Martian exobiology.

.Once we know what is there

- we may be able to protect Earth using quarantine.
- But with some scenarios, such as mirror life that can't be eradicated from the human microbiomes, quarantine of humans may not be enough to protect our biosphere

There are many potential scenarios here, in principle.

It's even possible to envision future scenarios where Mars remains

- restricted Category V in the backwards direction indefinitely, no life from Mars can be permitted to enter Earth's biosphere
- yet remains category II (no need for sterilization) in the forward direction also indefinitely.

We get such a scenario by combining the ideas of this section with the earlier section:

- Example future scenarios where unlike the case for the Moon, we might need to continue to protect Earth's biosphere, maybe even indefinitely, even if we prove there is no or minimal risk of forward contamination of Mars

Here is an example scenario:

- All Martian mirror life habitats on Mars are inhospitable to terrestrial life, so there is no risk of contamination by terrestrial life harming its scientific interest
- Martian mirror life however is able to survive in some conditions found on Earth
- Martian mirror life can convert normal to mirror organics

In this situation there might be

- no need to sterilize missions to Mars, because terrestrial life can't inhabit habitats for Martian mirror life
- but we couldn't return astronauts or unsterilized samples from Mars to Earth because it would convert terrestrial organics to mirror organics.

We would always need to break the chain of containment with Mars on returning to Earth.

In this hypothetical scenario, if in addition:

- Martian mirror life is harmless to humans,
- only potentially harmful to other organisms or our ecosystems as a whole (by gradually changing them to mirror organics)
-

then we get a scenario where hypothetically:

- humans can settle on Mars but can never return to Earth.

It's an unlikely seeming scenario but in our vast universe there might be a civilization in some galaxy that has encountered this scenario.

A laboratory with the samples handled telerobotically as a solution to all these human quarantine issues – however the other problems remain and the safest way to do telerobotics is in an orbital facility with the robotics controlled remotely from Earth

The simplest solution to all these human quarantine issues might be to require all sample handling to be done telerobotically, eliminating the risk for accidental contamination of technicians. The LAS study for a sample receiving facility from 2009, relies on telerobots to do almost all the sample handling (Beaty et al, 2009:75). So we could do it already in 2009, and telerobotic capabilities have improved since then.

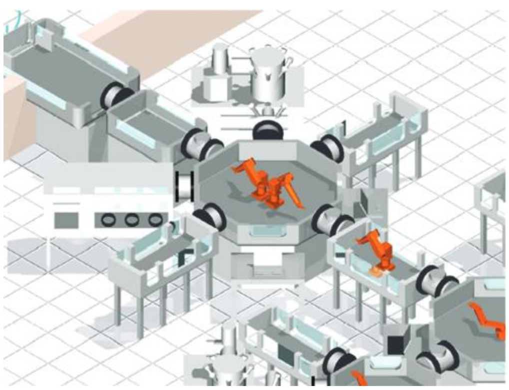


Figure 16: the LAS fully robotic floor plan for a Mars sample receiving facility.

Credit NASA / LAS (Hsu, 2009)

However there could still be risks from accidents when returning the sample to the lab, human error, even a crash of a plane into the facility. Also,m even with a telerobotic facility, filters have to be replaced.

Also a telerobotic facility doesn't solve the other issues we discovered, such as that the technology to contain the sample doesn't exist yet, and that the timeline to return the sample is so long, that we can‘t do it realistically before 2039 and with delays beyond 2039 likely (with the end to end requirement to start the build after the legal process).

This article recommends that the safest way to do such remote manipulation is by remotely operating telerobotic instruments in an orbital facility. This also seems to be the only legally practical solution if we wish to study unsterilized samples close to Earth as soon as the 2030s.

For details see **Recommendation** to return a sample for teleoperated ‘in situ’ study above Geosynchronous Equatorial Orbit (GEO) (below)

There is an alternative that perhaps could be considered that could solve all those problems too. This is a only a sketch for an idea, but could perhaps be worked up into something interesting. See

- Proposal: a sketch for a biosafe laboratory on Earth designed for 100% containment of even nanoscale mirror life using telerobotics, a sump heated to 300°C with heat and vacuum stable light oil, and built in heat sterilization at end of life of the facility - could this be a safe way to open “Pandora’s box”?

Zubrin's arguments in: "Contamination from Mars: No Threat" - not likely to be decisive in legal process - response of planetary protection experts in "No Threat? No Way"

Zubrin, founder and president of the Mars Society, is the only dissenting voice in the planetary protection literature on the need to protect Earth from Mars samples. This is in an article he wrote for the Planetary Report in July / August 2000 (Zubrin, 2000). Zubrin says:

> *"Of all the dragons infesting the maps of would-be Mars explorers, one stands out as not only illusory but hallucinatory. This is the "Threat of Back Contamination.""*
>
> *"The story goes like this: no Earth organism has ever been exposed to Martian organisms, and therefore we would have no resistance to diseases caused by Martian pathogens. Until we can be assured that Mars is free of harmful diseases, we cannot risk exposing a crew to such a peril, which could easily kill them or, if it didn't, return to Earth with the crew to destroy not only the human race but the entire terrestrial biosphere.*

The kindest thing that can be said about the above argument is that it is just plain nuts....".

He then outlines his main arguments against it. They are points to consider but there is another side to each one.

I will paraphrase his main points and link to sections in the current paper with responses to what he says:

- **That diseases are keyed to their hosts**, and so Mars life would not be able to survive on, or in a human body. That if there were indigenous Mars host organisms, they'd be as distantly related to us as elm trees, and Dutch elm disease can't infect humans. Also, there's no evidence for macroscopic hosts on Mars anyway, so how can Mars microbes harm humans?

  Yes Dutch Elm disease can't infect humans but Legionnaire's disease can, a disease of biofilms and amoebae, not adapted to humans. There are many other microbes that can harm us and are not keyed to us as hosts such as the microbes that cause tetanus, botulism, fungal diseases etc: For many more examples see: Could present day Martian life harm terrestrial organisms? (below).

- **Any Mars life that could survive on Earth is already here**, transported on meteorites - we receive half a ton of meteorites from Mars every year, and so Earth life is already exposed to Mars life.

  Most of that material is sterilized in the journey from Mars to Earth and comes from areas of Mars with a low chance of life – and meanwhile the dust, salts, ice etc never get here via asteroid impacts: Could Martian life have got to Earth on meteorites? Our Martian meteorites come from at least 3 m below the surface in high altitude regions of Mars

- **That Mars life would not be able to survive on Earth** because the environment is so different here.

  Martian extremophiles may well be able to survive on Earth too, see: Could present day Martian life harm terrestrial organisms?

- **That to sterilize a Mars sample would be like sterilizing a dinosaur egg**, a terrible loss to science.

  We don't have to sterilize the samples to protect Earth. See: **Recommendation** to return a sample for teleoperated 'in situ' study above Geosynchronous Equatorial Orbit (GEO)

- **That every time you turn over the soil you are returning past Earth life to the present** which could potentially harm us. So since we don't take precautions when we do that, we don't need to take precautions for samples returned from Mars.

Turning over soil is okay by Greenberg's “Natural Contamination Standard” (Greenberg et al, 2001).

See: Comet and asteroid sample returns are straightforward - but are unrestricted sample returns - sterilized during collection - or Earth has a similar natural influx

The November / December 2000 edition of the Planetary Report has the replies to him, by specialists in planetary protection, under the title ***"No Threat? No Way"*** (Rummel et al., 2000):

- ***A Case for Caution*** by John Rummel, NASA'S planetary protection officer at the time, and previously, NASA senior scientist for Astrobiology
- ***Hazardous Until Proven Otherwise***, by Margaret Race, a biologist working on planetary protection and Mars sample return for the SETI Institute and specialist in environment impact analysis
- ***Practical Safe Science*** by Kenneth Nealson, Director of the Center of Life Detection at NASA's JPL at the time.

As Margaret Race put it in her response:

> *"When I read the opinion piece by Robert Zubrin .... I didn't know how to react. As a biologist working on planetary protection and Mars sample return at the SETI institute, I wondered how an engineer and Mars enthusiast like Zubrin could make such irresponsible and inaccurate statements. Obviously, Zubrin is entitled to his opinion, even if it's based largely on misuse of facts. But what about the readers of The Planetary Report? Don't they deserve more than op-ed humour?"*

The other responses were similar in tone.

John Rummel, planetary protection officer for NASA makes the point that microbes that have not co-evolved with humans can be dangerous and uses Robert Zubrin's Dutch Elm disease example as a reminder that microbes which are not human pathogens can still cause damage t other organisms.

He uses the example of Radiodurans which is able to survive in reactor cooling ponds. There is no way radiodurans could have evolved in reactor cooling ponds. This shows that a microbe doesn't need to evolve in the same environment it later inhabits

> *'One canard to point out, however, is Bob's assertion that "microorganisms are adapted to specific environments," and thus Mars microbes would refrain from living on Earth. This is not a reliable speculation. A notable counterexample from Earth is Deinococcus radiodurans, an organism first isolated from nuclear power plants environments that did not exist prior to the 1940s. Where did this microbe come*

from? Deinococcus radiodurans has since been found in natural environments (dry lake beds) quite unlike Three-Mile Island. '

Margaret Race describes the basis for planetary protection in the 1967 Outer Space Treaty, and the recommendations of the study by the National Research Council, *"hardly an alarmist group"*. She also mentions a previous survey for the Planetary Report that found that out of 4,300 members of the Planetary Society, an overwhelming majority agreed to the statement:

"all materials brought to Earth from Mars should be considered hazardous until proven otherwise."

She likens our precautions for a Mars sample return to installing smoke detectors and fire extinguishers in a building, saying:

"He's confident in our impressive technological prowess; he's raring to go and doesn't want anything to slow down or stop our exploration of Mars - especially not burdensome regulations based on very small risks and scientific uncertainty. Yet when he suggests that there's no need for back contamination controls on Mars sample return missions, he's advocating an irresponsible way to cut corners. If he were an architect, would he suggest designing buildings without smoke detectors or fire extinguishers?"

Kenneth Nealson says that the technology for containing biohazards is not out of reach, and he also, already back in 2001, predicts that in the future we will be able to use *in-situ* searches, writing

"Second, a number of measurements could be made onsite (on Mars) that would help in the search for life. The technology of in-situ life detection has lagged behind many other efforts; now may be the time to push for the development of instruments capable of detecting without ambiguity the presence of life at a given site, or more particularly, in a given sample. Sending data back should be a major part of the planetary program, especially as we venture farther from Earth to where sample return is more difficult and expensive. To become more expert in this procedure while on Mars would seem a reasonable and useful endeavor. Why not be safe, have pristine samples to study, and take on our duty as responsible scientists and citizens? I believe that is not too much to ask; in fact, it is prudent and wise to follow such a course."

"Doing solid science in a clean and safe way will help ensure the future of the space program. Alternatively, denigrating those who would argue for safe measures regarding the unknown is ultimately irresponsible."

Zubrin's arguments are not likely to be persuasive for the legal process.

These complexities arise due to need to contain almost any conceivable exobiology – simplest solution to sterilize the samples

All these complexities arise because of the need to contain almost any form of biology, including for instance, mirror life. The requirements would be the same for an unsterilized sample from a Mars analogue planet anywhere in our galaxy, or the observable universe, if it could be returned to Earth.

If the samples are thought to be unlikely to contain life, the simplest solution is to sterilize the samples.

Sterilized sample return as aspirational technology demonstration for a future astrobiology mission – with the six months return journey used to sterilize the sample

The process is greatly simplified if the sample is seen as an aspirational astrobiological technology demonstration (Bada et al, 2009). It is then reasonable to sterilize the sample. Ionizing radiation is preferable as heat sterilization would destroy much of the interest of the sample (Board et al., 2002b:39).

Proposal: the six month return journey can be used to sterilize the sample with an on-board portable x-ray or a Cobalt 60 gamma-ray source.

An x-ray source gives the best dose uniformity ratio (DUR) < 1.8 and is more penetrating, but typically has high power requirements of the order of kilowatts (GIPA et al, 2018:24,36). A portable X-ray source is in use already on the ISS for a bone densitometer (Rutkin, 2014), operating at 35 kV and 80 kV (Vellinger et al, 2016).

The new technology of miniature field emitters using carbon nanotubes (Kim et al, 2016) reduces the operating current to microamps instead of milliamps, reducing power requirements to of the order of watts.

Cobalt 60 would require shielding to protect technicians working on the spacecraft and to protect spacecraft electronics, and though conceptually simpler, may add to the complexity and mass requirements.

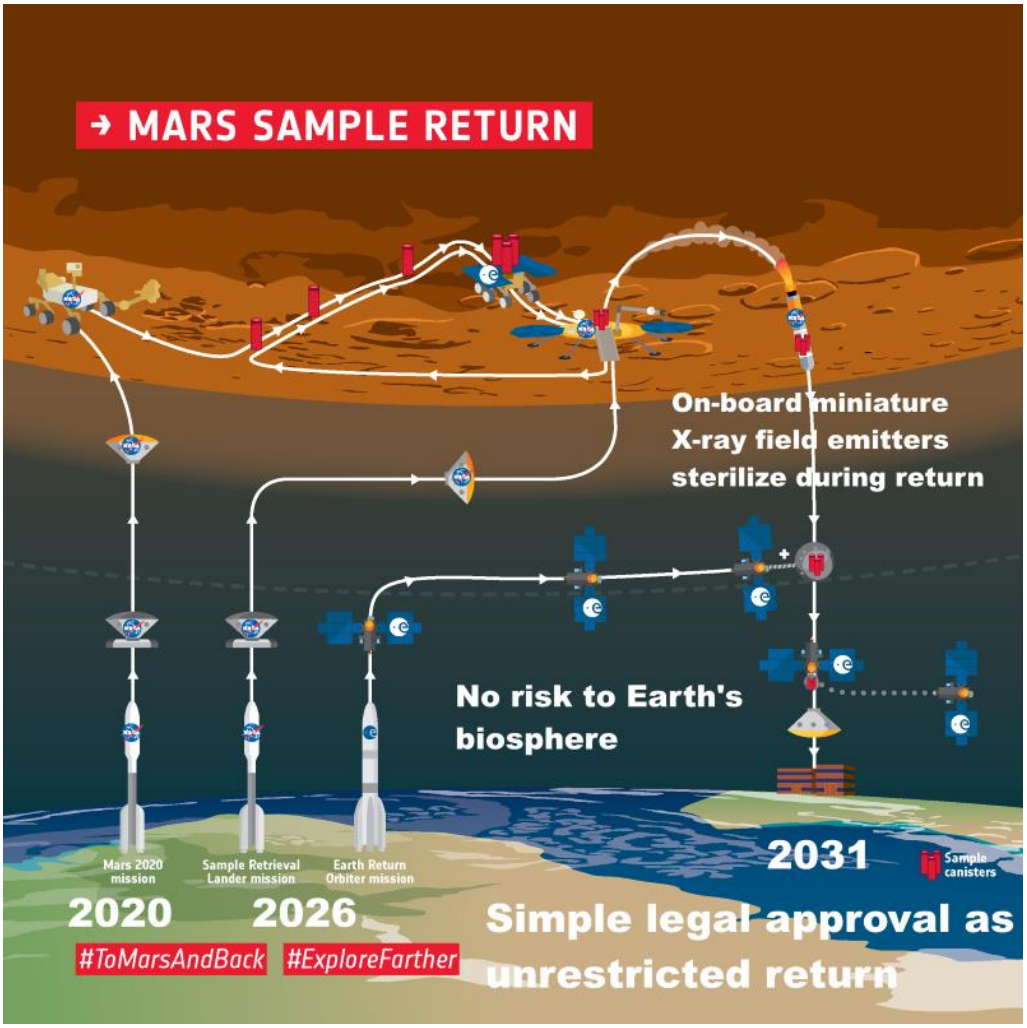


Figure 17: Text added to ESA graphic (Oldenburg, 2019) showing concept of sterilized return using an on-board X-ray source during the return mission

Alternatively the sample can be returned to a satellite in Earth orbit and sterilized there.

On the remote chance the sample contains life, an exposure level would be selected that leaves any native life adequately sterilized but easily recognizable. This should have minimal effects on the geological investigations.

Level of sterilization needed to protect Earth's biosphere is similar to ~100 million years of Martian surface ionizing radiation - and would leave present day life and past life still recognizable - if recognizable without sterilization

Curiosity measured 76 mGy / yr (milligrays per year) on the Martian surface (Hassler, 2014). Radiodurans can survive 5000 Gy with no loss of vitality (with a characteristic shoulder of resistance, with vitality starting to fall beyond those levels) (Battista et al, 1999). This means radiodurans would have no loss of vitality after over 65,000 years of Mars surface ionizing radiation. A hypothetical more resistant microbe could survive longer than this with no loss of vitality.

When radiodurans was first discovered in 1956, it could withstand ten times more radiation than the previous record holder. According to one theory, radiodurans may have evolved its resistance to repair damage from background radiation in permafrost or semifrozen conditions. In these cold conditions, microbes can enter cryptobiosis, a form of microbial “hibernation” where all measurable metabolic processes stop. Cryptobiosis can last for millennia in some cases and for a microbe in cryptobiosis, background radiation can lead to significant DNA damage (Makarova et al, 2001).

Radiodurans is also occasionally blown up into the stratosphere where it can be exposed to a thousand times the UV levels at ground level. There are many other suggestions for the origins of its radioresistance (Krisko et al, 2013), however we have nowhere on Earth with the high levels of ionizing radiation of Mars.

Miliekowsky et al. suggest we should consider the possibility that life on Mars could be even more radiation resistant than radiodurans after billions of years of evolution in conditions of such high levels of ionizing radiation. Mileikowsky et al provide figures for a hypothetical more resistant microbe on Mars, based on the assumption that it requires five times the dose to reduce numbers to 1/e (1/2.718, roughly a third). They are using a simpler model here without a shoulder of resistance. This means that their more resistant microbe would need 5000 Gy to reduce numbers by 1/3 instead of the 1000 Gy for radiodurans. They then did a detailed modeling of the effects of the radiation on the cells with a fit to data from experiments with D radiodurans R1 (Mileikowsky, 2000: p 401).

[In the rest of this section I add (million grays) after every occurrence of MGy to assist any readers that rely on a screen reader to read out the text on the screen]

We can use column 8 of table IIIa of Mileikowsky et al (Mileikowsky, 2000: p 400), which is based on an assumption of 194 mGy / yr (milligrays per year).

To reduce numbers to 1 in a million

- radiodurans requires 2.8 million years, total dose 0.54 MGy (megagray, or million grays)
- their hypothetical more radioresistant microbe requires 42 million years, total dose 8.24 MGy (million grays), or 15 times the dose for radiodurans.

Adjusted to 76 mGy / yr (milligrays per year) as measured by Curiosity, the figures are

- Radiodurans: 7.1 million years
- Hypothetical more radioresistant microbe: 107 million years

Meanwhile, according to Kminek et al, adjusted to 76 mGy / yr, to reduce many amino acids to a millionth of the original concentration requires (Kminek et al, 2006):

- 1.3 billion years of surface radiation, total radiation dose of about 100 MGy (million grays)
  (original figure was 500 million years based on 200 mGy per year).

Earth Planetary Science Letters article[Figure needs permission, redraw or new source]

Figure 18: Surviving fraction of amino acids after exposure to 1.3, 2.6 and 5.3 billion years.

Original figure is for 500 million years, 1 billion and 2 billion years of Martian surface cosmic radiation equivalent from a gamma ray source (Kminek et al, 2006:4). The 2004 paper assumes a dose of 200 mGy / yr. Adjusted to 76 mGy / yr as measured by Curiosity gives 1.3, 2.6 and 5.3 billion years.

Based on those figures we can calculate that the same amino acids are

- Reduced a thousand fold with half that dose, or 50 MGy (million grays) in 0.65 billion years,
- surviving amino acids are halved every 5.017 MGy (million grays) or every 65 million years
- reduced ten-fold every 16.7 MGy (million grays) in 217 million years.

We can use this to calculate the effect on amino acids of the 0.54 mGy needed to reduce radiodurans a million fold.

Based on Kimmeck et al's 5.017 MGy (million grays) to destroy half the amino acids, 0.54 MGy (million grays) would destroy

$$100 \times (1 - 0.5^{0.54/5.017})$$

= 7.2% of the same amino acids.

The 8.24 MGy (million grays) needed to reduce the hypothetical more radioresistant microbe a million fold would destroy

$$100 \times (1 - 0.5^{8.24/5.017})$$

= 68% of the same amino acids.

So this level of sterilization would destroy most of its amino acids, but about a third of the original amino acids would remain, and still leave biosignatures of life recognizable.

It would be possible to exceed these doses for an extra safety margin.

We have:

- **0.54 MGy** (million grays)
 (to reduce radiodurans a million fold) equivalent to 2.8 million years surface radiation,

- **8.24 MGy** (million grays)
 (to reduce their hypothetical more radioresistant microbe a million fold) equivalent to 42 million years surface radiation

- **100 MGy** (million grays)
 (to reduce amino acids to a millionth of the original amounts) equivalent to 1.3 billion years surface radiation

Somewhere within this range would provide a sweet spot of adequate sterilization while leaving present day life recognizable as such.

Even 1.3 billion years equivalent of radiation is unlikely to make a difference to the search for past life unless the samples are exceptionally well preserved, as this would be less than the exposure age of most surface deposits.

Geologists could allow for effects of the sterilization just as they do when analyzing Martian meteorites, which typically have ejection ages of up to 20 million years of cosmic radiation during the journey from Mars to Earth (Eugster et al, 2002).

Suggestion to use low power nanoscale X-ray emitters for sterilization during the six months return journey from Mars

The simplest way to sterilize the sample during the return journey from Mars might be to add sterilizing X-ray emitters to the Earth return orbiter or sample return capsule. The X-ray emitters would need to have low power requirements.

A field emission miniature x-ray tube using carbon nanotubes has been developed for dentistry, with a diameter of 0.7 cm and length of 4.7 cm. It has better stability, and a longer lifetime than a conventional x-ray tube, and doesn't have to be cooled (Kim et al, 2016).

[Figure needs permission, redraw or new source]

Figure 19: Low power and low mass X-ray tube based on carbon nanotubes, may have potential for sterilizing the sample during the return journey.

A, schematic layout of the X-ray tube, B, the components, and C, the completed unit. (from Figure 2 of (Kim et al, 2016))

The tube operates at 50 kV with an emission beam current of 140 µA (Kim et al, 2016) or 7 watts. It should have a power requirement of the order of watts compared to 1.2 kW for the maximum for a typical dental x-ray system (Preva , n,d,:39). The dose at 3 cm is 8.19 Gy per minute (Kim et al, 2016)

At that rate an 8.24 MGy (million grays) dose to reduce the hypothetical more radioresistant microbe a million-fold would take a little under 700 days of operation while the 0.54 MGy (million grays) to reduce radiodurans a million-fold would take a little over 45 days.

The power requirement of watts is low enough however that multiple units could be used to increase the dosage and to supply sufficient X-rays to the complete sample container depending on what dose is required.

It would also be necessary to take account of attenuation of the X-rays by the titanium sample tube walls which could lead to a need for increased doses.

If the power requirements are too high to sterilize the samples during the return journey, the sterilization could be done in a receiving satellite in Earth orbit before delivering the samples to Earth.

Experimental data on effects of sterilizing doses of gamma radiation – preserves the geological interest of rock samples - need to test effects of X-rays

Allen et al did an experiment to simulate the effect of sterilization of Mars samples (Allen et al, 1999). They tested the minerals salt, halite, carbonaceous chert, Mars soil simulant (weathered volcanic ash), carbonaceous chondrite meteorite, plagioclase, olivine, pyroxene,aragonite, montmorillonite clay, quartz and gypsum.

They used a dose of 30 Mrad which they considered to be enough to sterilize all life, even radiodurans, using a Cobalt 60 source. That’s equivalent to 3 MGy (million Grays)

(one Mrad is 100,000 Gy).

There was no effect on radiometric dating as the isotope ratios of Strontium 87 / 86, Samarium 149 / 152 and Samarium 150 / 152 were not changed. There was no change in the rock composition or crystal structure including the interplanar spacing of crystals. None of the crystals were destroyed and there was no evidence of dehydration of gypsum to anhydrite. There were no changes to the spectra of pyroxene, aragonite, gypsum and Mars soil simulant. The irradiation had no effect on the basal spacing of montmorillonite, which is extremely sensitive to temperature and degree of hydration.

The irradiation did change the colour of quartz crystals, from clear to a deep brown colour and darkened. This colour change was most noticeable between 0.3 and 3 Mrad. Also halite turned deep blue. It also altered the thermoluminescence properties of quartz and halite. They concluded

> *"If samples returned from Mars require sterilization, gamma irradiation is an attractive option"*

More research is needed for the lower frequency more penetrating X-rays. Also it's necessary to test the higher levels needed to sterilize not just radiodurans but hypothetical more radioresistant microbes from Mars.

X-rays, at a lower frequency, might have less effect on the rock samples. Also, since X-rays are tunable unlike gamma rays from Cobalt 60, experiments could be made to find the optimal operating voltage for sterilization while reducing the impact on geological studies.

The geology should be fine after sterilization. But might Perseverance find present day life or well preserved past life that could be seriously damaged by sterilization?

Present day life might even be viable before sterilization (after all that's why you do it). While past life might be changed significantly if we can return well preserved life that is little damaged or might be easier to recognize as such.

Until we can send in situ life detection – major challenge to find samples from Jezero crater to help decide central questions in astrobiology - most past biosignatures degraded beyond recognition – nearly all organics abiotic - past and present day life low concentration and patchy distribution - especially challenging if Martian life never developed photosynthesis or nitrogen fixation

The situation for astrobiology is very different from geology. Any sample from Mars will be of some geological interest. It's a case of using the geological context to select the ones of most interest. The main problems for geological samples are to avoid too great a change in the temperature, and the redox state of its surroundings after sample collection (Grady, 2020).

Collecting astrobiological samples would be easy if we were searching for life on an Earth-like planet. Almost any sample with organics would have present day life in it, or at least, evidence of present day life processes.

However on Mars nearly all the organics will be infall from meteorites, comets, interplanetary dust and in situ abiotic processes .(Mulkidjanian, 2015) (Westall et al, 2015) (Franz et al, 2020). So, most samples we might return of present day Martian organics are likely to be of no astrobiological interest. Even if there is present day life there, in microhabitats in Jezero crater, it still might not be able to make use of much of the organics from infall and abiotic processes if there isn't enough by way of water activity, nitrogen, and so on. So, returning organics without life doesn't tell us much about whether there is present day life there or not.

It's going to be an especially hard challenge to find samples of past life. Even on Earth, we have very little by way of organics from the most ancient stromatolites, and finding those was a huge challenge (Allwood, 2009). It is especially hard to find terrestrial organics that predate photosynthesis. The problem is that most past organics are destroyed by geological processes and ionizing radiation from radioactive elements in the rocks.

On Mars also, most organics would be destroyed over billions of years, though some of the processes are different on Mars. Amongst other things, past organics would be destroyed, or degraded beyond recognition by such processes as ionizing radiation, radioactive decay of elements in the rock, and reactive chemistry of the hydrogen peroxide and perchlorates (Grotzinger, 2013) (McMahon et al, 2018)..

So we expect most organics found by Perseverance to be of no astrobiological interest. Not just low astrobiological interest, but of no interest at all, except to give us more knowledge about the extent to which Jezero crater was habitable to life in the past.

See

- Most Martian organics are expected to be from non living processes even if Mars has present day life and had abundant past life

There may well be samples there of great interest to astrobiology. The problem is, how to identify them.

Jezero crater predates the evolution of photosynthesis on Earth, and any life there may also predate the evolution of photosynthesis on Mars, and it's possible that Martian life never developed photosynthesis (Summons et al, 2011:21). See:

- Possibility that past life in Jezero crater, or even modern Martian life, never developed photosynthesis

It may well also predate nitrogen fixation. See:

- Possibility that past life in Jezero crater or even modern Martian life never developed nitrogen fixation – or that microbes in oxygen rich surface layers never developed nitrogen fixation

This makes sample selection a challenge since Perseverance has only limited in situ life detection capabilities. It would miss most biosignatures or the biosignatures of life in small quantities.

A sample with well preserved past organics and no life in it might still be of astrobiological interest if it is well preserved and comes from a habitable environment. It could be evidence that there is either no past life on Mars or that it couldn't make use of that habitat. For instance we might be able to get evidence about whether there was widespread photosynthesis at the time of Jezero crater from a good sample from a habitat that photosynthetic life could use.

However the problem then is that there are many processes that can destroy evidence of past life. A sample of rocks formed in the past on Mars is certain to help to clarify the geological conditions at the time, but it may well be impossible to use the same sample to tell us anything about the astrobiological past.

Although the main focus of the discussions in astrobiological papers is past rather than present day life, the same considerations also apply to present day life. Without the ability to detect in situ life, and limited knowledge about where to look for it, it is hard to intelligently select the best samples to return.

So this is very unlike the situation for geology, it is a major challenge for Perseverance to return a sample of any astrobiological interest. This is why astrobiologists have recommended in situ searches first. The only astrobiologist to recommend a sample return I found was Chris McKay who suggested a simple scoop of dirt and return similar to China's proposed mission (Jones, 2021) as a technology demo for astrobiology.

See

- Several studies by astrobiologists concluded we need capabilities to identify life in situ, for a reasonable chance to resolve central questions of astrobiology

There is almost no support in the published astrobiological literature for sample return on its own as a strategy to look for past or present day life on Mars. However now the decision is made, we need to do what we can to make the best use of the opportunity.

There may be ways to increase the low chance of returning samples of astrobiological interest. But first we need to understand the challenges.

Most Martian organics are expected to be from non living processes even if Mars has present day life and had abundant past life – and most organics found so far by Curiosity and Perseverance resemble meteorite organics

Most Martian organics are expected to come from meteorites, interplanetary dust, comets and indigenous organics from abiotic processes including abiotic photosynthesis (Mulkidjanian, 2015) and electrochemical reduction of mantle materials (Westall et al, 2015) (Franz et al, 2020).

Perseverance detected organics for the first time in December 2021 through its Sherloc Raman Spectrocopy instrument. These are simple aromatics indistinguishable from organics from meteorites, and similar to most of the organics found by Curiosity (JPL, 2021s).

Perseverance may find much more interesting samples for astrobiology. But suppose Perseverance found a sample similar to the Tissint Martian meteorite on Mars. It has organics with carbon 13 to carbon 12 isotope ratios significantly lower than for the Martian atmosphere (Lin et al, 2014). Carbon 12 is the light stable isotope of carbon which gets taken up preferentially by biological processes through kinetic fractionation, because it leads to lower energy costs than the heavier stable isotope carbon 13 (USGS, n.d.). The carbon 13/12 ratio for the Tissint meteorite is similar to coal, petroleum and other sedimentary organics on Earth, while the lower range of Martian atmospheric ratios is similar to the values for Earth's atmosphere (Lin et al, 2014:fig 6).

Carbonaceous chondrites can also have lower levels of Carbon 13, but would typically lead to lower concentrations of organics than for the Tissint meteorite (Laborator Ecole Polytechnique Fédérale de Lausanne, 2014)

.

As Philippe Gillet said, director of EPFL's Earth and Planetary Sciences (Laborator Ecole Polytechnique Fédérale de Lausanne, 2014)

> *"So far, there is no other theory that we find more compelling,*
> *…*
> *"Insisting on certainty is unwise, particularly on such a sensitive topic. I'm completely open to the possibility that other studies might contradict our findings. However, our conclusions are such that they will rekindle the debate as to the possible existence of biological activity on Mars – at least in the past."*

However this is not proof of life, as there are other processes that lead to lower isotope ratios for Carbon 13. Abiotic methane from hydrothermal vents can have carbon 13 depleted to as low as -50 ‰ (parts per thousand) (McDermott et al, 2015).

Although the Tissint research is suggestive, after years of study with our best laboratories on Earth, the most important question remains unresolved. We don't know whether the Tissint organics come from life or inorganic processes.

Curiosity's detection of organics depleted in Carbon 13 could be from biologically produced methane which then interacted with UV in the atmosphere - but samples of those organics would give no other biosignatures to distinguish between the hypotheses

Perseverance doesn't have the capability to test for carbon 13 depletion of the organics it finds in situ, but Curiosity does, and Curiosity has found samples depleted in carbon 13, in research published in 2022.

Perseverance may be able to return samples similar to the ones that Curiosity has found to be depleted in carbon 13.

However, these would be a very weak biosignature since if the signals are due to past life, the organics are produced only indirectly as a result of interactions of biologically produced methane with UV in the atmosphere, and wouldn't be expected to contain traces of the life itself.

In detail, House et al (House et al., 2022) reports that Curiosity found low levels of Carbon 13, reduced -70 ‰ , in organics which may all be from the same connected ancient surface in Gale crater. They also found that the Martian atmosphere is enhanced in Carbon 13 compared to Earth by +46‰. They ruled out hydrothermal vents as an explanation because -70‰ depletion is too much to be explained in that way (House et al., 2022).

These organics were associated with sulfur and they found that the sulfur was also depleted in the heavier isotope sulfur 34 compared to abundances of sulfur 32.

The analysis involved heating the organics, and the methane was released when the organics were destroyed on heating to ~ 850°C (pyrolysis) (House et al., 2022)

However biologically produced methane is only one of three suggested processes

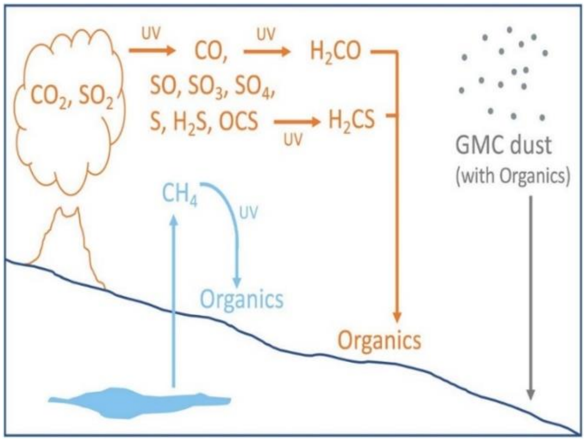


Figure ??: Processes that could explain the Carbon 13 depleted organics found by Curiosity (figure 5 of House et al., 2022)

- Biological methane from the subsurface reacted with UV to produce organics
- Carbon dioxide and sulfur dioxide from volcanoes, and water vapour, reacted with UV to produce organics
- The organics were deposited from a galactic molecular cloud when our solar system passed through it.

House et al say (House et al., 2022)

> ***With present knowledge, it will be difficult to determine which of the three scenarios most accurately depicts the events that unfolded on Mars billions of years ago.***

They say Curiosity will have further opportunities to sample this layer in 2022, and they suggest that the Curiosity discoveries may help NASA's Perseverance team select samples to return to Earth.

However, with their first, biological hypothesis, the organics found by Curiosity are produced indirectly. It's just the result of methane from past life interacting with UV in the atmosphere that produced these organics, so the deposits wouldn't preserve the original organics, so there isn't any potential here for multiple biosignatures.

The only possible biosignature in these organics is Carbon 13 depletion, and even with the samples returned to Earth, this would be hard to distinguish from methane produced by the other processes they proposed.

If Perseverance returns samples similar to the Curiosity carbon 13 depleted organics, or the Tissint meteorite or ALH84001, this won't resolve the question of whether they were produced by life – a more unambiguous sample is needed

If Perseverance does return samples of organics with depleted carbon 13 similar to the Tissint meteorite, or even more depleted ratios similar to the samples found by Curiosity, it may still not resolve the question of whether Mars had past life.

It would be the same situation if Perseverance found a sample similar to ALH84001. It has multiple lines of evidence that suggest the presence of life, including apparent microfossils with associated organics, yet they are not conclusive. See:

- Difficulties of recognizing microfossils even with associated organics – example of ALH84001

Unlike the usual situation for geological puzzles, finding the geological context for ALH84001 or the Tissint meteorite might not do much to resolve the question of whether the organics are from life, if indeed that is what they are. What is needed are multiple independent biosignatures or clearer less ambiguous biosignatures than the ones found in those meteorites.

The debates would be likely to still continue over geological or biological explanations which couldn't be resolved by geological context.

Perseverance is able to detect some unambiguous biosignatures with its Raman spectroscopy, a new capability. But the biosignatures would need to be exceptionally well preserved to be spotted in this way. See:

- Perseverance could detect distinctive biosignatures like chlorophyll and carotene - but only for exceptionally well preserved life

Astrobiologists recommend searches with a suite of instruments able to detect multiple biosignatures in situ, to increase confidence in whether a sample has potential life in it (Westall et al, 2015). However Perseverance doesn't have this capability indeed it doesn't have any dedicated life detection instruments. For more on this see:

- Several studies by astrobiologists concluded we need capabilities to identify life in situ, for a reasonable chance to resolve central questions of astrobiology

On Mars, even a thriving past ecosystem might leave no biomass to be preserved, for instance if the conditions were acidic:

- We don’t know which geological contexts on Mars best preserve past life (if it’s there) - many Martian processes can destroy organics, or wash them out, and even a thriving past ecosystem might leave no biomass, for instance in acidic conditions

There may be well preserved past organics in Jezero crater, but many conditions need to come together for this to happen, including:

- the ecosystem deposited biomass originally,
- it’s been recently exposed to the surface (or the organics will be destroyed by ionizing radiation)
- no other processes destroyed the biosignatures either while below the surface or once exposed.

Amongst all the organics produced through abiotic processes, there may be samples with multiple well preserved biosignatures in Jezero crater, even on the surface and within reach of Perseverance.

However, apart from some special cases such as exceptionally well preserved chlorophyll or carotene, Perseverance doesn’t have the in situ capabilities needed to distinguish the Tissint meteorite or ALH84001 from potentially more interesting samples for astrobiology on Mars.

The organics in the Tissint and ALH84001 meteorites are likely to be easier for astrobiologists to study than most samples Perseverance finds on Mars. That’s because Martian meteorites we have all come from over 3 meters below the surface (Head et al, 2002).

Perseverance samples surface rocks and has no capability to drill to depths of meters.

Astrobiologists have stressed the importance of the ability to drill well below the surface to search for past life on Mars. The reason is that most surface organics from past life would be destroyed by cosmic radiation – and may be mixed with infall of organics from other sources, unless exposed recently by geological processes.

The processes on Mars expected to destroy most surface organics from past life

Although cosmic radiation has little effect over centuries, it’s an exponential process (Kminek et al, 2006), To show how this works, if it takes 50 million years to destroy half the molecules of some particular amino acid, a period of time ten times longer, 500 million years of cosmic radiation will reduce quantities to a thousandth ($\sim 2^{10}$) of the original concentration. A period of time 20 times longer, 1 billion years of cosmic radiation will reduce that amino acid to a millionth, and 40 times longer, 2 billion years will reduce it to a trillionth ($\sim 2^{40}$) of the original concentration.

Meanwhile, processes on Mars can introduce surface abiotic organics into deposits of past life organics.

Deposits of organics from past life can also be altered chemically, for instance by perchlorates or hydrogen peroxide, radioactive decay of elements in the rocks (Grotzinger, 2013) (McMahon et al, 2018)...

Over billions of years, any surface organics from Martian past life can be gradually changed beyond recognition by this process of destruction of the original organics and slow introduction of new organics from abiotic sources.

Possibility that past life in Jezero crater life, or even modern Martian life, never developed photosynthesis

In the scenario where Martian life never developed photosynthesis (Hays et al, 2017) , its distribution is likely to be even more heterogeneous (patchy) than in Earth's deserts.

Molecular clocks suggest photosynthesis evolved on Earth 3.4 to 2.9 billion years ago (Fournier et al, 2021).

There's also possible evidence of stromatolites on Earth dating back to 3.5 billion years ago (Baumgartner et al, 2019).

So the evidence is unclear, the Jesero crater deposits could have formed either before, or after the evolution of oxygenic photosynthesis on Earth. In any case there's no particular reason to expect photosynthesis to arise at the same time on Mars as on Earth.

In the current paper we suggest that it's possible that the frequent freeze thaw cycles lead to faster evolution so may permit photosynthesis to evolve faster.

Another possibility is that photosynthesis was transferred from Earth to Mars. This would be challenging though perhaps not impossible in the early solar system when there were large meteorite impacts capable of transferring life between the planets more easily. See:

- Could life get transferred from Earth to Mars? With Earth's high gravity and thick atmosphere the challenges are far greater but may be more possible in the early solar system with impacts large enough to blow out part of Earth's atmosphere

If Mars developed a biosphere it's possible it never progressed to photosynthesis (Summons et al, 2011:21).

> ***At life's origin, the dominant energy source was unlikely to have been sunlight. Energy flowing from chemical and thermal disequilibria and particularly from the***

interaction of hot rocks with water is more likely. Perhaps the same was the case for early Mars.

…

If Mars evolved a biosphere, it may not have progressed to photoautotrophy or a dependence on photoautotrophy as it did on Earth. Thus, in the consideration of Martian environments conducive to producing molecular biosignatures, targeting depositional environments that had a strong chemical energy flux and sustained redox gradients for long periods by biogeochemical cycling is a most promising strategy.

Westall et al suggest evolution of photosynthesis would have been more challenging on Mars than on Earth. Photosynthesis developed on Earth in shallow coastal areas with direct access to sunlight. These conditions were probably common on early Mars but modelling suggests the water was covered in ice most of the time because of the lower levels of sunlight.

This would have hindered evolution of photosynthesis as they might not have been continuously habitable for photosynthesis for long enough for microbes to develop the capability. (Westall et al, 2013:894)

Alternative to photosynthesis - chemosynthesis – perhaps using hydrogen sulfide or hydrogen including hydrogen from radiolysis in rocks – with much lower levels of biomass than a photosynthesis based ecology

If Jezero crater past life predated photosynthesis, it might use chemosynthesis to fix organics, using reactions with hydrogen, or hydrogen sulfide, such as

$$CO_2 + 4H_2S + O_2 \rightarrow CH_20 + 4S + 3H_2O$$

This is one of the reactions used by life in hydrothermal vents (NOAA, n.d.witd)

For the reactions that involve hydrogen, the hydrogen could come from radiolysis – the radiation from radioactive elements in rocks can split subsurface water into oxygen and hydrogen. This is a process used by terrestrial microorganisms in the “Subsurface Lithoautotrophic Microbial Ecosystems” (SLIMEs) (Nealson et al, 2005).

This energy source could make it possible for life to be distributed more widely, wherever there was enough water to be used in this way (Tarnas et al, 2018) As the lead author Jess Tarnas put it

> ***"We showed, based on basic physics and chemistry calculations, that the ancient Martian subsurface likely had enough dissolved hydrogen to power a global subsurface biosphere,"***

However this life might occur at very low concentrations in the rocks. If Mars did lack oxygenic photosynthesis at the time, this would limit the ability to take up carbon dioxide from the atmosphere, and limit the distribution of life at high enough concentrations to detect after degradation.

Sleep et al estimate that before photosynthesis, the burial rate of organics on Earth would be less than 0.56 Tmol per year compared to 10 Tmol a year with photosynthesis. The actual burial rate would likely be much less as ecologies tend to stabilize at much less than the maximum sustained yield of organics with most of the available energy going to maintain the ecology rather than to generate excess organics for burial (Sleep et al, 2007).

Possibility that past life in Jezero crater or even modern life never developed nitrogen fixation – or if it did, that nitrogen fixation was never taken up by microbes in oxygen rich surface layers

Martian life may also be limited by availability of nitrogen. Nitrogen levels in the atmosphere for present day Mars are low, and may be too low for nitrogen fixation (it's close to the limit of what may be possible and may be too low). If nitrogen fixation isn't possible on present day Mars, this would limit life to habitats with sufficient nitrates available, assuming that Martian life like terrestrial life requires nitrogen. See:

- Sources of nitrogen on Mars as potential limiting factor – unless Martian life can fix nitrogen at 0.2 mbar

Nitrogen fixation originated in anoxic conditions on Earth, in nitrogen poor conditions. It was only taken up by aerobic microbes later (Onstott et al, 2019). In the past, Mars may have had oxygen rich surface layers similar to those for Gale crater lake (Doyle, 2017) (Lanza et al, 2014) (NASA, 2017).

If nitrogen fixation followed a similar evolutionary path on Mars, the Martian life might have never developed nitrogen fixation in the past. Or perhaps early Martian life did develop nitrogen fixation, but the ability to fix nitrogen was never taken up by aerobes. If so, nitrogen from the atmosphere may be only available to life in deposits from anoxic environments in early Mars.

In these two scenarios, past life would be either

- limited to deposits where nitrogen was available, or
- also found in anoxic environments where it could access nitrogen from the atmosphere

Even if Martian never developed nitrogen fixation and never developed photosynthesis in either the past or the present, this scenario need not mean a lifeless Mars.

Past life and even present day life could still be there, but be challenging to detect because it would be limited to habitats with chemical sources of energy and with sources of nitrogen.

Present day and past life may be patchy or inhabit millimeter scale features

Surface organics on Mars are also constantly destroyed by its abundant perchlorates and other reactive chemistry. These could remove traces of past life, and also, recently deposited traces of present day life.

Most of the rock on Mars is volcanic in origin. Microbes may inhabit millimeter scale features in the rock. Cockell used terrestrial obsidian to gain insights into colonization of Martian rocks. Although obsidian hasn't been directly detected on Mars, the processes are similar. He found that many cubic centimeter samples are lifeless. He recommended a minimum sample size of ten cubic centimeters from a Martian rock, for a reasonable chance to detect life in it.

As he put it: (Cockell et al, 2019c)

> *Every sample Is a 'microbial island'*

Mars was far more habitable for terrestrial life at the time when the deposits in Jezero crater formed than it is today. However even then, past Martian life may be similarly patchy, in microbial islands, if it remained at such an early stage of development, perhaps without photosynthesis (Hays et al, 2017) or nitrogen fixation.

Also, we don't know in advance where to look in the geological record for past life (Beaty, 2019) (Cockell et al, 2019c). Figure 21 gives some idea of the complexity of the processes inferred for Gale crater and Jezero crater is likely to be as complex as this. Even if we know the geology very well, we still may not know where to look for life in the geology of the landscape.

[Figure needs permission, redraw or new source]

> Figure 21: sketch of major processes and environments inferred from observed carbon and oxygen isotope ratios found in samples taken by Curiosity at Gale crater. Similar processes can be expected at Jezero crater.
>
> Blue arrows indicate flow of groundwater and brown arrows indicate reactions or transport of carbon or oxygen bearing materials. Some of these processes may be

ongoing today including oxygenation of organics delivered to Mars (exogenous organics) and photolytic CO_2 reduction (abiotic photosynthesis).
[Figure 3 of (Franz et al, 2020)]

Any past or present day life is likely to be patchy (heterogeneous) and may be highly localized. Without robust in situ biosignature detection, Perseverance may well return samples of abiotic organics formed through these processes even when there are other organics in nearby rocks or even the same rock from the same geological deposit, created by past or present day life.

If Mars has present day life - it's likely to be in low concentrations as for hyper-arid terrestrial deserts, and may colonize temporary habitats slowly over thousands of years

Present day life in Mars analogue hyperarid deserts on Earth is low biomass, and non uniform (heterogenous), often in isolated patches of life surrounded by regions with minimal or no life. For instance in the hyper arid core of the Atacama desert, microbial life is rare except in halite crusts [salts] that it colonized (Vítek et al, 2010). It's possible, maybe likely, that this passage describing Mars analogue hyperarid deserts could also apply to Mars, if there is present day life there (Wierzchos et al, 2012):

> *In desert zones, besides the scarcity of water, microorganisms also need to withstand solar fluxes, including lethal UV light, high and low temperatures and their rapid fluctuations, high rates of water evaporation, prolonged periods of desiccation, oligotrophic conditions [low levels of nutrients], and frequently high salinity levels such as those in evaporitic rock habitats. Even brief exposure to solar radiation can cause cell death within a few hours. Despite these numerous hurdles for life, researchers have been able to detect the presence of microorganisms in all of Earth's deserts.*
>
> *It has thus become apparent that through a long process of evolution microbes have developed colonization strategies, with their survival in the extreme desert habitat dependent upon a delicate balance between favorable and less favorable conditions. Since any disturbance in this balance could have lethal consequences, these microhabitats generally sustain low levels of biomass.*

Most of the Martian environment is even more inhospitable than these deserts. Yet, as on Earth, life may still be there, dependent on a fragile and delicate balance. Some microhabitats transition to no longer habitable while new microhabitats form as local conditions change. This fragile balance could mean that only some of the potential microhabitats are inhabited and the levels of biomass are likely to be low.

The process of colonization of new habitats may take thousands of years. There is evidence that species of lichens and mosses are still in the process of recolonizing Antarctica since the last ice age, with the species diversity dependent on the distance from the nearest geothermally active sites that provided refuges during the ice ages (Fraser et al, 2014)

By analogy with hyper arid deserts, Mars may have life within the rocks, or beneath them. There may also be life in the surface layers of the dirt.

This image shows some of the possible habitats for rock dwelling endoliths (that dwell inside rocks) and epiliths (that dwell on the surface of rocks)

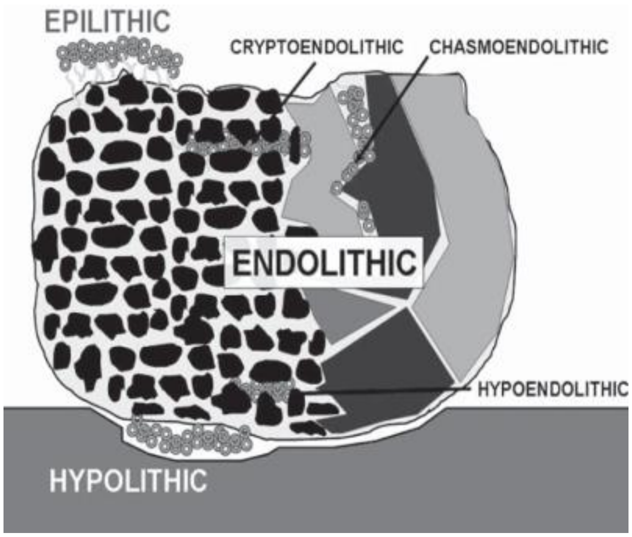


Figure 20: Potential habitats for endoliths on Mars as on Earth's hyperarid deserts - epiliths on the surface of rocks, cryptoendoliths within the rocks, hypoliths beneath rocks, and chasmoendoliths in cracks in the rocks. From (Wierzchos et al, 2012)

The cryptoendoliths can occur in transparent rocks like quartz, or porous rocks like sandstone. Other habitats include micropores in salt deposits (Wierzchos et al, 2012).

In addition, some, or many potential habitats on Mars may remain uninhabited because

- Martian life never evolved the necessary capabilities to take advantage of that habitat, or
- Life never got to that particular habitat since the habitat formed, or
- Life got there but was sterilized by a later event.

We don’t know which geological contexts on Mars best preserve past life (if it’s there) - many Martian processes can destroy organics, or wash them out, and even a thriving past ecosystem might leave no biomass, for instance in acidic conditions

We don’t yet know which geological contexts on Mars will best preserve past life (if it’s there) – as there are many ways it can be destroyed or never be deposited originally

We also don’t yet know which Martian processes are most conducive to preserving fine structure and organics from past life.

The preservation of biosignatures of past life depends on the history after deposition (Grotzinger, 2013) (McMahon et al, 2018).

- if the organics remain close to the surface for hundreds of millions of years before they are buried, or are temporarily exposed to surface conditions at some later stage in their history, the organics will be degraded by cosmic radiation.
- Later flooding can wash out organics,.
- If organics encounter warm conditions they can flip to the mirror form of the molecule and so lose the chirality signal through racemization.
- The organics can be further modified by chemical reactions, for instance with perchlorates, hydrogen peroxide and oxygen

Preservation of the biosignatures also depends on the conditions in which the organics are deposited (Hays et al, 2017).

- The very conditions that make a habitat suitable for life, such as the flowing water that creates chemical redox gradients in a hydrothermal vent which life exploits for energy, can also degrade the biosignatures that help us to recognize that life.
- Similarly the water that deposits the clays and other minerals that help preserve the biosignatures can also in turn destroy those biosignatures.

So we may need to find a sweet spot, a deposit that was both habitable, but also not degraded too much by the fluids that made it habitable (Hays et al, 2017), for it to be preserved well enough to be recognized

Cockell et al summarize some of these processes that can lead to return of a lifeless sample from Mars (Cockell et al, 2019b):

a. *Did life arrive or originate on Mars?*
b. *Does (part of) the sample derive from a habitable environment?*
c. *Does (part of) the sample derive from an inhabited niche within that environment?*
d. *Was the biomass impinging on the sample appreciable?*
e. *Did biosignatures form within the sample and endure to the present?*

f. Can the biosignatures now be unequivocally detected?

If the answer to all those is "Yes" then

g. Sample revealing life on Mars.

[Figure needs permission, redraw or new source]

Figure 22: Scenarios that can lead to the return of a lifeless sample from Mars, including the case of a sample that actually did contain life but it is no longer recognizable. [Figure 1 of (Cockell et al, 2019b)]

Cockell suggests we aim to identify and map out samples of organics without biosignatures as we search for the ones that do have recognizable signatures of life

Cockell et al say (Cockell et al, 2019b) that the processes that could preserve life on Mars are different from Earth because Mars

- Has no tectonic subsidence or burial
- A more iron rich crust
- High concentrations of oxidizing agents such as perchlorates
- Tendency for acidic chemistries for the life (which leads to less preservation of organics) and lower temperatures (which leads to less biomass)

They say a thriving ecosystem could leave no biomass on Mars. However, if Mars did have past life on a planetary scale, it is unlikely that nothing has been preserved of past ecosystems anywhere on Mars. So, a collection of many lifeless samples from many different contexts would suggest that Mars never had life.

Cockell et al say a more likely scenario is that past life signatures are found but are close to the detection limit or are ambiguous, leading to the situation where some researchers are of the view that life was detected in the samples, while others say it wasn't detected, as happened with ALH84001 (Cockell et al, 2019b).

Grotzinger, Project Scientist for the Curiosity Mars Science Laboratory mission, suggests we may need to find a "magic mineral" on Mars that preserves ancient life. He says that for Earth this key mineral was chert. The discovery in 1954 that this mineral preserves the cellular structures of microbes was the key to unlocking the secrets of early life on Earth. There may be similar key discoveries that open up a window into past life on Mars (Grotzinger, 2013) (Grotzinger, 2014).

For a clear signal, for past life, the sample has to be deposited, and then avoid conditions that could destroy or degrade it. For the best chance of detecting past life on Mars, we need: (Grotzinger, 2013):

1. **Life there in the past**, In the best case for detecting life, life was present in almost every potential habitat. However if, say, it was not capable of nitrogen fixation or

photosynthesis, it might occur in only a few locations, so we will need to learn where to look for it.

2. **Remains of life accumulated**, Likely locations may include the bed of a lake, a delta, or the detritus from a flash flood.
3. **The remains were preserved quickly,** caught in clays or salt, or the microbes rapidly entombed in fast forming rocks like chert.
4. **Ideally, plunged rapidly into freezing conditions**, because in warmer conditions, the organics deracemize (flip into their mirror images) which makes it harder to distinguish life from non life.
5. **Buried quickly**, ideally within a few tens of millions of years, to a depth of several meters for protection from the cosmic radiation degradation.

Then once it is safely buried, it has to survive for billions of years and then be returned to the surface. This means that over those billions of years it

1. **Wasn't washed out with later floods, or altered or destroyed by the perchlorates and other chemicals,** or returned to the surface temporarily for more than brief time periods.
2. **Wasn't mixed** with other sources of organics, or if it was, it was mixed in a way that is easy to disentangle
3. **Was returned to the surface rapidly** (perhaps as a result of a meteorite strike or fast weathering of the rock by the Martian winds), and did this in the very recent geological past.

Need many example samples as we study factors that lead to lifeless samples

Cockell et al recommend multiple samples of each sample type for the best chance of returning life. They suggest we look at a variety of hydrological contexts, not just locations with most water, as high levels of water flow often reduce biomass and biodiversity. They warn that high temperatures and salts can also make water uninhabitable. They recommend a sampling strategy that can help to further our understanding of the processes on Mars that lead to lifeless samples rather than a single minded focus on an attempt to return a sample with biosignatures in it.

> *We recommend that researchers preparing for the analysis of Martian samples should place more emphasis on distinguishing different scenarios that lead to lifeless samples as opposed to a single-minded search for biosignatures. Sample collection strategies for Mars exploration would benefit from being designed around the collection of a sufficient number and diversity of samples to understand the factors that give rise to lifeless samples*

If Mars is lifeless we can't decide this with just one mission, but our first samples can expand our knowledge of the potential microhabitats that it hasn't colonized. To do this we need to target potential habitats or microhabitats past and present.

There may be ways to improve the chance of returning recognizable past or present day life if it is there. Over the entire surface of Mars it's likely there are many samples we can find that will tell us much about past life on Mars, but with a first expedition with no in situ life detection capabilities we are essentially running blind.

However, given the decision to prioritize sample return over in situ searches, Perseverance's target, Jezero crater (NASA, 2020prls), has high potential.

Meanwhile ESA's Rosalind Franklin when it is sent to Mars in 2022, will target Oxia Planum, another site with complex multi-layer phyllosilicate clay deposits which gives another opportunity to find out about present day and past life, especially as it has the ability to drill to a couple of meters (ESA, 2019op).

In Jezero crater, the carbonate deposits especially could trap organics quickly and stabilize them and protect them from leaching. The delta deposits also may favour accumulation of large amounts of organics (Horgan et al, 2020).

Jezero crater also contains hydrated silica, identified from orbit. This has high preservation potential, however this depends on what form this takes. Amongst other possibilities the hydrated silica could have formed volcanically, or as a result of fluids causing chemical changes in olivine rich rock or delta deposits, or been transported there by the river or wind, or it could have formed in situ in a lake (Tarnas, 2019).

We need to manage expectations here. Astrobiologists will be under great pressure to make deductions from inadequate samples from Mars. However,if Perseverance returns samples of organics equivalent to the Tissint meteorite or ALH84001, or most likely more degraded than those samples because of the surface ionizing radiation, perhaps even with apparent microfossils as for ALH84001, astrobiologists won't be able to make any conclusive deductions..

Several studies by astrobiologists have concluded that we need capabilities to identify life in situ, and to drill, to have a reasonable chance of resolving central questions of astrobiology (Paige, 2000), (Bada et al, 2009), (Davila et al, 2010).

Perseverance lacks these capabilities. They will be important for future astrobiological missions to Mars (Hays et al, 2017) which would explore the surface salts, the surface and near subsurface brines and ices, caves, the deep subsurface, and other possible refugia for extant life (Carrier et al, 2020:804). See

- Several studies by astrobiologists concluded we need capabilities to identify life in situ, for a reasonable chance to resolve central questions of astrobiology

However even with these limitations we may still be able to do things to improve the potential to return samples of past or present day life.

It is especially important to try to return samples with clear biosignatures because Perseverance's sample tubes are not 100% clean.

Limitations on cleanliness of the Mars sample tubes with estimated 0.7 nanograms contamination per tube each for DNA and other biosignatures and a roughly 0.02% possibility of a viable microbe in at least one of the tubes – higher levels of sterilization needed unless Perseverance returns exceptionally well preserved life

Sadly, if unambiguous biosignatures like chlorophyll or carotene or even viable life is found in the sample cached by Perseverance, there will be some issues still confirming that it is from Mars and not contamination.

The Mars sample tubes themselves are not 100% sterile of terrestrial biosignatures or viable terrestrial spores. The engineers were worried that putting the tubes in a bag to keep them sterile would risk jeopardizing the mission, in case the bag couldn't be opened (Redd, 2015).

Instead, after baking and sterilization, the sample tubes were exposed to the atmosphere in a clean room. They then had to be handled by technicians when they were placed in the rover. This decision made 100% sterilization impossible. So NASA went for less strict requirements.

For living cells, the requirement is no more than a 0.1% chance of a single live terrestrial organism in each sample tube. They estimate that they achieved a much more stringent 0.00048% (Boader et al, 2020: table 6).

If their estimate is accurate, this makes it no more than a 0.02% chance of finding a single live terrestrial organism in at least one of the 38 tubes (NASA, 2020prst). This means that if a viable microbe is found, the significance level is about 3.09 sigma. At that level some would claim a discovery of life, but it could be challenged, especially if the microbe is a species known from Earth.

If a microbe is found which seems to use terrestrial biology but the species is novel, again a claim to have discovered life on Mars could be challenged. The vast majority of microbial species haven't been characterized or sequenced or cultivated in the laboratory, the problem of "microbial dark matter" (Dance,2020). Clean room samples usually have numerous unrecognized microbial species (Weinmaier et al, 2015).

However, if a microbe is found with a clearly non terrestrial biology that would be a sure sign that it came from Mars.

The sample tubes are also permitted to have up to 1 nanogram of potential biosignature organics per gram of returned sample of organics. They test for DNA, the most common amino acids used by terrestrial life, glycine and alanine (other amino acids not measured, assumed to be less abundant), the most common lipid palmitic acid (other lipids not measured) and so on.

They tested for 16 "Tier 1" compound, most of them biomolecules(Boader et al, 2020: table 1) (Summons et al, 2014:991).:

- DNA,
- dipicolinic acid (spores),
- n-acetylglucosamine (bacterial and fungal cell walls),
- glycine and alanine (amino acids),
- palmitic acid and squalene (for lipids),
- pristane (as a hydrocarbon from petroleum contamination not found in meteorites),
- chlorobenzene and dichloromethane (risking confusion with Martian organics),
- naphthalene (example of a PAH, found in fossil fuels, and one of the constituents of ALH84001),
- urea (as representative of nitrogenous compounds and important for prebiotic chemistry),
- acetic acid (as a short-chain carboxylic acid),
- glycerol (as a polyhydroxy compound),
- pyruvic acid (as a hydroxy carboxylic acid), and
- n-heptacosane (as a linear hydrocarbon, a common industrial contaminant)

The estimated level achieved was 0.7 nanograms (Boader et al, 2020: table 6).

They emphasized that their goal is to set limits not only for the compounds they tested but to ensure that

> *"all related compounds (e.g., all proteinogenic amino acids, common lipids, nucleotides, sugars, hydrocarbon biomarkers, etc.) should be at similar or lower levels"*
> (Summons et al, 2014:991)

This limit of 1 nanogram per sample tube is for the particular most abundant organic measured (Summons et al, 2014:991). So for instance their aim with the limit for alanine and glycine was to achieve similar limits individually for any amino acid. The 1 nanograms is not a limit for the total of all amino acids.

Details and the motivation are given in the proposals from the 2014 Organic Contamination Panel (Summons et al, 2014:991 and table 5) and the final list of organics measured in (Boader et al, 2020: table 1)

They do have a limit on the total organic carbon per sample tube. For this, the requirement was 10 nanograms per tube. Their best estimate of the level of total organics achieved is 8.1 nanograms per tube (Boader et al, 2020: table 6),

This means each sample tube could have up to 0.7 nanograms of contamination from DNA, or Glycine, or some other organic biosignature. The paper doesn't give a detailed breakdown of the estimated level achieved for each organic.

The aim here was to make sure that typical levels of organics detected in Martian meteorites we already have could be detected in returned Mars samples.

However ancient organics on Mars are likely to be severely degraded by cosmic radiation (Kminek et al, 2006) and hard to distinguish from organics from infall from meteorites, comets, and interplanetary dust (Goetz et al, 2016:247) (Frantseva et al, 2018) as we saw in:

- The processes on Mars expected to destroy most surface organics from past life

They didn't test for chlorophyll or carotene, which are amongst the top candidates of the more complex molecules that could resist ionizing radiation from past life according to astrobiologists, and that could be preserved on Mars if it has related life.

So we can't give levels for these, except that they have to be less than the 10 nanograms limit for all organics (estimate of 8.1 nanograms achieved), but if either carotene or chlorophyll is found in small quantities, it would again be hard to rule out terrestrial contamination.

See:

- Perseverance could detect distinctive biosignatures like chlorophyll and carotene - but only for exceptionally well preserved life

An ultramicrobacteria by definition has a total volume of less than 0.1 cubic microns in its mature state, while an ultramicrocell is defined as a viable cell of the same volume that grows to normal sized cells when cultivated, found in old or starved cultures of normal bacteria (Duda et al, 2012) (Nakai, 2020). Some may be much smaller down to a volume of 0.02 cubic microns (Duda et al, 2012).

There are 10,000 microns in a centimeter so a cubic centimeter of water, one gram, has the same volume as at least ten trillion ultramicrobacteria. So a nanogram (billionth of a gram) of water has the same volume as at least 10,000 ultramicrobacteria or ultramicrocells.

Assuming Martian microbes have a density similar to water. the estimated level of 8.1 nanograms per tube for total organics is enough mass for 81,000 ultramicrobacteria, and it's enough mass for 160 million of the hypothetical minimal volume RNA world nanobes based on their estimated volume of 50,000 nm^3 (Board et al, 1999: 117), or 0.00005 cubic microns. Even a picogram is enough mass for 160,000 of those hypothetical RNA world nanobes.

So, there is a small but non zero possibility of a false positive detection of a viable microbe, and a high possibility of detection of biosignatures such as DNA that could incorrectly suggest the presence of Martian life.

A false positive could delay the process of determining that there is no Martian life in the sample, indeed it might be impossible to prove the absence of Martian life that might have similar biology to terrestrial life because of the permitted levels of terrestrial contamination in the samples.

See:

- Permitted levels of contamination could make it impossible to prove absence of Martian life in Perseverance's sample tubes – leading to an unnecessary requirement to sterilize returned samples indefinitely

The largest amino acid by mass is Tryptophan W with a molecular mass of 204.22 g/mol (NCBI, 2022t) and the smallest is Gycine with a molecular mass of 75.07 g/mol (NCBI, 2022g), So the estimated 0.7 nanograms per organic per tube, if applied to amino acids would permit between 2 trillion and 5.6 trillion terrestrial amino acids depending on its molecular mass.

In situ instruments astrobiologists wish to send to Mars some day are designed to achieve far higher sensitivities, for instance the Astrobionibbler is able to detect a single amino acid in a gram of sample (Schirber, 2013).(Noell et al, 2016). This reflects the expected difficulty of finding the signature of degraded past life in samples.

If samples of degraded past life are returned, or well preserved past life in low concentrations, higher levels of sterilization for the sample tubes are likely to be needed to study them adequately.

Meanwhile for Perseverance, to have a realistic possibility of detecting Martian life, against the background signal of the permitted organic contamination, significant amounts of exceptionally well preserved past life is needed, or viable or well preserved present day life.

Could Perseverance's samples from Jezero crater in the equatorial regions of Mars contain viable or well preserved present day life?

There is much discussion about whether life is possible in equatorial regions of Mars such as Jezero crater. The question is not yet resolved. A minority of scientists think the Viking landers detected life already in the equatorial regions in the 1970s. Whether or not they did, there are many other possibilities now for life there.

This has been invigorated by

- Discovery of patterns resembling circadian rhythms in the Viking labelled release results, offset by two hours from the temperature peaks – such a long delay is hard to explain using abiotic processes
- The discoveries of RSLs (Recurrent Slope Lineae) or dark streaks that form on sun facing slopes in spring and grow seasonally and may be at least partially explained by flowing salty brines as well as dust flows, with the brines potentially habitable.
- the discovery by Curiosity of salty brines that form briefly overnight in surface layers of sand dunes. They are at times warm enough for terrestrial life but too salty and at other times have enough water activity for terrestrial life but are too cold – however they may be habitable to more versatile Martian life, and even terrestrial biology may be able to survive there in biofilms that can create their own microclimate by retaining water from the night through to warmer conditions.

Let's look at this research in detail:

Puzzles from the Viking landers – why some think Viking detected life already in the 1970s – evolved gases in the labelled release experiment offset from temperature fluctuations by as much as two hours, more typical of a circadian rhythm than a chemical reaction

The equatorial region of Mars is dry with no surface deposits of ice, making it a challenging location for life. However, the night time humidity can reach close to 100% and there are salt deposits that can capture water from the atmosphere. Reanalysis of the Viking mission data has led some to consider the possibility that the Viking landers found life in equatorial regions of Mars in the late 1970s. The most striking discovery was of circadian type rhythms offset from temperature fluctuations by as much as two hours. Miller et al found that: (Miller et al, 2002).

- The detector is only one inch from the experiment so the temperature fluctuations at the detector should be synchronized closely to the temperature fluctuations of the experiment through radiation and convection.
- The gases released would take only about twenty minutes to reach the beta detector from the experiment.
- The temperature change is about 2º C which is enough of a difference for circadian rhythms in various organisms including bread molds.
- Though the labelled release of gases is delayed by up to two hours as the temperature rises, it follows it almost exactly as it falls which is another characteristic of circadian rhythms.
- The gas release follows the larger scale temperature changes but not smaller changes, again a feature of circadian rhythms.

[Figure needs permission]

Figure 23: The labelled release (in black) is almost synchronized with the temperature fluctuations (red), but delayed by two hours.

From (Miller et al, 2002: Figure 7 (lower))

[Figure needs permission]

Figure 24 The labelled release (in black) is smoother and there is uncorrelated noise in both graphs. A chemical reaction would be expected to follow variations in the temperature (shown in red) more exactly..

From (Miller et al, 2002: Figure 8 (lower))

None of the explanations for the Viking results are totally satisfactory, whether life or non life. Perhaps the most favoured chemical explanation for the Viking results is the reaction studied by Quinn et al in 2013. They suggested that the perchlorates in the soil were decomposed through gamma radiation to hypochlorite (ClO^-), trapped oxygen, and chlorine dioxide. Then the hypochlorite reacted with the ^{14}C-labelled alanine to produce chloroalanine which then decomposed to produce the ^{14}C-labelled CO_2. (Quinn et al, 2013).

Any abiotic explanation has to explain the two hour offset of the circadian rhythms. There are other anomalies to explain too.

- Two of the labelled release experiments got inactivated after storage in darkness for several months
- Activity of the soil is significantly reduced if heated first to 50 °C.

Levin and Straat in a paper published in 2016 review some of the issues they have found with this and other proposals (Levin et al, 2016).

It is either life or very complex chemistry.

Whatever the answer about whether Viking found life on Mars in the 1970s, there does seem to be potential for microhabitats for life in equatorial regions of Mars. This may be especially so for native life that may have different capabilities from terrestrial life such as the ability to grow at colder temperatures.

Could spores from nearby habitats explain the Viking results?

There might be habitable brines in the Recurrent Slope Linea, dark streaks that form on hillslopes in spring, extend through the summer and broaden, then fade away in autumn. Some may be based on dust flows, but others seem likely still to have a wet formation mechanism. Some RSL's occur in equatorial regions and are hard to spot from orbit, so it's not impossible that there could be some close to Perseverance. Some were found close to Curiosity's landing site after the rover landed on Mars. Perhaps spores from these brines could explain the results?

See Could local RSL's be habitable and a source of wind dispersed microbial spores? Both dry and wet mechanisms leave unanswered questions - may be a combination of both or some wet and some dry

Then in addition, we now know that ultracold perchlorate brines can be found in many of the sand dunes in Gale crater and probably also in Jezero crater.

So could these be habitable to Martian life? If so they might be a source for spores for the Viking experiments, and whether they are relevant to Viking, they are of interest in their own right as a possible habitat in Jezero crater.

Detection by Curiosity rover of liquid water as perchlorate brines in Gale crater sand dunes and similar conditions are predicted in Jezero crater dunes

Curiosity found clear though indirect evidence of salty brines in Gale crater. These brines may be based on calcium perchlorate as the salt, which can form liquid brines at cold temperatures below -70 °C.

Curiosity detected these brines through the DAN experiment (Dynamic Albedo of Neutrons) (NASA, n.d.dan) which is able to detect subsurface hydrogen from water. At the same time, the REMS instrument detected a lower night time humidity over sand, suggesting that the water from the air is taken up within sandy soil at night (Martin-Torres et al, 2015). This all adds to strong evidence for liquid water in Gale crater, and especially in the sand dunes.

This figure shows data points from Curiosity plotted against time of day and season

[figure needs permission]

Figure 25: Indirectly detected surface brines mapped according to time of day and time in the Martian year. The light blue patches are liquid on the Martian surface and the dark blue patches later in the day are liquid at a depth of 5 cm.

Black lines frame the time when liquid water in the form of these brines is stable. Sunrise and sunset times shown with the red lines. (Martin-Torres et al, 2015)

These brines seem at first to be outside the range of habitability for Earth life. At times in the daily cycle they are warm enough but have too little water activity and at other times they have enough water activity but at -73 °C are far too cold for even the most cold tolerant Earth microbes to flourish (Martin-Torres et al, 2015).

[figure needs permission]

Figure 26: Inferred temperature / water activity measurements superimposed on the phase diagram for calcium perchlorate. The blue dots cover the region where liquid water could form, water activity about 0.525, temperature about 200°K or -73°C (Martin-Torres et al, 2015)

So, can these brines form in Jezero crater too? The answer seems to be that they likely can. Gale crater is one of the driest, warmest regions of Mars and the brines are likely to form more easily in more humid locations further from the equator (Martin-Torres et al, 2015)

Jezero crater is further from the equator at 18.45°N (NASA, n.d.WiP) compared to Curiosity's landing site at 4.589°S (NASA, n.d.WiC). Although Curiosity's landing site was lower in elevation at -4,400 meters below Martian "sea level" (NASA, n.d.cls), compared to -2,700 meters for Perseverance (DLR, n.d.), Jezero crater is more humid.

Calcium perchlorate brines are likely to be stable on the surface for five hours in the morning in Jezero crater compared to three hours for Gale crater. The relative humidity in the atmosphere is expected to reach ~100% an hour or so before dawn, compared to ~90% for Gale crater (Chevrier et al, 2020: figure 7).

How Martian life could make perchlorate brines habitable when they only have enough water activity for life at -70 °C – biofilms retaining water at higher temperatures - chaotropic agents permitting normal life processes at lower temperatures – and novel biochemistry for ultra low temperatures

The inferred temperature and water activity for the brines found by Curiosity are well outside the normal habitability minima of 0.6 a.w. (water activity) and -20°C for terrestrial life (Rummel et al , 2014). However terrestrial life never encounters these conditions of extreme cold and high concentrations of perchlorates. Any Martian life would have evolved to live in these conditions, so it may be able to inhabit microclimates outside the known range of terrestrial life.

There are several factors that Martial life could exploit that may make these brines more habitable for Martian life, and possibly for terrestrial life too.

Microbes can create and modify microclimates, so the microclimate inferred by REMS from the surface humidity may not be identical to the microclimate experienced by microbes in the sand.

Nilton Renno, who runs the REMS weather station on Curiosity and who previously showed habitable salty brines could form briefly in polar conditions (Fischer et al., 2013) (Renno, 2014):, remarked that biofilms could modify the habitability of the brines Curiosity found, by retaining moisture through to daytime. (Nilton Renno cited in Pires, 2015) He said:

> ***"We had made simulations that imitated the conditions of Mars in my laboratory and the results showed us how small amounts of liquid water can exist on the surface of the polar region of Mars," he said. "Now we have the proof that liquid water is possible even in tropical region. It is an important discovery."***
>
> ***Life as we know it needs liquid water to survive. While the new study interprets Curiosity's results to show that microorganisms from Earth would not be able to survive and replicate in the subsurface of Mars, Rennó sees the findings as inconclusive. He points to biofilms—colonies of tiny organisms that can make their own microenvironment.***

Rettberg et al. raised this as a question in 2016, to paraphrase (Rettberg et al, 2016: section 2.1),
:

- Can multispecies colonies form biofilms to help them tolerate environments they couldn't survive in individually?
- Can organisms replicate there if the temperature and water activity separately reach levels suitable for life, but not both at once, for instance by storing water when it is cold and then using it to replicate as the brines warm up?

Nilton Renno's biofilms can be one way to store water from conditions of lower temperatures through to warmer times in the night - day cycle.

Let's try to expand on the idea. If Mars did develop biofilms, perhaps originally in more habitable brines, parts of the biofilms could be blown around in the dust storms. It's possible that given billions of years of evolution, Martian life in the brines would develop spores, or multicellular propagules similarly to fungal hyphal fragments.

In the low Martian gravity, even with the thin atmosphere, a half millimeter diameter propagule could contain as many as 40 million viable cells, assuming each cell is 1 micron in diameter. Martian life could then evolve adaptations to survive in dust storms, for instance, perhaps using the extraordinarily hard substance chitin which is produced by lichens, but use it to protect from the impacts of saltation (bouncing dust grains). For details see

- Could Martian life be transported in dust storms or dust devils, and if so, could any of it still be viable when it reaches Perseverance?

- Native Martian propagules of up to half a millimeter in diameter (including spore aggregates and hyphal fragments) could travel long distances with repeated bounces (saltation) - if they can withstand the impacts of the bounces

- Martian life could also use iron oxides from the dust for protection from the impact stresses of the saltation bounces - or it might use chitin - a biomaterial which is extremely hard and also elastic and is found in terrestrial fungi and lichens

These propagules could modify the local microclimate immediately as soon as they encounter it, as they take up water from the brines at night and use the water at warmer temperatures in day time. Ground temperatures in Gale crater frequently rise above 0 °C in the daytime.

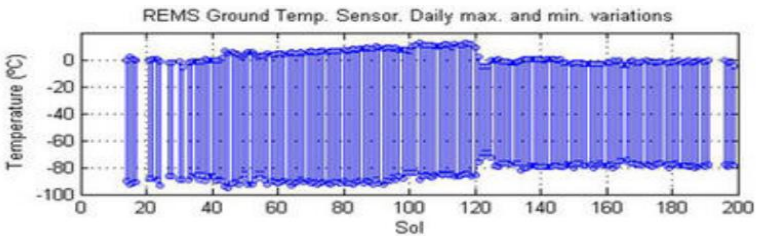


Figure 27: Ground temperatures on Mars as measured using REMS for the first 200 days of the Curiosity mission (NASA, 2013stmgc)

The ground temperatures in Jezero Crater may rise to 10°C or more at midday in mid summer (solar longitude Ls180) and a near surface biofilm exploiting the liquid perchlorates found by Curiosity could potentially retain liquid water through to these temperatures.

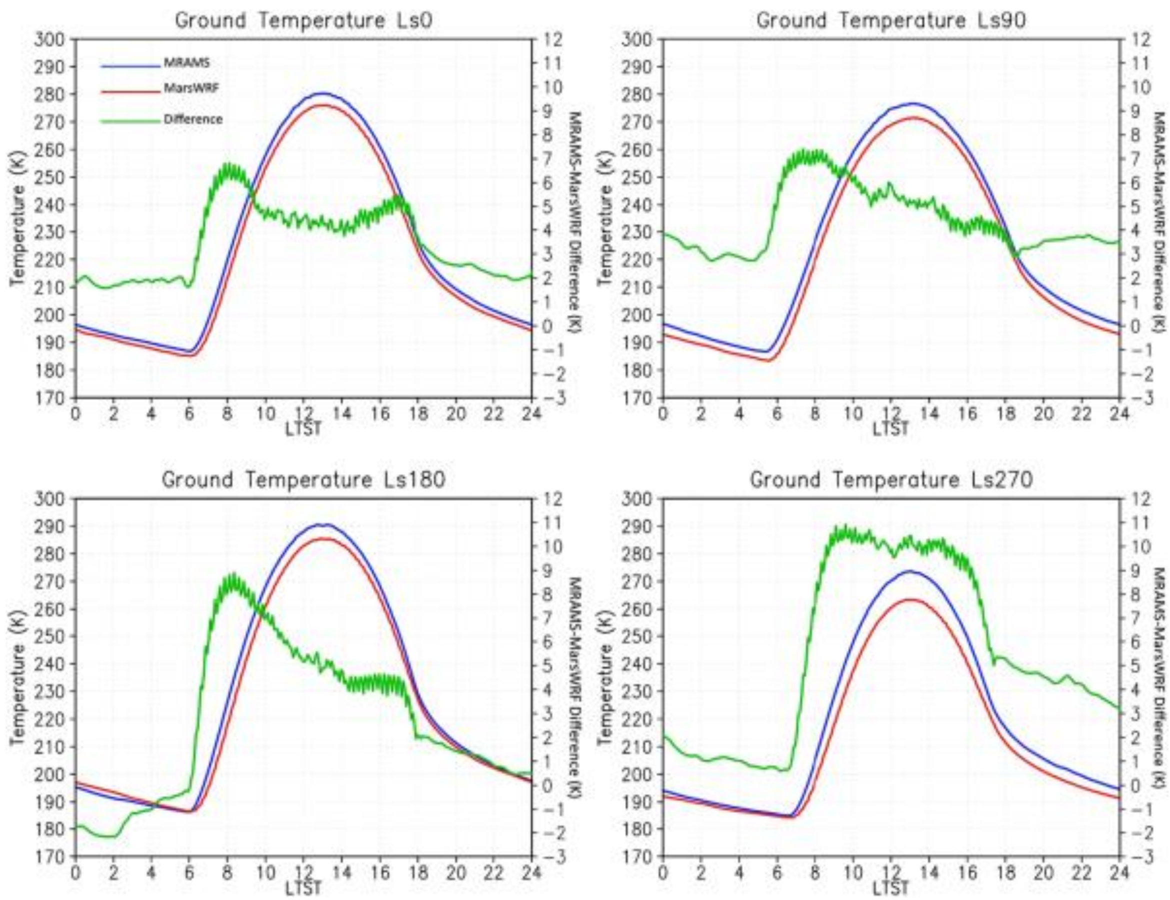


Figure 28: Predicted ground temperature for Jezero Crater using the Mars Regional Atmospheric Modeling System (MRAMS) and the Mars Weather Research and Forecasting model (MarsWRF). From (Pla-García, 2020: Fig. 3)

It is also possible that Martian life could exploit lower temperatures than terrestrial life. The -20°C limit is not well determined even for terrestrial life. Rettberg et al also ask (paraphrase) (Rettberg et al, 2016: section 2.1),

- How strict is the lowest temperature for replication for Earth life? Have the experiments exploring the lower temperature limits of replication been carried out over long enough timescales to study extremely slowly replicating organisms?

It is hard to study replication at such low temperatures because of the long generation times. Experiments with yeast show doubling at -18 °C, with a doubling time of 30 days. One experiment showed ammonia oxidation at -32 °C sustained for 300 days, the duration of the experiment. Since cell division would be so very slow at those temperatures, the researchers couldn't tell whether this is just maintenance metabolism, or whether it actually did support very slow cell division (Rummel et al , 2014)

Then, chaotropic agents can help microbes be active at lower temperatures by disrupting the hydrogen bonding of water molecules with each other. Common examples include ethanol, urea, butanol etc. The Mars surface has many chaotropic agents which could reduce the minimum temperatures for cell division, including $MgCl_2$, $CaCl_2$, $FeCl_3$, $FeCl_2$, FeCl, LiCl, chlorate, and perchlorate salts (Rummel et al , 2014).

Typically, these chaotropic agents reduce the lowest temperatures for cell division for many microbial species by 10 °C to 20 °C. However Rummel et al couldn't find any experiments testing their action at temperatures low enough to reduce the lowest temperature limit for cell division for life (Rummel et al , 2014:897). The lower the temperature, the harder it is to do the experiments to test the effect on cell division, due to the longer replication time of life at such low temperatures.

Even -40 °C (i.e. a 20 °C reduction below -20 °C) is a long way from the inferred temperature of -73 °C for the brines unmodified by biofilms with water activity 0.525 in Figure 26. However, one study did find that chaotropic agents can increase the ability of microbes to survive freezing to -80 °C with no loss of viability, when frozen for 24 hours, compared with 60% loss of viability without. The test used the microbial propagules of xerophilic fungi (low water activity) (Chin et al, 2010) but didn't test for cell division at low temperatures.

More radically, Dirk Schulze Makuch et al suggested that native life on Mars might have evolved to use the cold brines on Mars with a novel cold adapted biochemistry, using perchlorates or hydrogen peroxide internally, in place of the chloride salts in our cells (Schulze-Makuch et al, 2010a).

First, with a H_2O - H_2O_2 intercellular fluid, metabolic activity could continue down to -56°C (217°K). A mix of two salts typically has a lower freezing point than either individually. If the cells used perchlorates internally, the limit might be reduced further, to -70°C which is similar to the temperature inferred for the brines observed by Curiosity (Schulze-Makuch et al, 2010a).

Houtkooper et al suggest this novel biochemistry as a possible explanation for some of the puzzling Viking results. The organics might react with the hydrogen peroxide and decompose easily, when heated or even when hydrated with too much water, making the discovery of life-originated organics challenging (Houtkooper et al, 2006).

Also, the water activity may not be such a fixed limit. Microbes in a deep-sea hypersaline anoxic basin (DHAB) can flourish at a water activity as low as 0.4, well below the usual limit of 0.6 (Steinle et al, 2018) (Merino et al, 2019: Salinity and Water Activity)

Some microbes are known to release ice-binding proteins (IBPs) slowly into the surrounding environment which prevent the formation of ice. Garcia-Descalzo et al have proposed that these could act as cold-brine shock proteins'' that could help to make the microenvironment around the microbes more habitable. They also wonder whether bacteria unable to achieve cell division in the cold and salty conditions could, without reproducing, slowly modify them to become more habitable (Garcia-Descalzo et al, 2020:1072). They have not yet confirmed this experimentally but so far have only studied one species of terrestrial microbe, Rhodococcus sp. JG3 (Garcia-Descalzo et al, 2020).

The low temperature brines found by Curiosity remain liquid because of a high concentration of perchlorates. So, could Martian life make use of perchlorates in such high concentrations? These concentrations aren't found on Earth and Heinz et al, say that there has been little by way of studies to find the limits for concentrations for terrestrial life (Heinz et al, 2020) . They report the discovery of fungi that can grow in a 23% weight by weight solution of sodium perchlorate, or 2.4 M (Heinz et al, 2020) which corresponds to a water activity of about 0.8 (from figure 5 of (Toner et al, 2016)).

Heinz et al suggest (Heinz et al, 2020):

> ***It is plausible to assume that putative Martian microbes could adapt to even higher perchlorate concentrations due to their long exposure to these environments occurring naturally on Mars, which also increases the likelihood of microbial life thriving in the Martian brines.***

Some Martian brines could be oxygen-rich, permitting aerobes or even primitive sponges or other forms of multicellularity - Stamenković's oxygen-rich briny seeps model

Mars may have oxygen in the brines even with the low levels of oxygen in the Martian atmosphere. Colder water takes up more oxygen from the atmosphere, and concentrations may reach high levels in the coldest brines (Stamenković et al, 2018)

The lowest oxygen level they found with their modelling was 2.5 millionths of a mole per cubic meter (0.0008 mg per liter) in the tropical southern uplands where temperatures are high, the atmospheric pressure is low, and based on their worst case scenario for uptake of water in their brine with lowest solubility, sodium perchlorate. This is already enough to support some aerobic microbes. However, they give reasons for believing that their more optimistic best case calculations are close to the true situation.

With their best case, extremely cold brines in polar regions can potentially reach oxygen saturation levels similar to those needed for primitive sponges (Stamenković et al, 2018)

Indeed, in principle, extremely cold calcium perchlorate brines in polar regions could reach oxygen saturation levels of up to 0.2 mole per liter (6.4 mg per liter) at -133 °C. This is similar to oxygen levels typical of warm sea water on Earth. Magnesium chlorates could reach concentrations ten times larger (Stamenković et al, 2018)

These findings show that in the extremely cold conditions of the Martian brines, even with the low levels of oxygen in the Martian atmosphere, the oxygen concentrations in brines may in best case be similar to the warmest seas on Earth.

Speculatively, some of the higher levels of oxygen might be accessible to Martian life with a greater range of tolerance to temperature than terrestrial life. Or the very coldest brines might act as a reservoir of oxygen rich brines accessed by deeper and warmer layers. Stamenković put it like this in an interview (Walker, 2019):

> ***The options are both:***
>
> - ***first, cool oxygen-rich environments do not need to be habitats. They could be reservoirs packed with a necessary nutrient that can be accessed from a deeper and warmer region.***
>
> - ***Second, the major reason for limiting life at low temperature is ice nucleation, which would not occur in the type of brines that we study.***

Whether or not such high levels of oxygen are useful to Martian life, there may well be oxygen levels suitable for aerobic microbes. The research doesn't investigate the timescale for uptake of oxygen, for instance whether the temporary brines found by Curiosity could take up oxygen overnight (Walker, 2019).

Life could also exploit enhanced humidity in micropores in salt deposits - but these may be rare in Jezero crater

Humidity is also increased by spontaneous capillary condensation of water vapour in micropores in salt deposits. This process enables communities of cyanobacteria and heterotrophic microorganisms to thrive in the hyperarid core of the Atacama desert (Vítek et al, 2010). Cassie Conley and, separately, Paul Davies have suggested these micropores as potential habitats on present day Mars (Conley, 2016) (Davies, 2014).

Salt deposits however may be rare in Jezero crater. The deposits of most interest to astrobiology in Jezero crater consist mainly of clays and carbonates, and it doesn't have the large bright salt deposits of Mount Sharp (Lerner, 2019).

Melting frosts - and potential for a temperature inversion to trap a near surface cool humid layer at dawn as the air warms, perhaps permitting thin films of water to form briefly

Gilbert Levin and his son Ron Levin suggest that a cool humid layer could be trapped near the surface as dawn approaches, in a temperature inversion, overlain by a layer of warm air. This might lead to thin films of water that form briefly in the early morning then evaporate. Chris McKay, agrees that this process could form a layer of liquid though it may not last long (Abe, 2001).

The Viking 2 lander (NASA, 1997) and Phoenix lander (NASA, 2008mfosm) both imaged frosts on the surface. The other rovers haven't photographed them but there are estimates that a few tens of microns of frost could have formed in Gale crater at night (Martínez et al., 2016). That's enough to be useful water for a microbe as it melts. There is possible direct detection of frosts in Gale crater a few microns thick (Gough et al., 2020).

Experiments with black yeasts, fungi and lichens in Mars simulation conditions suggest life could use the night time humidity directly without liquid water

Then it's also possible that life on Mars is able to make use of the night time humidity without any liquid water present on the surface

Rettberg et al ask, to paraphrase (Rettberg et al, 2016: section 3.1),
:

- Is water vapour alone sufficient for replication?

Some terrestrial lichens and microbes in desert regions and frozen regions are known to use the humidity of the air directly to grow, without the presence of water.

Some experiments have suggested that this may also be possible on Mars. Some lichens (de Vera et al, 2014) , cyanobacteria, a black yeast and microcolonial fungi (Zakharova et al, 2014) have been shown to be able to use the humidity of the air directly in Mars simulation conditions.

For Zakharova et al's experiments they used the black yeast *Exophiala jeanselmei*, and the black fungi *Cryomyces antarcticus* and *Knufia perforans*. The temperature was varied between −55°C (218°K) at night and 15°C (288°K) in day time and humidity from very low levels in daytime to 70% at night at a pressure of 0.7% standard atmosphere (700 Pa), The fungi showed no sign of stress (no heat shock proteins) however they showed less metabolic activity to start with, recovering normal levels after 7 days. All the fungi grew in biomass showing the ability to survive and reproduce. The researchers speculated that this might involve some new unknown metabolic pathway.

The black yeast they studied in this experiment, *Exophiala jeanselmei,* normally inhabits rocks. However it is closely related to opportunistic human pathogens (Zakharova et al, 2014) and is occasionally an opportunistic pathogen itself (Urbaniakt al, 2019). This leads to the question of whether a Martian black yeast could be a pathogen of humans. See Could present day Martian life harm terrestrial organisms? below

The black fungi and black yeast they studied have stress resistant proteins that are so effective that similar black yeasts and fungi are able to survive in reactor cooling ponds, indeed the high doses of ionizing radiation in this habitat enhance their growth capacity. Those proteins may help explain why they were able to withstand Martian surface levels of UV (Zakharova et al, 2014).

De Vera et al's lichen experiment simulated temperature changes of 294°K (21°C) in the daytime to 223°K (-50°C), at night and humidity changes from 75% at night to 0.1% in daytime. This experiment used a constant pressure of 1% reducing to 0.8% by day 6.

When the lichen was exposed to full surface Martian UV levels the fungal component of the lichen deteriorated and the algal component had reduced photosynthesis (de Vera et al, 2014).

However it was a different story when the lichen was exposed to the lower levels of UV expected in fissures and cracks. After an initial shock response, with reduced photosynthesis, the lichen adapted to the experimental conditions, with increasing yields for as long as the experiment continued. It was able to photosynthesize at rates higher than for wild lichens in Antarctica (de Vera et al, 2014).

The pressure of 0.7% to 0.8% was above the triple point of water and matched pressures measured by Curiosity in Gale crater. One surprise from this experiment is that the fungal component, which is an aerobe, was able to maintain its viability in the semi shade protected condition, suggesting that the algae may have been able to provide enough oxygen for the closely connected fungus (de Vera et al, 2014).

In another experiment, twenty species of microbe from soil and permafrost samples were able to grow at Mars surface pressure conditions (Schuerger et al, 2016) but no fungi were able to grow in these experiments. This only tested anoxic low pressure conditions, the microbes were grown on agar mixed with their soil, in 0.1% agar/soil mixtures

[Figure needs permission]

Figure 29: colony forming units showing growth in conditions of Martian surface atmospheric pressure.

[figure 1 from (Schuerger et al, 2016)]

Schwender's comprehensive review in 2020 (Schwendner, 2020) doesn't mention any newer experiments testing growth of microbes or lichens in Mars surface simulation conditions. Presumably there hasn't been anything more published from 2014 through to 2020, or it would have been mentioned.

These suggestive early experiments leave many questions open about whether terrestrial life could survive Mars surface conditions using just the humidity of the atmosphere. More research is needed on these topics.

Surface conditions of ionizing radiation, UV radiation, cold and chemical conditions don't rule out the presence of life

Curiosity measured levels of ionizing radiation similar to the interior of the ISS. This is only sterilizing on timescales of millions of years. Radiodurans is able to withstand 65,000 years of Mars surface ionizing radiation with no loss of viability. None of the microbes die and they have a remarkable capability to repair even double strand breaks in the DNA, and without forming a new organism. Radiodurans can repair 100 double strand breaks per chromosome without any loss of viability or mutation of its genome (Minton, 1994).

Radiodurans can do this despite never encountering such conditions in the wild. It even responds to ionizing radiation with increased growth and it's possible that some microbes are radiotrophic, able to use melanin to get energy for growth from ionizing radiation (Dadachova et al, 2008). Martian microbes are likely to be even better able to cope with ionizing radiation than this since they evolved in conditions where high levels of ionizing radiation resistance is a major evolutionary advantage.

Rummel et al concluded that levels of ionizing radiation are not used to distinguish the special regions on Mars (where terrestrial life potentially could flourish) (Rummel et al , 2014).

> **Finding 3-8**: From MSL RAD measurements [Curiosity's Mars Science Laboratory Radiation Assessment Detector], ionizing radiation from GCRs [Galactic Cosmic Rays] at Mars is so low as to be negligible. Intermittent SPEs {Solar Particle Events] can increase the atmospheric ionization down to ground level and increase the total dose, but these events are sporadic and last at most a few (2–5) days. These facts are not used to distinguish Special Regions on Mars.

UV radiation can be blocked by a shadow, even a shadow of a pebble, or a few millimeters of dirt can protect life from UV. Also some lichens and cyanobacteria are able to withstand the levels of UV found in partial shade on Mars using protective pigments used to protect them against UV in high mountains and in Antarctica. Again these are not used to distinguish the special regions (Rummel et al , 2014).

> **Finding 3-7**: The martian UV radiation environment is rapidly lethal to unshielded microbes but can be attenuated by global dust storms and shielded completely by < 1 mm of regolith or by other organisms.

As for the low temperatures of the Martian surface, many microbes can survive extreme cold by cooling in a glassy state without forming the damaging crystals of water ice. (Rummel et al , 2014).

Perchlorates are much less reactive in colder conditions and they are used by some microbes as oxidants, as a source of energy. (Rummel et al , 2014).

It's a similar situation for other challenges life would face on the surface of Mars. None of the surface conditions are such as to make it impossible, at least with current knowledge, that present day life may survive on the surface of Mars in some niches (Rummel et al , 2014).

Sources of nitrogen on Mars as a potential limiting factor – potential for Martian life to fix nitrogen at 0.2 mbar – and “follow the nitrogen”

Nitrogen may be the main limiting factor for life on Mars after liquid water. There are many sources for the other necessities of life.

There are many sources of energy on Mars. The blue-green algae chroococcidiopsis is one of the main candidates for a Mars analogue terrestrial microbe (Billi et al, 2019a). It can use light as a source of energy, and as a prime producer, could survive almost anywhere on Mars with access to liquid water, sunlight, and some protection from UV (Billi et al, 2019b), and access to trace elements and nitrogen.

Alternatively, non photosynthetic life can use chemical redox gradients as a source of energy, for instance surface layers of sand dunes are superoxygenated while the slowly moving sand dunes constantly bring subsurface reducing layers to the surface (Fisk et al, 2013)

Basalt is a good source for trace elements for life and is common on Mars (McMahon, 2013)

Liquid water is available in the temporary brines, or as night time humidity.

This leaves nitrogen. There is 0.2 mbar of nitrogen in the Mars atmosphere, which is far less than the 780.90 mbar in Earth’s atmosphere. This makes nitrogen fixation a major challenge.

If life on Mars can't fix nitrogen, any life there may depend on naturally occurring nitrates, which may be a limiting factor for life. See:

- Possibility that past life in Jezero crater or even modern Martian life never developed nitrogen fixation – or that microbes in oxygen rich surface layers never developed nitrogen fixation

Curiosity has discovered surface nitrates in low concentrations (~0.01–0.1 wt % NO_3) in Yellowknife bay drill sites. This overlaps the lowest end of the range for nitrates in the Atacama desert (~ 0.1 to greater than 1 wt% NO_3) (Stern et al, 2015). These concentrations are consistent with impact generated nitrates from early Mars (Stern et al, 2015).

Mars could also have an abiotic nitrogen cycle with photochemically produced HNO_3 fixed in thin (0.2 to 5 nm) pure water metastable interfacial films, potentially supporting up to one kilogram of fixed nitrogen per square meter (Boxe et al, 2012)

There may also be a possibility that Martian life could fix nitrogen even at these low pressures. In laboratory experiments, some cold tolerant microbes from Antarctica are able to fix nitrogen at a partial pressure of 0.2 mbar similarly to the partial pressures on modern Mars, as reported in unpublished research (Mancinelli, 1993) following (Klingler et al, 1989). Sakon et al also found that some cold tolerant life (psychrophiles) can still fix nitrogen at these low partial pressures when the temperature and UV flux of Mars is simulated (Sakon et al, 2005) (Sakon et al, 2006).

These experiments involve a partial pressure of nitrogen, in an atmosphere of other gases at normal terrestrial atmospheric pressures. Follow up experiments are needed to duplicate the Martian atmospheric pressure in a Mars simulation chamber and find out if these microbes from Antarctica can still fixate nitrogen at the same partial pressure of 0.2% at a total pressure of 0.6% (Sakon et al, 2006).

More experimentation is needed on the possibility of nitrogen fixation in Mars conditions.

Nitrogen might or might not be a limiting factor for life.

On Earth nitrogen is returned to the atmosphere by denitrification, the opposite of nitrogen fixation. Later in this current paper we explore a possible feedback process that could keep nitrogen levels on Mars at low levels, with just enough nitrogen for nitrogen fixation but so little nitrogen that it proceeds only slowly.

- Interactions of nitrogen cycle with swansong Gaia - if life returns more nitrogen to the atmosphere when Mars is wetter, the Swansong Gaia cycle is reinforced

If life on Mars can fix nitrogen it could be found anywhere not limited by the presence of nitrates or other forms of fixed nitrogen, but if it can fix nitrogen only slowly, it may be present in low concentrations in regions where there is no fixed nitrogen available already.

Or Martian life might be limited to habitats that already have nitrates or other sources of nitrogen. Nitrogen can also be a clue to the presence of traces of life organics, for instance, in amino acids. In that case, a preliminary detection of nitrogen in a potential habitat could be a clue to follow up to look for biosignatures. With all these scenarios, nitrogen is an interesting element to look for on Mars.

Several authors have suggested variations on a “follow the nitrogen” strategy to look for life. Capone et al. say that in a planet with both land and oceans, the presence of nitrogen in any form on the land is hard to explain without life supplied chemistry, because nitrates tend to dissolve in the presence of small amounts of water, and nitrogen has few geological reservoirs, apart from a few clays with ammonium substituted for potassium. Except for nitrates in very dry places, most would end up in the oceans (Capone et al., 2006).

> ***The presence of nitrogen in any form should be a signal to planetary scientists to take notice. It may well be that the form and amount of nitrogen could constitute a roadmap for understanding whether chemical or biological processes were involved in its deposition. At least on a body that has had a separation of continental and oceanic components, the existence of nitrogen on continents is not easy to explain without special life-supplied chemistry.***
> ***…***
> ***Thus, our recommended approach might be to search for the nitrogen; characterize and quantify it; if its abundance and chemistry cannot be explained by abiotic processes, do not leave until it is explained; and when it comes to sample return—bring back anything that is enriched in nitrogen!***

Shannon put's it like this (Shannon, 2006)

> ***An oasis of liquid water on Mars would still be presumed sterile if no nitrogen—and therefore no nitrogen containing organic compounds—were detected. In this way, nitrogen might be a better target than the water itself.***

Bada et al suggested a "follow the nitrogen" strategy to look for biosignatures such as chirality by first looking for specific nitrogenous compounds used by life such as amino acids which may be preserved as components of stable fatty acids (such as lipids), sugars, and peptidoglycans (mureins, constituents of cell walls) (Bada et al, 2009).

All life on Earth requires nitrogen. Also, there are theoretical reasons for expecting alien organic life to use nitrogen, as the weaker nitrogen based amide bonds are essential for the processes by which DNA is replicated. Mars, compared with Earth, has little nitrogen, either in the air or in the soil.

Jeff Bada explained it like this, interviewed for an article for NASA's Astrobiology Magazine (Schirber, 2013).

> ***"Organic nitrogen compounds are central for biology as we know it. No other class of compounds plays a more important role."***
>
> ***"Nitrogen atoms in nucleobases and amino acids form hydrogen bonds that stabilize the structure of both nucleic acids and proteins. Without these hydrogen bonds there would be no helical structure to DNA and RNA and no alpha-helix structure for proteins."***

[Also the nitrogen-hydrogen bonds are easy to break helping enable the chemical processes of life]

"No other atom than nitrogen can form the diverse set of hydrogen-bonded compounds found to be fundamental in the biologically central processes of replication and catalysis

Could Martian life be transported in dust storms or dust devils, and if so, could any of it still be viable when it reaches Perseverance?

Life on Mars might be adapted to spread in the dust using spores and other propagules. Hardy propagules could be transported over shorter distances by dust devils and over longer distances by the Martian dust storms. These would be of astrobiological interest even if they are only viable after transport for short distances. Propagules damaged by UV or UV radiated perchlorates in the dust could still give a method to detect life in remote regions of Mars. They may also be a backwards contamination issue if viable Martian life can be transported over great distances.

NASA have the survival of terrestrial microbes in the dust as one of their many knowledge gaps for human missions to Mars *(Race et al, 2015: 34)*. Then as we'll see, Martian life could be better adapted to transport in Martian dust than terrestrial life.

One experiment by Wadsworth et al, suggests that survival of terrestrial microbes would be challenging. When perchlorates on Mars are irradiated by UV, this reduces survival, probably because it produces the more toxic chlorites and hypochlorites. Perchlorates are widespread in the dust (Wadsworth et al, 2017).

However they only studied one species, bacillus subtilis. They commented:

> *"The bacterial model we tested wasn't an extremophile so it's not out of the question that hardier life forms would find a way to survive."*

Also their result only applies to dust irradiated by UV. A microbial spore imbedded in a dust grain would be shielded from UV by iron oxides in the dust (Sagan et al, 1968). Research with simulated wind blown Martian dust suggests microbes can indeed get attached to a dust particle and get blown in the winds (van Heereveld et al, 2017) (Osman et al 2008).

Martian life could also be protected within fragments of biofilms blown in the wind.. Dried Chroococcidiopsis biofilms can withstand three years of desiccation and the equivalent of several hours of UV radiation in equatorial conditions on Mars. Mosca et al suggest that such biofilms could be transported from niches that have become unfavourable to more favourable niches on Mars, if such a biofilm ever evolved in Mars history. (Mosca et al, 2019)

Billi et al, suggest that a biofilm of chroococcidiopsis could withstand up to 8 hours of full sunlight on Mars. This is enough time to be transported 100 km at 5 km/sec in a dust storm. (Billi et al, 2019a). This is based on experiments in the BIOMEX experiment on the outside of the ISS. They found that a dried biofilm of chroococcidiopsis could survive 469 days of Mars surface UV in a layer of about 15–30 microns (0.015 to 0.03 mm) when mixed with regolith and with the UV attenuated to conditions of partial shade on Mars (Billi et al, 2019b).

Then dust storms cut down the UV radiation giving additional protection to any spores. Native Martian life might be adapted to spread in dust storms, as this may be the best time to spread. Dust could travel hundreds of miles per day during a dust storm.

Since Curiosity is not solar powered, it was able to operate during dust storms and gave a direct observation of UV from the surface during the 2018 global dust storm. Curiosity found that the UV radiation fell by 97% and remained low for several weeks (Smith, 2019).

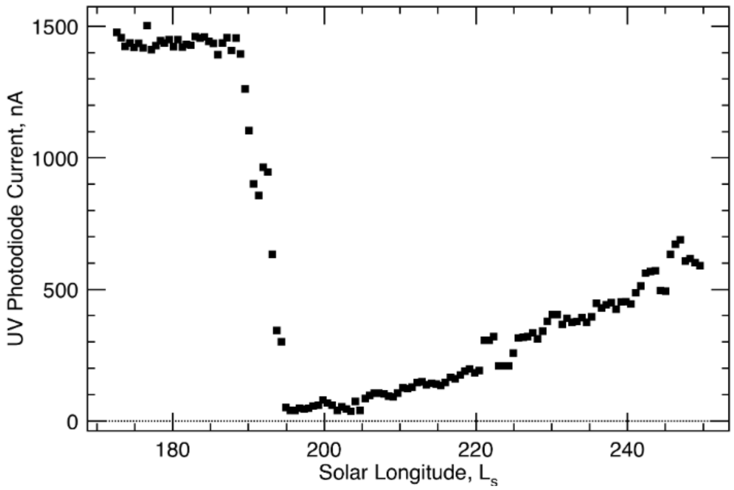


Figure 30: UV measurements by upward pointing photodiodes on the REMS instrument suite on Curiosity. At the height of the 2018 dust storm, the UV fell by 97% at the onset of the dust storm.

Simultaneous with these measurements, Curiosity's mastcam used direct imaging to measure an increase in dust optical depth at 880 nm of τ =8.5, which works out at 0.02% of normal light levels (Figure 5 of Smith, 2019)

These occasional global dust storms can cover much of the planet (Shirley, 2015). They typically start in the south, in the southern spring or summer, encircle the planet in southern latitudes then extend north across the equator. These could transport spores to the location of the rover from almost anywhere on Mars.

Native Martian propagules of up to half a millimeter in diameter (including spore aggregates and hyphal fragments) could travel long distances with repeated bounces (saltation) - if they can withstand the impacts of the bounces

The sand dunes have typical grain sizes of 0.5 mm, or 500 microns. The Martian winds, though far to weak in the thin atmosphere to lift an autumn leaf, can pick up a grain of 500 microns size in bouncing motion, saltation, with each bounce in a strong wind taking the grain a distance of order a few meters and lifting it to a height of order a few 10s of cms (Kok, 2010:fig.3b). This refines the calculations of which (Almeida et al, 2020) which gave larger estimates but didn't include splashing of the surface particles by the impacting saltating particles.

Typically on Earth the fluid threshold to initiate saltation is only slightly more than the impact threshold to keep it going (ratio 0.82 for loose sand). However on Mars the low gravity and lower vertical drag leads the particles to travel higher and longer.

Because of this effect, the impact threshold on Mars is approximately an order of magnitude lower than the fluid threshold (Kok, 2010:fig.1). This means that once a particle is detached from the surface due to the fluid threshold, perhaps in a local gust of wind, it will then continue to bounce across the dunes until the wind speed drops to below the much lower impact threshold.

As an example, saltation occurs in Proctor crater with wind speeds at only a third of the fluid threshold, however the instantaneous wind speed will occasionally exceed the fluid threshold and then the dust grains will keep going until the wind speed falls to below the impact threshold (Kok, 2010:4).

A propagule of 500 microns, typical in size for saltation on Mars(Kok, 2010:fig.3b) can contain many spores. At a maximal packing density of $\frac{\pi}{3\sqrt{2}} \approx 0.74048$, which is the densest packing density possible for congruent spheres (Hales, 1998) (Hales et al, 2017), a single grain of 500 microns diameter can contain $\frac{\pi}{3\sqrt{2}} \times \frac{4}{3}\pi \times 250^3 = \frac{\pi^2 \times \sqrt{2} \times 250^3}{9} =$ 24. 2 million spherical spores or microbes in dormant state at 1 micron diameter.

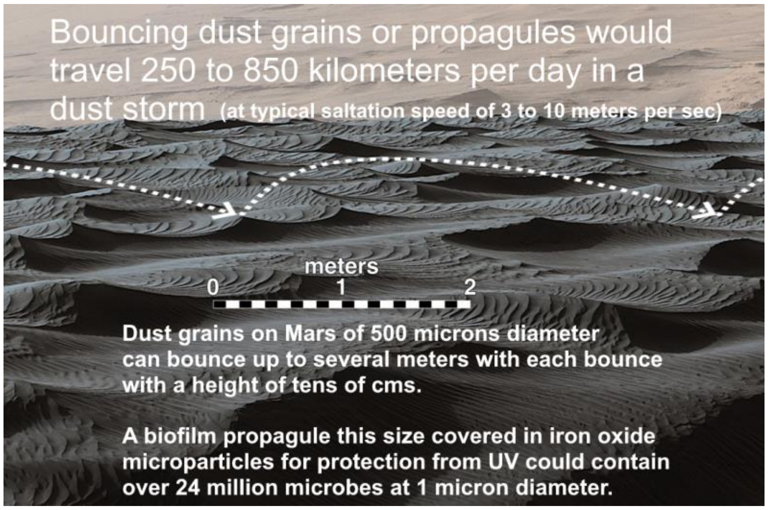


Figure 32: Bouncing dust grains or propagules would travel 250 to 850 kilometers per day in a dust storm (at typical saltation speed of 3 to 10 meters per sec).

Dust grains on Mars of 500 microns diameter can bounce up to several meters with each bounce with a height of tens of cms. A biofilm propagule this size covered in iron oxide microparticles for protection from UV could contain over 24 million microbes at 1 micron diameter.

Artist's impression of a typical bounce based on figure 2b from (Almeida et al, 2020) superimposed on photograph of the top of a large sand dune taken by Curiosity on December 23, 2015 (NASA, 2016rssys)

These saltation simulations assume the propagule has the same density as the Martian sand, or 3.2 kg / m^3. A Martian biofilm propagule at the same size of 500 microns diameter would be likely to have a third of the density and so could be carried further in the winds. However, covered in iron oxides to protect it from the UV radiation, It might look similar to a dust grain to our rovers on Mars.

Propagules transported in this way would need to be able to resist the mechanical stress of saltation, which can damage the viability of the spores. In one Mars simulation experiment, with spores of b. subtilis, in grains of Icelandic granite to simulate Martian granite, half of the spores were killed in a minute and these spores were completely destroyed (Bak et al., 2019).

However this is for unprotected spores. In Bak et al.'s experiment, there was a long tail of spores that remained viable for days. These may be spores that were protected in cavities in the dust grains (Bak et al., 2019).

The number of viable spores was reduced by more than three orders of magnitude after 5 days of the experiment (Bak et al., 2019:4).

However at a saltation particle speed of 3 to 10 meters per second for a particle of 400 microns in diameter (Kok, 2010:fig.3a), a propagule can travel 250 to 850 kilometers a day. So a similar spore on Mars could travel 1,300 - 2150 km in 5 days (rounded to nearest 50 km) before the numbers of viable spores are reduced a thousand-fold.

This suggests that a small proportion of spores of b. subtilis can be transported great distances on Mars in dust storms and remain viable. Also, though b. subtilis is highly resistant to radiation and oxidizing chemicals, only one terrestrial species was tested, and spores on Mars would have evolved to resist Mars saltation and might survive for more than 5 days in these conditions.

Martian spores could evolve extra protection such as a shell of agglutinated iron oxide particles to protect themselves from UV

Terrestrial spores are already adapted for protection from UV which they encounter especially high in Earth's atmosphere and in mountainous and polar regions. Spores are protected from reactive chemicals by multiple coat and crust layers (Cortesão et al, 2019).. This makes them far more resistant to oxidizing agents, bactericidal agents, chlorites, hypochlorites etc than vegetative cells (Sella et al, 2014).

Spores are also more UV resistant than vegetative cells. Some terrestrial spores can withstand many hours of UV radiation on Mars, including one strain still viable after 28 hours of simulated direct UV radiation in Mars simulation surface conditions (Galletta et al, 2010).

If there is wind dispersed life on Mars it would be likely to have evolved similar levels of protection from UV or be even more hardy than these terrestrial spores. Adapted to such extreme conditions, Martian spores could have more layers of protection than terrestrial spores.

Martian spores, like terrestrial microbial spores, could be protected from UV in cracks in the dust - but might they evolve to take that strategy a step further and somehow utilize the UV radiation blocking properties of the dust more directly?

Some agglutinated foraminifera accumulate grains to protect themselves, but external rather than internal to the cell wall. Some foraminifera also form external cysts at other stages in their life cycle, for feeding, prior to reproduction, during growth phases and protection from mechanical and chemical disturbances.

Foraminifera can build agglutinated external sediment cysts quickly, within hours, fixed by organic material produced by the foraminifera.

This suggests that a Martian microbe could also potentially build an agglutinated external cyst rapidly if the conditions require it (Heinz et al, 2005).

[Figure needs permission]

Figure 31 Cyst (left) and free species (right) of Ammonia beccariiin a Petri dish (Atlantic Coast, YeuIsland, intertidal zone). From plate 1 of (Heinz et al, 2005).

Perhaps (a suggestion) native microbial Martian life might evolve the ability to cover itself with a layer of fine iron oxide dust cemented together with organics to protect itself from the UV and the reactive chemicals during wind dispersal in dust storms. This could be in addition to the normal cyst which is formed by thickening a cell wall, or it could be a replacement for it.

The suggestion is that in the unusual conditions on Mars with the UV, the reactive chemicals in the dust and the abundant iron oxide dust, perhaps a Martian microbe might develop an external agglutinated cyst from the dust grains and then for further protection develop an endospore within it. In this way it could combine the protection of endospore and exospore in the same resting state.

Then Martian life might develop colonial ways of surviving in dust storms. The particles in storms are large enough so that the winds could transport larger clusters or aggregates of microbes (Board, 2015 :12). Martian microbial life could have evolved larger bacterial fruiting bodies similarly to those of the myxobacteria with some bacteria altruistically developing into non reproductive cells to protect the spores inside (Muñoz-Dorado et al, 2016).

Multicellular life could reproduce by fragmentation in dust storms, similarly to fungi reproducing through hyphal fragments or red macroalgae (rhodophyta) which often propagate using multicellular propagules.

These propagules would be fragments of the parent plant, a vegetative multicellular structure, that breaks off from the parent thallus and gives rise to a new individual (Cecere et al, 2011).

Perhaps bacteria that form Martian biofilms could use similar strategies. The evolution could begin with fragments of a biofilm accidentally broken off in winds due to the impact splashes of sand grains bouncing on the dunes (a process known as “saltation”) and the natural movements of the dunes. The bacteria might then evolve strategies to create propagules extended above the surface that then detach in response to the stronger winds in dust storms or dust devils.

Martian life could also use iron oxides from the dust for protection from the impact stresses of the saltation bounces - or it might use chitin - a biomaterial which is extremely hard and also elastic and is found in terrestrial fungi and lichens

Then, perhaps (a suggestion), Martian dust grain sized propagules could evolve to be better able to resist saltation than a terrestrial spore that has never encountered these conditions. It might have a strong outer crust of non-reproductive cells or iron oxide nanoparticles glued together with organic secretions.

The hematite (iron oxide) has a Mohs hardness of 5 to 6.5, similar to steel, compared to 3.5 to 4.5 for siderite (iron carbonate), and 3 for calcite (calcium carbonate) (King, n.d.).

Martian life might have evolved biomaterials with much stronger protective layers than iron oxide. Chitin, or some similar material, is a likely biomaterial to find on Mars if it followed a similar pattern of evolution, as it is an essential component of the cell walls of fungi and the fungal component of lichens (Lenardon et al, 2010). The same material is also used in insect exoskeletons and jaws. Chitin could add to the strength of the outer layer of a propagule of Martian life, such as a fungus.

Chitin has a Mohs hardness of 7 - 7.5 (Zhang et al, 2020) similar to quartz (King, n.d.). Chitin nanofibers have a Young's modulus of elasticity of more than 150 GPa (Vincent et al, 2004), higher than copper or titanium alloy and not far below wrought iron or steel (Engineering ToolBox, 2003).

Saltation is a major challenge for terrestrial spores on Mars. However, on the basis of current knowledge, a small proportion of terrestrial spores could potentially remain viable after they are carried thousands of kilometers imbedded in a crack in a dust particle, and we can't rule out the possibility that returned Martian dust could potentially contain viable spores or larger propagules of Martian life shielded from the UV and resistant to the stress of saltation.

There would be great evolutionary advantages for any Martian life that developed the ability to spread in dust storms perhaps using organics like chitin combined with nanoparticles of iron oxides. Potentially these could be transported from almost anywhere in Mars in the global dust storms that occur every few years.

Potential for spores and other propagules transferred from distant regions of Mars similarly to transfer of spores from the Gobi desert to Japan

If the Viking results were the result of life, they may not indicate life actually growing in the dirt sampled by the landers. They could be explained by spores transported from other parts of Mars.

Even if the brines found by Curiosity are potentially habitable in microhabitats due to biofilms - still many may in actuality be uninhabited. Repeating what Cockell et al said about terrestrial basalt samples (Cockell et al, 2019)

> *Every sample Is a 'microbial island'*

He was referring to the heterogeneity of the habitability of an individual rock sample due to natural features including cracks and cavities. However, in a rather similar way, every sample of the salty brines might also be a microbial island because it needs a biofilm to make it habitable. It might depend on the chance of whether a sufficiently large biofilm propagule has encountered that particular salty brine patch in the sand - especially if they need a biofilm to spread before they become easily inhabitable by single microbes.

Terrestrial life in extreme cold conditions in the McMurdo dry valleys can take thousands of years to colonize a habitat (Fraser et al, 2014). Martian life might also grow slowly, propagating in brines below the usual terrestrial cold limits for life. Martian life might also take millennia to colonize a new microhabitat.

New habitats would form as the sand dunes migrate bringing new materials to the surface (Fisk et al, 2013). In the very hostile conditions on Mars, perhaps potential microhabitats might remain uninhabited not just for thousands but even for millions of years after they first form. In this way some of the brines may be inhabited by biofilms and some not, as happens with endoliths in deserts.

Even so, we can hope to find viable propagules in the dirt, so long as the nearby life does spread through spores or propagules, which could be transported in the Martian dust storms or by the dust devils. There may be many viable spores in the dust for each one that encounters a habitat it can grow in.

Even soils near the cold - arid limits of life have a few thousand cells per gram of desert soil. In one example, direct microscopic cell counts using a fluorescent stain (DTAF) found between 1,400 and 5,700 cells per gram in dry soil in the McMurdo dry valleys in Antarctica. This was in University Valley, so cold and dry that most of the microbes were either dormant or not viable (Goordial et al, 2016). If a little dust from a nearby habitat even with as little as a few thousand cells per gram as this is blown over the surface of Mars there could still be many cells in each gram of surface dirt.

Proposed surface microhabitats on Mars that could achieve higher densities of life and be a source for propagules in the dust – including brines that form rapidly when ice overlays salt at high latitudes, caves that vent to the surface, fumaroles, and fresh water melting around heated grains of dust trapped in ice layers through the solid state greenhouse effect

We have already looked at several ways that life might be possible even in Jezero crater in Could Perseverance's samples from Jezero crater in the equatorial regions of Mars contain viable or well preserved present day life?

These include:

- How Martian life could make perchlorate brines habitable when they only have enough water activity at -70 °C – biofilms retaining water at higher temperatures - chaotropic agents permitting normal life processes at lower temperatures – and novel biochemistry for ultra-low temperatures
- Life could also exploit enhanced humidity in micropores in salt deposits - but these may be rare in Jezero crater
- Melting frosts - and potential for a temperature inversion to trap a near surface cool humid layer at dawn as the air warms, perhaps permitting thin films of water to form briefly
- Experiments with black yeasts, fungi and lichens in Mars simulation conditions suggest life could use the night time humidity directly without liquid water

However if there is Martian life in any of these potential microhabitats it may be in low concentrations, in only some of them, or not easily dislodged by the dust storms.

The dust storms on Earth transport microbes considerable distances, such as from the Gobi desert to Japan (Maki et al, 2019). Some of the more distant habitats on Mars might be more productive of spores than the ones local to the equatorial regions.

The higher latitudes with surface ice may be more habitable than equatorial regions and may be a promising location for microhabitats for present day life. This includes salt lying on ice (Fischer et al., 2014) which can form liquid brines within hours, and could lead to microhabitats throughout the higher latitudes of Mars. This could be an explanation of the droplets seen on the legs of the Phoenix lander which grew, merged, and eventually vanished, believed to have fallen off the leg (Gronstall, 2014)

Figure 33 Possible droplets on the legs of the Phoenix lander - they appeared to merge and sometimes fall off. In this sequence of frames, the rightmost of the two droplets - highlighted in green on this black and white image - grows and seems to do so by taking up the water from its companion to the left, which shrinks (Gronstall, 2014)

In December 2013, Nilton Renno and his team used the Michigan Mars Environmental Chamber (Fischer et al., 2013) to simulate the conditions at the Phoenix landing site. They were able to trigger formation of droplets similar to the ones on the Phoenix lander's legs. In their experiment, salty brines formed within a few tens of minutes when salt overlaid ice (Fischer et al., 2014). The team concluded that suitable conditions for brine droplets may be widespread in the polar regions

Nilton Renno, talking about these droplets, said (Renno, 2014):

> *"This is a small amount of liquid water. But for a bacteria, that would be a huge swimming pool - a little droplet of water is a huge amount of water for a bacteria. So, a small amount of water is enough for you to be able to create conditions for Mars to be habitable today'. And we believe this is possible in the shallow subsurface, and even the surface of the Mars polar region for a few hours per day during the spring."*

There are many other potential sources of liquid water on or near the surface of Mars once we expand the potential origin of a spore to anywhere on Mars. This includes some possibilities even for fresh liquid water.

Fresh water is stable against freezing and boiling over 29% of the surface of Mars, but it is not stable against evaporation because the partial pressure of water vapour in the Martian atmosphere is two orders of magnitude too low (Martinez et al., 2013:2). However fresh water could form temporarily in special conditions, if there is some buffering of the water vapour.

This could happen after rapid melting of ice, faster than the evaporation rate, which may be possible, on: (Martinez et al., 2013:2.1).

> ***"slopes facing the sun, under clear sky and calm wind conditions, at locations with low surface albedo and low soil conductivity"***

Fresh liquid water is also possible through the solid state greenhouse effect at a depth of about 5 cms below the surface of optically transparent ice or snow.

This process can melt a layer 1 mm thick, at surface temperatures on Mars as low as 180 K (-93 C), and remain liquid through to the next day and gradually increase to a depth of centimeters and decimeters (Möhlmann et al, 2009) (Martinez et al., 2013:2.2.2) (Martinez et al., 2013:3.1.2).

This solid state greenhouse effect has been suggested as one possible cause for the southern hemisphere dune streaks in Richardson crater. These form in the debris of the Martian CO_2 geysers in early spring and then extend down slopes at a rate of up to several meters a day, and then fade away in autumn, not unlike the RSLs. (Martinez et al., 2013:3.1.2).

There are two types of these flow-like features. The ones which form in Richardson crater in the southern hemisphere are particularly promising because both the current models involve liquid water in some form and what's more, in the models, these features start off as fresh water trapped under ice (the other possibility is Undercooled Liquid Interfacial water, physisorbed monolayers of unfrozen water on the surface of pebbles and rocks, (Martinez et al., 2013:2.2.1)).

The similar looking Northern hemisphere flow-like features have an alternative dry formation mechanism involving dust and dry ice, and form at (Martinez et al., 2013:3.1.2).

This solid state greenhouse effect should occur not only in the flow-like features, but anywhere on Mars with optically clear snow or ice. This would not be easy to spot from orbit, it's essentially a cryptic habitat. It could also potentially be a widespread habitat as a source for spores and propagules.

There are many other potential surface and near surface habitats that could perhaps be a source for propagules from distant habitats transported in the dust (Martinez et al., 2013).

There could also be warm habitable subsurface caves filled with water or sulfuric acid (Boston, 2010) and these might be connected to the surface. Mars could also have volcanic vents that vent water rich gases (fumaroles). Though no active fumaroles have been detected, there may be ones that were recently active and these could have been a habitat for life in the past and may still be more habitable due to the chemical gradients and other alterations such as leached chemicals and minerals from the basalt (Cockell et al, 2019a).

Another suggestion is that in colder regions of Mars, the humid air from the fumaroles might form ice towers, like the ice fumaroles on mount Erebus in Antarctica. These ice towers would hide the thermal signature of the fumarole and would be at a temperature of only a few degrees above the surrounding surface.

The proposed ice fumarole habitats for life are in dark caves beneath the towers. The terrestrial analogue caves are dark and most microbes use chemical redox gradients for energy. With terrestrial fumaroles, the air inside has 80% to 100% humidity. Hoffmann has suggested searching for these ice towers on Mars. The suggestion is to look for circular hot spots a few degrees warmer than the surroundings and up to 100 meters in diameter. In the low Martian gravity these towers could be up to 30 meters high. The terrestrial towers often collapse and then reform over a timescale of decades (Cousins et al, 2011), (Hoffmann et al, 2003).

Searching for distant inhabited habitats on Mars through presence or absence of spores in the dust

For a very rough estimate to get started, let's suppose that the Martian dust storms achieve a thorough mixing of the surface layers of dust over most of the surface of Mars.

Let’s suppose uniform mixing over the surface of Mars, which has a total surface area of 114 million square kilometers (NASA, n.d. mbtn).

To achieve a few spores per gram of dust in the dust storms with uniform mixing requires about 114,000 square kilometers of distant habitat similar in cell counts to the University valley soils of between

To achieve one spores per gram of dust after uniform mixing requires

- 100,000 square kilometers of distant habitat similar in cell counts to the University valley soils of between 1,400 and 5,700 cells per gram (Goordial et al, 2016).
- Between 1,140 and 114,000 square kilometers for RSLs based on measurements of a Mars analogue of the RSLs, an Antarctic dry valley water track, found of the order of 1,000 to 100,000 cells per gram by direct visual count, again using fluorescence based staining with DTAF (Chan-Yam et al, 2019: Fig. 4).

Some of these proposed habitats could be more habitable than the RSLs, especially if made more habitable through biofilms.

- less than a tenth of a square kilometer of the Martian surface, if any areas reach a billion cells per square kilometers - exposed to the dust storms and after uniform mixing.

More productive distant habitats, nearby habitats or larger areas of habitat might lead to larger cell counts.

If there are enough viable cells in the dust, these might be enough to explain the Viking measurements without the need for any nearby habitat.

These are just ballpark figures and a crude estimate. The mixing would surely be non uniform.

- Cell counts depend on the direction of the winds and distance of the habitat, as for Earth (Fisk et al, 2013).
- spore counts might vary seasonally as for Earth (Fisk et al, 2013)
- also differ for each dust storm depending on where the dust comes from.

However it seems not impossible that distant habitats on Mars could be sources of viable as well as non viable spores in the dust globally, as for Earth (Fisk et al, 2013).

A dust sample could also help with non detection of life on Mars. If there we don't detect any spores or propagules in a sample of a few grams of the dust or the surface soil, this might be useful to provide preliminary limits on the habitability of the surface dusts and salts on Mars.

Non detection of spores in the dust wouldn't be enough to prove that there is no life in these surface layers anywhere on Mars.

However, non detection can give preliminary data. Some possible reasons for non detection could be:

- any inhabited area is small or far away or both
- propagules are easily destroyed, for instance by saltation or UV, or the reactive chlorites and hydrogen peroxide
- any extant life or protolife exposed to the surface is at an early stage of development and doesn't yet have viable spores
- extant life is in habitats rarely exposed to the winds e.g. beneath the surface of rocks or at the poles just below the surface of the ice.
- The winds for the dust storms didn't blow spores in the direction of the sample collector (even if the habitat is local to Jezero crater)
- Spores form rarely, seasonally or even synchronized on multi-year periods

We couldn't deduce which of these or other hypotheses is the reason, immediately from non detection of spores in the dust, together with study of the dust itself.

However, it would be a useful first step narrowing down possibilities, towards designing future experiments to find out whether there are any inhabited surface habitats on Mars and how extensive they are, if they exist.

Could local RSL's be habitable and a source of wind dispersed microbial spores? Both dry and wet mechanisms leave unanswered questions - may be a combination of both or some wet and some dry

The Recurring Slope Lineae (RSL's) remain a leading candidate for potentially habitable brines. Some may be explained as dry granular flows but this explanation has some difficulties and liquid water is still likely to play a role. They are characterized in planetary discussions as *“an Uncertain Region that is to be treated as a Special Region until proven”* (Rettberg et al, 2016).

Many RSLs are known in the equatorial regions. No papers were found reporting RSLs from Jezero crater, but they can be hard to spot from orbit.

In the case of Gale Crater, one site with RSLs was discovered after the rover landed. It was close enough for Curiosity to reach, but it is not sufficiently sterilized to study them close up, leading to debates about how close is safe to go (JPL, 2016).

Perseverance is also not sufficiently sterilized to approach an RSL. Could spores be transported to Perseverance’s location from a nearby RSL in the winds?

These dark features extend down sun-facing slopes when local temperatures rise above 0 °C in spring, broaden during the summer, and fade away in autumn (McEwen, 2011). Hydrated salts are detected as the features broaden, suggesting that thin layers of liquid brines may flow below the surface (Ojha et al, 2015).

In the wet-formation mechanism, the features themselves are an indirect effect of the presence of shallow subsurface brines. The amounts of water involved are small but not zero in this model. Soil thermal responses limit unbound water in RSLs to 30 grams of water per kilogram of soil (Edwards et al, 2016).

The dry granular flow mechanism arose from a study of RSLs in Eos Chasma. These RSL’s terminate at slopes that match the stopping angle for granular flows of cohesionless dust. Even these RSLs still have hydrated salts, and the seasonal patterns still suggest some role for water in their formation though the research ruled out substantial quantities of crust-forming evaporitic salt deposits (Dundas et al, 2017).

Difficulties with the dry granular flow mechanism include the seasonality and especially the rapid fading away of streaks at the end of the season, Dust streaks usually fade over decades. Also there is no explanation in this model of how dust is resupplied year after year.

Resupply is also a major difficulty for the wet formation mechanism. RSLs in the Valles Marineris seem to traverse bedrock rather than the regolith usual for other RSLs, and if water is involved in their formation, substantial amounts must be resupplied to sustain lengthening throughout the season (Stillman, cited in David, 2017).

Stillman in 2018, suggests some of these features may be caused by dry granular flow, and others by a wet-dominated mechanism (Stillman, E., 2018:81). So it remains possible that some RSLs are habitable, and if so, perhaps life could get to Perseverance from any nearby RSLs though at present it seems an unlikely scenario.

Another suggestion is that the darkening of the soil is the result of deliquescence of salts in perchlorate and chloride containing soils. Their association with gullies could be the result of earlier flows during a wetter period on Mars leading to precipitation of salts that now darken seasonally. There would then be no need for a salt recharge mechanism, and some salts like calcium perchlorate could deliquesce rapidly in less than 3.5 hours, a similar process to the deliquescence observed by Curiosity but on a larger scale with more of the salts (Heinz et al, 2016).

More distant RSLs could be a source of propagules for the sand dunes, transported by the Martian dust storms from almost anywhere on Mars.

Could Perseverance find recognizable well preserved past life?

The three main possibilities here are

- macrofossils,
- microfossils (which might or might not be associated with organics), and
- biosignatures of past life.

Searches for macrofossils of microbial mats or multicellular life - Knoll criterion and difficulties of recognizing life by its structures

A complex multicellular animal or plant would most likely be easy to recognize, but Martian life may never have developed as far as multicellularity. Even if there is multicellular life now, Jezero crater corresponds to a time on Mars long before development of multicellularity on Earth.

There is a case for Martian multicellularity however, even as early as the deposits in Jezero crater. On Earth, oxygen may have triggered the explosion of terrestrial multicellular life (Parfrey et al, 2011). Curiosity's Chemcam instrument found manganese oxides which suggest that at the time of Gale crater lake, three billion years ago (NASA, 2017), the water was oxygen rich (Lanza et al, 2014).

Present day Mars may also have conditions for oxygen rich brines anywhere on the surface, by taking up oxygen from the atmosphere, a process that happens most easily in cold conditions. Extremely cold brines in polar regions could reach oxygen saturation levels similar to those needed for primitive sponges (Stamenković et al, 2018) as we saw in

- Some Martian brines could be oxygen rich permitting aerobes or even primitive sponges or other forms of multicellularity - Stamenković's oxygen-rich briny seeps model (below)

The case can be argued both ways, that the harsh conditions of early Mars could have slowed down evolution, or that the ionizing radiation and the frequent "snowball Mars" phases, combined with the oxygen rich atmosphere, could have triggered a more rapid evolution on Mars, and possibly even complex multicellular life billions of years before it became common on Earth. So, we shouldn't rule out the possibility of complex life on Mars quite yet.

It's important to realize how limited our exploration has been.

- Two stationary landers, Viking I and II,
- One stationary lander with a rover of limited range, Pathfinder + Sojourner
- Three rovers, Opportunity, Spirit and Curiosity with maximum travel distance of 45.16 km for Opportunity (NASA, 2019merm)
- Perseverance, travel distance over 10 km in 2022 (NASA, 2020wip)

The surface area of Mars is the same as the total land area of Earth. If we explored Earth with the same capabilities looking just for fossils using remotely controlled rovers, landed in deserts perhaps, we probably wouldn't have found any macro fossils of past life yet. To find anything, we would need to know where to go or what to look for.

Detection of early microscopic life on Earth and even macrofossils such as stromatolites is often controversial and requires multiple lines of evidence before it is accepted. Sometimes features in terrestrial geology that were previously accepted as the result of life processes become proven to be abiogenic (Javaux, 2019).

So far there has been no clear evidence of multicellularity on Mars. But we may have found ambiguous evidence.

If there was life in Jezero crater then it is possible that Perseverance finds macrofossil evidence in the form of microbial mats, maybe even stromatolites. Curiosity, Opportunity and Spirit may already have found microbial fossils on Mars.

This is a potential microbial mat or more generally, MISS (microbially induced sedimentary structures) found by the geobiologist Nora Noffk in a careful analysis of Curiosity photos based on her expertise studying the corresponding structures on Earth:

[Figure needs permission]

Figure 34 the geobiologist Nora Noffke says these look like trace fossils of microbial mats. (Bontemps, 2015) (Nofke, 2015).

They resemble structures associated with microbes on Earth as the terrestrial structures change with time as the mats after growing, dry up, and crack and then grow again. Indeed they resemble a MISS that she discovered in Australia, from 3.48 billion years ago (Nofke et al, 2013).

These structures could be the result of other processes, for instance some of them could be the result of erosion by the wind, by salt, or water, but interviewed by NASA Astrobiology Magazine she says (Bontemps, 2015)

> *"But if the Martian structures aren't of biological origin, then the similarities in morphology, but also in distribution patterns with regards to MISS on Earth would be an extraordinary coincidence."*

She says (Bontemps, 2015)

> "All I can say is, here's my hypothesis and here's all the evidence that I have, although I do think that this evidence is a lot."

She suggests a four step process of confirmation similar to the methods used to confirm terrestrial microbial mats (Nofke, 2015:21-2).

1. **Detection** of past aquatic environments suitable for microbial mats and where they could also be preserved to the present. The waves and currents need to be not too weak or too strong, which she refers to as the "hydraulic window for mat development". She suggests searching for rock bed thicknesses between 2 and 20 cm with evidence of currents, e.g. ripple marks of less than 12 cm crest to crest distance, and for exposed rock surfaces not littered by loose sediment, suggesting recent wind erosion. When a structure is found that resembles a fossil mat, then we move to the next stage.
2. **Identification** - using many photographs taken in different lighting conditions and from all sides to bring out the 3D structure of the surface. Then close up images, and measurement of the thicknesses of potential features (e.g. mat chips and roll up structures).
3. **Confirmation** - to look for microstructures such as aligned grains and seven other mat layer textures. Then to look for minerals that may be due to microbial mineral activity, distributed in ways that may mirror ancient textures of the mats themselves, and section the structures and examine the mineralogy of sections.
4. **Differentiation**, to differentiate from other structures formed in the same conditions that aren't microbial mats.

Differentiation won't be easy. Terrestrial sedimentary structures are well understood ,but for Mars this would require better understanding of how Martian sediments are deposited and altered, and so is something that can only be accomplished in the future. She puts it like this (Nofke, 2015:22).:

Because much of Mars' early history and the former depositional and diagenetic processes are still unknown, this last step clearly can only be ac-complished in the future

This is an example of a striking fossil of early life from Earth which is now confirmed (a stromatolite not a microbial mat):

[Figure needs permission]

Figure 35: These unusual cone shaped structures are now known to be very early stromatolites from 3.4 billion years ago. (Allwood, 2009)

However, it took a great deal of work and evidence, particularly the evidence of organics caught up in the material of the stromatolite fossil itself, before they were accepted as such.

There are many suggestions for Martian fossils from enthusiasts after examining the photographs, and perhaps some of them are indeed fossils (Davidson, 2004).

However, as for Nofke's potential microbial mats, interpreting them needs caution, until we understand the physical processes on Mars much better. We have to use the Knoll criterion named after Andrew Knoll who is on the Curiosity mission science team (Knoll, 2013).

Andy explains how the "Knoll Criterion" came about like this: whenever scientists pick metrics to identify life that are based on the life we find on Earth, some hopeful people insist that life may be different on another planet, and so those metrics may not apply. However, Andy counters, "modern Mars exploration is geological principles and practices exported to another planet."

So, he inverted the approach to identifying fossils, explaining "we should really understand what chemical and physical patterns can be generated by physical processes alone." Then, using process of elimination, anything that can not be explained by chemistry and physics is likely to be biological, whether on Earth, Mars or elsewhere.

If it can't be explained by chemistry or physics it is likely to be biological. Sadly though, this means that our rovers may be discarding many actual fossils because a genuine fossil might look identical to a feature that can be formed by physical processes, or we might just not know the limits of what physical processes can do on Mars and err in the direction of caution.

Difficulties of recognizing microfossils even with associated organics – example of ALH84001

It's the same situation with microfossils, as astrobiologists found with the minute structures in ALH840

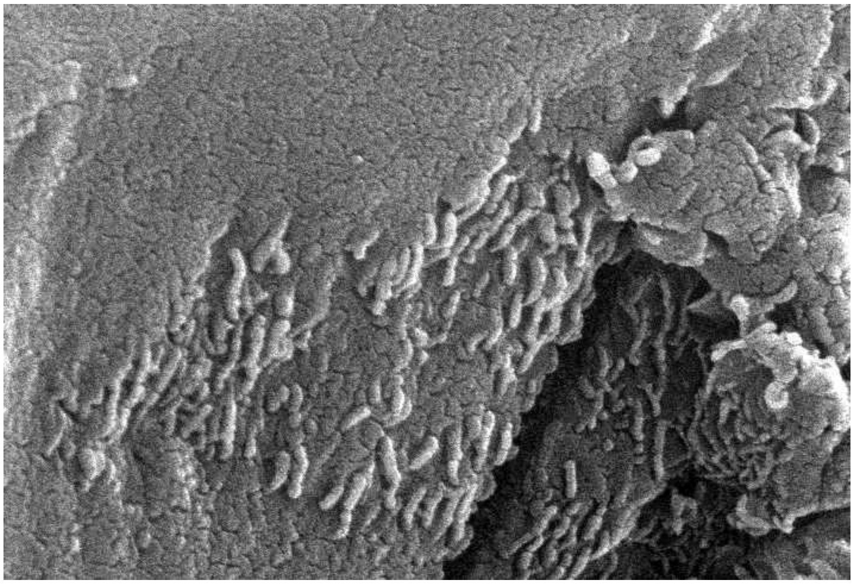


Figure 36: The structures in these photos are between 20 and 100 nm in diameter (Treiman, n.d.), well below the resolution of a diffraction limited optical microscope of 200 nm and the smaller structures are too small for DNA based life.

The jury is still out on whether the structures in ALH84001 were the result of life or not. The strongest argument in favour of life originally was the presence of pure crystals of magnetite which are used by many ancient microbes to sense the direction of up or down through magnetic field dip. However it was soon discovered that similarly pure magnetite can be produced abiotically (Schwandt et al, 2004). This led to questions about some of the supposed ancient terrestrial magnetite biosignatures. Some of these could be abiotic too (Till et al, 2017).

But this doesn't prove that the magnetite crystals **are** abiotic. Just that they could be. There are several other apparent biosignatures in this meteorite, but they **could** all be biotic.

Steven Benner and Paul Davies think the structures in ALH84001 just ***might*** be fossils, because, though too small for terrestrial life, they are just the right size for simpler RNA world cells from an earlier form of life without the large ribosomes and without proteins (Benner et al, 2010:37).

> *"The most frequently cited arguments against McKay's cell-like structures as the remnants of life compared their size to the size of the ribosome, the molecular machine used by terran life to make proteins. The ribosome is approximately 25 nanometers across. This means that the "cells" in Alan Hills 84001 can hold only about four ribosomes - too few ... for a viable organism.*
>
> *"Why should proteins be universally necessary components of life? Could it be that Martian life has no proteins?*

... Life forms in the putative RNA world (by definition) survived without encoded proteins and the ribosomes needed to assemble them. ... If those structures represent a trace of an ancient RNA world on Mars, they would not need to be large enough to accommodate ribosomes. The shapes in meteorite ALH84001 just might be fossil organisms from a Martian "RNA world".

The structures and organics in this meteorite give us the closest we've ever got to something that could be extraterrestrial life, in a real world situation, and actually accessible to study in our laboratories. This controversy made it clear to astrobiologists how difficult it is likely to be to prove that an ancient structure resembling microbes is life.

As Harry McSween put it in 1997 (McSween, 1997)

"this controversy continues to help define strategies and sharpen tools that will be required for a Mars exploration program focused on the search for life."

Since we already have these structures in a Martian meteorite, they may well occur in some of the returned samples. It is possible that more samples will help resolve the question, but they may well leave it as undecided as before.

There are many ways that structures like this can form. For instance there are natural organic amphiphiles (like soap bubbles) that naturally self-assemble into cell-like vesicles when placed in water which might well create structures that look like fossils of life on Mars (Lerman, 2004).

If Martian life has never developed multicellularity, it is not likely that we will find life through structure alone. Even the presence of organics, and even if the structures are associated with organics, it won’t prove that they are life.

Perseverance could detect distinctive biosignatures like chlorophyll and carotene - but only for exceptionally well preserved life

The instruments that Perseverance bring to Mars with Raman spectroscopy and fluorescence spectrometers are able to distinguish aromatic from aliphatic organics. These spectrometers could detect the characteristic signatures of some distinctive biomolecules like chlorophyll and carotene. These are preserved for some time in Martian surface conditions of ionizing radiation and UV. The biosignatures of chlorophyll and carotene are detectable even half a year after being directly exposed to the UV, in samples mixed with regolith (Baqué et al, 2020), so could be detected if freshly exposed, perhaps by the rock abrasion tool (Stromberg et al, 2019). This is a possible method for detecting present day Martian life if it uses the same biomolecules for photosynthesis as terrestrial life

However, apart from such easily recognized organics as chlorophyll, Perseverance's in situ instruments are not reliably able to distinguish biotic from abiotic organics (Hays et al, 2017) (Fox et al, 2017)

It is the same issue with returned samples. Even with all the capabilities of terrestrial labs, detecting biosignatures will not be easy, especially if the samples are degraded or mixed with abiotic organics.

Exceptionally well preserved past life might be recognizable, for instance, degradation products of carotenoids have been detected in 1.54 billion year old rock samples on earth (Fox et al, 2017)

A clear signature of chirality would help, but that's easily erased in past organics through racemization (flipping of a molecule into the opposite sense) as the past conditions are likely to be warm, and the racemic signature is not preserved for long except in very cold conditions. To avoid this racemization, the life would need to encounter cold conditions soon after burial and then remain cold until soon before it is sampled.

Also although modern life is strongly chiral, early life could be chirality indifferent, "ambidextrous". In 2014, Joyce found an RNA enzyme consisting of 83 nucleotides that lets "left handed" L-RNA catalyze the replication of right handed D-RNA and vice versa which would permit replication of RNA world life in a chirality indifferent organism (Joyce, 2007) (Sczepanski, 2014) (Singer, 2014). Also modern life often produces some organics of opposite chirality (Fox et al, 2017).

Also, though this is never known to happen in terrestrial conditions, potentially extraterrestrial processes could produce natural abiotic compounds nearly all of one chirality (enantiopure). In one experiment a small excess of serine near its solid - liquid eutectic point in water was amplified to a 99% excess (Fox et al, 2017). The authors suggest that similar chiral amplifications may have occurred before the origin of terrestrial life (Klussmann et al, 2006) - if so such processes might operate with prebiotic chemistry on Mars.

So, an absence of any chiral signature doesn't need to mean an absence of life. If native Martian life is "ambidextrous" then it might have no chiral signature, but have roughly equal amounts of ordinary and mirror organics.

Then in the other direction, many meteorites have a chiral excess resembling life, sometimes a strong one. This figure shows one of many chiral imbalances from water modified carbonaceous chondrite meteorites, in this case for isovaline which doesn't occur naturally in terrestrial biology so is not likely to be due to contamination:

[Figure needs permission]

Figure 37: Chiral imbalances in meteorites, in this case for isovaline in carbonaceous chondrites. Liquid water may play a role in amplifying the excess, since the Type 1 meteorites are most altered by water and the Type 3 ones are least altered.

[From: Fig. 11 Enantiomeric excesses (in percent) of isovaline measured in CI, CM, and CR carbonaceous chondrites correlate with the degree of aqueous alteration of the samples" (Burton et al, 2012:5468)]

Another study found large chiral excesses of sugars in the Murchison meteorite, including a D excess of the rare sugar Arabinonic acid which typically has an L excess in soil (Cooper et al, 2016).

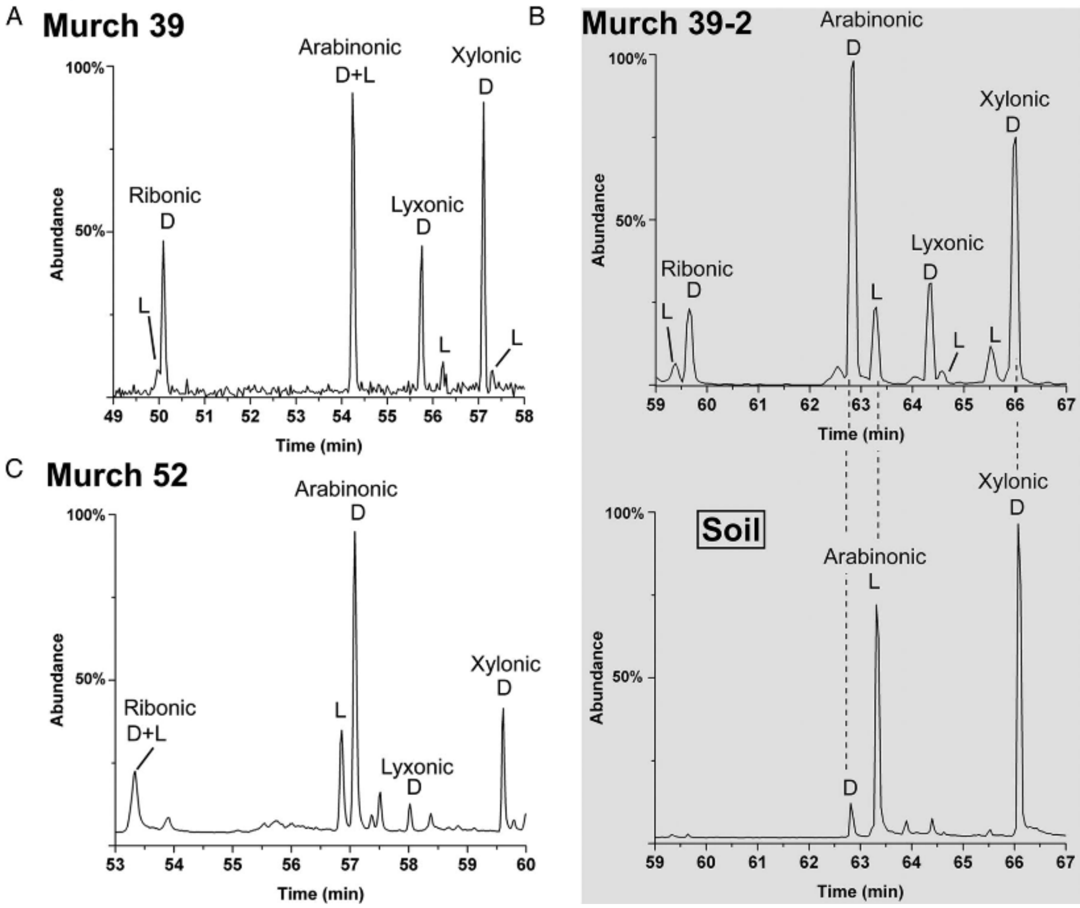


Figure 38: chiral imbalances in five carbon sugar molecules in the Murchison meteorite, the imbalance favours arabinonic D, so is in the opposite direction from soil samples.

[From: Figure 4, The 5C sugar acid enantiomer analysis of the Murchison meteorite (Cooper et al, 2016)]

Combine this with deracemization and mixture of any organics of past life with the large amount of meteorite infall organics expected on Mars (Goetz et al, 2016:247) (Frantseva et al, 2018), a chiral excess in a Martian sample due to past life is likely to be weak and not by itself a particularly clear biosignature.

The same is true for the carbon 12 / 13 ratio. Carbon 12 is preferentially taken up by biological processes through kinetic fractionation. Carbon 13 is also stable but incurs more energy costs and so, a C12 excess is a possible signal for life.

However we already have a Martian meteorite with a C12/13 ratio resembling life, the Tissint meteorite, and this was not considered conclusive despite multiple apparent biosignatures (Lin et al, 2014). The problem is that ratios resembling biotic processes are produced naturally, for instance in hydrothermal vents (Westall et al, 2015:1006) (McDermott et al, 2015).

There are other biosignatures to look out for, for instance, a large excess of an amino acid that on Earth anyway is typically only found as the result of life processes. Another potential biosignature is the relative abundance of the more complex amino acids compared with the simplest one, glycine. Abiotic samples normally have a much higher abundance of the simpler and easiest to synthesize molecules. An abundance of more complex amino acids, would suggest the organics are a result of life rather than abiotic chemistry (Creamer et al, 2017:1331).

There are many possible signatures to look out for. However, definite proof of past life is likely to take a while and require relatively good quality undegraded samples and the use of multiple biosignatures (Westall et al, 2015).

If there is a significant possibility of such exceptionally well preserved past organics, or past life, then that's a reason to hold back from sterilizing the returned sample, if possible.

Modern miniaturized instruments designed to detect life in situ on Mars - could also be used to examine returned samples in an orbital telerobotic laboratory

There are many astrobiological instruments we can send to Mars. These in situ instruments can also be of interest for studying a sample returned to an orbital laboratory in the Earth Moon system, which we look at in more detail in the section below: **Recommendation** to return a sample for teleoperated 'in situ' study above Geosynchronous Equatorial Orbit (GEO)

To date, the only life detection instruments sent to Mars were the instruments on board the two Viking landers in the 1970s. Since then a couple of life detection instruments got close to being accepted on the manifest of ExoMars but sadly never got into the final mission (now renamed as the Rosalind Franklin rover). Many more are at an advanced stage and could be flight ready soon.

If some time in the near future there is a call for instruments to send to Mars to search for life in situ, there will be many responses from astrobiologists. The new instruments are low mass, have low power consumption, and could be sent in a suite of many astrobiological instruments in one unit.

Two of these instruments were nearly sent to Mars. UREY (Bada et al, 2008), one of the instruments proposed by astrobiologists, was selected for ExoMars and then descoped when NASA withdrew from the project, which reduced the launch mass. The Life Marker Chip which uses polyclonal antibodies to find life organics (Davila et al, 2010), had a target mass of less than 1 kg (ESA, n.d.LFM), and was also selected for ExoMars and then descoped. Another version of it, LDChip300 was tested in the very dry core of the Atacama desert and was able to detect a previously unknown layer of microbial life at a depth of 2 meters below the surface. It is able to detect proteins and peptides at parts per billion, or at a concentration of between 10,000 and 100,000 cells per millilitre (Parro et al, 2011).

UREY has now been updated as Astrobionibbler, a “lab on a chip” with microfluidic supercritical water extraction, able to detect a single amino acid in a one gram sample. This has a target mass of 2.5 kg (Schirber, 2013) (Noell et al, 2016).

The Viking labeled release experiment was able to detect microbial respiration from a few cells, for instance in Antarctic soils, even when unable to reproduce or form colony forming units (Levin et al, 1981). A proposed update would detect whether the emitted carbon 14 in emitted methane or carbon dioxide depends on the chirality of the organics added to the sample (Anbar et al, 2012).

Another proposed experiment to detect extraterrestrial life would use microbial fuel cells. These can check for redox reactions directly by measuring the electrons and protons they liberate. This is sensitive to small numbers of microbes and has the advantage that it could detect life even if not based on carbon or any form of conventional chemistry we know of (Abrevaya et al, 2010)).

We can also use fluorescence imaging. Aromatic amino acids (incorporating a ring of six carbons) fluoresce when stimulated with deep UV at wavelengths less than 250 nm. Chlorophyll and some other biological organics also autofluoresce. We could also use fluorescent dyes that bond to specific macromolecules such as lipids, proteins and nucleic acids (Hand et al, 2017),

We can also use this autofluorescence to directly search for the activity of swimming microbes (Hand et al, 2017). Then it would be useful to send an off-axis holographic microscope to let the focus be adjusted after the image is taken making it easier to image individual microbes in a liquid medium (Lindensmith et al, 2016), Raman microspectroscopy synchronized with visible light can do a chemical analysis of the microbes directly (Hand et al, 2017), and superresolution optical microscopy can go beyond the usual optical resolution limit of 200 nm to observe nanobacteria (Hand et al, 2017). Then there's the possibility of a miniature variable pressure electron microscope that combines imaging with in situ chemical analysis (Gaskin et al, 2012) and SETG, a complete end to end gene sequencer small enough to hold in the palm of your hand (Mojarro et al, 2016).

A suite of several instruments would be needed as often multiple biosignatures are required simultaneously to detect life with confidence (Westall et al, 2015). This is feasible because of the miniaturization of these instruments. The mass set aside for Moxie, at 17.1 kilograms, the experiment to generate oxygen on Mars (NASA, n.d.MOXIE), would be enough mass for six or seven in situ life detection instruments similar in mass to the Astrobionibbler. Moxie is a useful experiment, also of interest for robotic missions, especially to generate oxygen for fuel on the surface to use for surface operations and return to orbit. But the comparison is interesting, the mass could be found for in situ life detection if that was the priority for a future mission.

In some future astrobiological mission to the surface we can do in situ testing using a suite of instruments such as these. In this way, we will be able to make informed decisions about which samples are of most interest to astrobiology. See

- Several studies by astrobiologists concluded we need capabilities to identify life in situ, for a reasonable chance to resolve central questions of astrobiology

Without these preliminary measurements, it is inevitable that it will be a matter of luck whether the selected samples are of astrobiological significance. We may however be able to increase the odds of returning life.

Sampling recommendations to improve chances of returning present day life, unambiguous past life, and material of astrobiological interest - including air / dust / dirt sampling additions to ESA's Sample Fetch Rover and modifications of Perseverance's caching strategies

There may be ways the mission planners can increase the probability of returning present day life, and also past life in a well preserved state. This could also impact on decisions about whether to sterilize all the samples on the return journey or to return unsterilized samples initially to above GEO. See:

- **Recommendation** to return a sample for teleoperated 'in situ' study above Geosynchronous Equatorial Orbit (GEO)

ESA's next Mars rover, the Rosalind Franklin rover, is scheduled to launch to Mars in 2022, arriving in 2023, and has the ability to drill to a depth of two meters, to find layers more likely to contain well preserved past life (Callaghan, 2020). Sadly, Perseverance is not able to do this.

However, both missions bring new instruments to Mars and new capabilities. Both the ESA rover with its drill and Perseverance itself may make discoveries over the next several years that may help Perseverance to select its rock samples more intelligently, and increase the possibility of returning present day life.

Perseverance is optimized to cache surface rock samples and samples of regolith (NASA, n.d. WISO) (NASA, 2020tesgs). Perseverance is now on Mars and can't be modified.

However, ESA's Sample Return Orbiter for Mars won't launch until the late 2020s, and is still at the concept stage, along with is Sample Fetch Rover, and NASA's Orbiting Sample Container and Mars Ascent Vehicle, so these spacecraft could all potentially still be modified. See:

- Need for legal clarity before launch of ESA's Earth Return Orbiter, Earth Entry Vehicle, and NASA's Mars Ascent Vehicle.

This article recommends Martian dust as an interesting target for both astrobiology and geology. The dust consists of small geological rock samples from random locations on Mars.

A dust sample can help with studies of spore dispersal in dust storms and habitability of the dust, and variation in habitability during dust storms which shield from UV light. A dust sample might even return wind dispersed spores if such exist on Mars.

The original Decadal review in 2012 recommended one dust sample (Board et al., 2012:159) but this was dropped. The Perseverance mission is likely to have some adventitious dust samples especially on the outside of the sample tubes left on the surface for some years before collection (Grady, 2020). However it would be of great interest to preserve a larger sample of dust, and to be able to study how the dust varies during dust storms and seasonally, and to search for spores in the dust.

This article proposes as one way to do this that the ESA fetch rover could take a couple of spare sample tubes and leave them open on the surface during dust storms to capture airfall dust. For a more elaborate proposal, it could take a rotary air pump spore collector adapted to Martian conditions to collect dust into a sample tube. See:

- **Recommendation:** Extra sample of air and airfall dust to search for Martian life, assess forward contamination issues for terrestrial microbes, dust dangers for astronauts, and to return a random sample of wind-eroded rock from distant parts of Mars

Although Jezero crater is of great interest for past life (NASA, n.d. PRLS), Perseverance is not likely to return rocks with past organics preserved as well as the Mars meteorites we already have. Our Martian meteorites are known to come from at least 3 meters below the surface protected from cosmic radiation before they were ejected from Mars (Head et al, 2002).

The organics in Mars meteorites have been controversial despite multiple apparent biosignatures. Astrobiologists warn that if Perseverance returns samples as ambiguous as these meteorites, they are not likely to resolve central questions in the field of astrobiology (Bada et al, 2009).

Surface organics preserved from past life are likely to be too degraded to have recognizable biosignatures. See

- The processes on Mars expected to destroy most surface organics from past life

Also

- Perseverance could detect distinctive biosignatures like chlorophyll and carotene - but only for exceptionally well preserved life

Past life organics would be degraded quickly by surface processes, with a ten-fold reduction of the number of amino acids in the sample for every ~200 million years of exposure to surface levels of ionizing radiation, see calculation in:

- Level of sterilization needed similar to ~100 million years of Martian surface ionizing radiation - and would leave present day life and past life still recognizable - if recognizable without sterilization

Perseverance could however take rock samples with potential for greater astrobiological interest from freshly excavated craters or wind eroded features. We will see that some of those could have very young surface exposure ages. These might potentially be significantly more interesting than the Martian meteorites if they contain relatively undamaged samples of organics from habitable environments of early Mars with the geological context well understood. See:

- **Recommendation:** use of Marscopter and Perseverance to help identify young craters with sharp rims to help sample subsurface organics excavated by meteorites

Also

- Exposure of organics through wind erosion - for samples of less degraded past life

It would also be of great interest to attempt to sample the brines detected by Curiosity. Faster moving sections of sand dunes may also be of astrobiological interest in a search for redox gradients that life in the dunes might use, as the winds mix lower reducing layers into surface oxidising layers (Fisk et al, 2013).

These samples would be of interest for the search for present day life. See:

- **Recommendation:** modify ESA's sample fetch rover to grab a sample of the near surface temporary brine layers from sand dunes - perhaps Perseverance may be able to do this too with its regolith bit

Near certainty of a young crater of 16 to 32 meters in diameter less than 50,000 years old within 90 days travel of the landing site - to sample for past life less damaged by cosmic radiation

In the section Level of sterilization needed similar to ~100 million years of Martian surface ionizing radiation - and would leave present day life and past life still recognizable - if recognizable without sterilization we found that a dose equivalent to 100 million years of surface radiation would be needed to sterilize a hypothetical surface organism ten times more hardy than radiodurans, which would reduce the amino acids three fold. After 200 million years of radiation the amino acids would be reduced ten-fold. Many biosignatures would be much harder to recognize.

The biosignatures are also likely to be mixed with abiotic organics and degraded by other processes such as the surface chemistry.

A young crater a few millions or tens of millions of years old could expose organics with much less deterioration by cosmic radiation than this sterilization dose, and than normal surface samples. Some of the youngest craters on Mars are only a few years or decades old. Mars Reconnaissance orbiter frequently detects new craters from orbit that weren't present in earlier photographs.

It would be useful to find a crater that can excavate to at least the two meters drill depth of the ESA's Rosalin Franklin rover (Callaghan, 2020).

A typical small crater of 16 to 32 meters in diameter can excavate the surface of Mars to more than 2 meters. A study by Daubar et al, of new craters that formed in the last few decades (from before and after images) found that this size of crater excavated the surface to depths of between 2 and 9 meters (based on seven newly formed craters at this size in Figure 4 of Daubar et al, 2014).

This is deep enough to find organics not significantly damaged by cosmic radiation even after three billion years. It may also be able to penetrate below the average depth of impact gardening by meteorites. The fines dominated regolith in Jezero crater is estimated as 2 to 5 meters thick, and the Jezero mafic unit (likely volcanic in origin) has no noticeable regolith (Schuyler et al, 2020).

In the size range 16 to 32 meters the crater rate is about 2.57 craters per square kilometer every ten million years (1.9 + 0.67 for first two rows in table 1 of Hartmann et al, 2017).

Perseverance is expected to travel an average of 200 meters per day (NASA, 2020plpk). So the total number of craters accessible to it in n days is:

$$2.57\,\pi\,(0.2n)^2$$

In 25 days of travel, or 50 days round trip, Perseverance can travel 5 km and access on average 202 craters less than 10 million years old. There is a 64% chance one of those is less than 50,000 years old

If Perseverance spends half the time on direct travel to a destination and the other half of the time on science and diversions to scientifically interesting targets, then in 360 days it can access any site within 90 days travel or 180 days round trip. In 90 days, it can travel 18 km and access 2616 craters in this size range on average.

We can calculate the probability that there is at least one crater of this size younger than a given age x as

$$100\ -100\,(\frac{10\,million\ -\ x}{10\,million})^n$$

where n is the number of craters accessible to Perseverance, in this case n=2616.

We find that there is a 50% chance of finding a crater less than 2,650 years old within 90 days travel, a 2.6% chance of finding one less than 100 years old and a half percent chance of finding one less than 20 years old in this size range.

With this same limit of 90 days travel, or 180 days round trip, there is a 99.96% chance of finding at least one crater in this size range less than 30,000 years old. There is a 99.9998% chance of finding a crater less than 50,000 years old in the size range 16 to 32 meters.

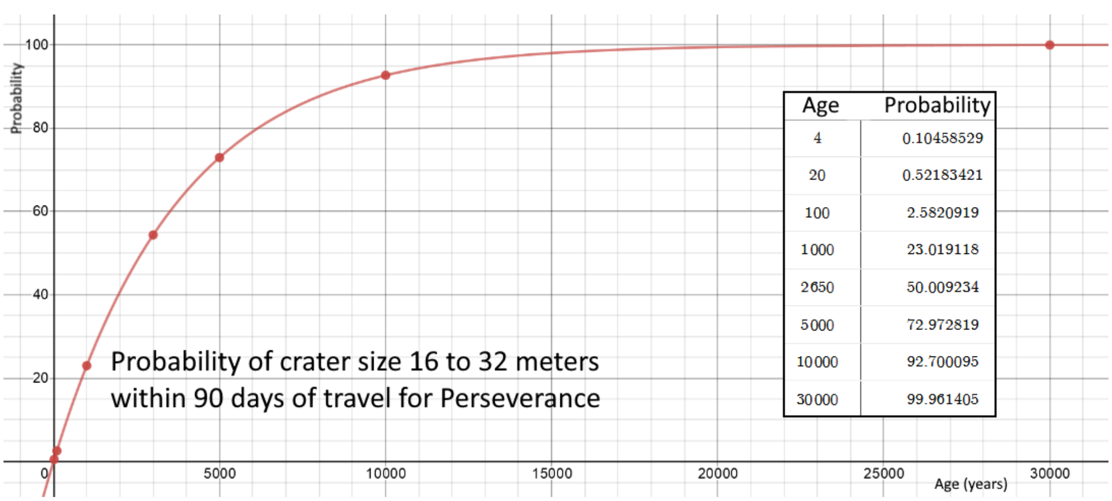


Figure 39: Probability of crater size 16 to 32 meters within 90 days of travel for Perseverance - graph available online from Desmos here

Larger craters are also possible. Hartmann et al say to use table 2 of (Hartmann, 2004) for crater sizes above 32 meters, which adds 0.44 for craters in the range 31.2 to 500 meters to the previous figure of 2.57. There is an overlap due to differing bins in the tables from 31.2 to 32 meters which would contribute less than 0.06 so 2.95 is a reasonable estimate. That would put Perseverance within reach of 3,003 craters in the range 16 to 500 meters that formed in the last 10 million years

Using the 0.44 craters per square kilometer and 18 kilometers travel distance in 90 days, Perseverance is potentially within range of 448 craters of 31.2 to 500 meters that would likely have excavated the surface to well over 5 meters depth (see Figure 4 of Daubar et al, 2014). .

Probability of a new crater within reach of Perseverance forming during the mission to sample newly exposed subsurface organics

The probability of a newly formed crater during the mission is low. Based on our calculation in the last section of 3,0003 craters of 16 meters in diameter or more within 18 kilometers distance, or 90 days travel, there is only one chance in a thousand that a new crater of 16 meters or larger size forms in the next 4 years, within 90 days' travel of Perseverance. However, if we consider craters smaller than 16 meters in diameter, it may be useful to monitor for newly formed craters in the vicinity of the rover.

Even a 4 meter diameter crater can excavate to a depth of half a meter or more (Daubar et al, 2014: Fig. 4), and may be of interest especially if it impacts on a deposit of interest for the search for past life.

Adding craters of 8 - 16 meters, some of which may excavate the surface to over a meter depth, adds an extra 22 craters per km² every ten million years to the previous 2.57 (6 + 16 from third and fourth rows of table 1 of Hartmann et al, 2017) for a total cratering density of 24.95.

Using the same calculation as before, this brings 25396 craters ($24.95\,\pi\,(18)^2$) within reach of 90 days travel (18 km) and a 1% chance that a crater of this size forms in the next 4 years within 90 days travel of Perseverance. That's calculated as before as

$$100 - 100\,(\frac{10\,million - x}{10\,million})^n$$

where x is the desired age range and n the number of craters of this size accessible to Perseverance.

If the search is expanded to a 360 day round trip non-stop, or two years travel, assuming half the time spent on science, there are 101,584 available above 8 meters in diameter and a 4% chance of a crater within reach forming within 4 years.

Adding craters of 4 - 8 meters, likely to excavate to a depth of half a meter, adds an extra 152 (110+42) to the crater count for a total of 155 craters per km². That puts 631083 craters in reach with a 22% chance of one forming in the next 4 years within 180 days travel of Perseverance and a 47% chance of one forming at the same distance in the next decade.

Adding 2 - 4 meters adds an extra 690 (240+450) for a total of 845 craters / km² which puts 3440420 in reach and there is then a 75% chance of a crater forming in the next 4 years at this size. and a 97% chance for the next decade.

In short, there is a significant chance that a new crater of interest forms during the mission. However, Perseverance would most likely need a large detour to visit such a new crater.

This approach could also be of interest for planning future missions. Of the hundreds of newly formed craters discovered from orbit, some are likely to be within other regions of astrobiological interest such as deltas, lake beds and the large northern hemisphere salt flats explored by the Phoenix rover. Even a mission such as ExoMars (ESA, 2019edu), with the ability to drill 2 meters could benefit from the serendipitous exposure of new material accessible without drilling, and with low surface exposure ages.

Dating young craters from orbit through fresh appearance with sharp rim - and absence of interior craterlets or few craterlets

Unless we are lucky enough to spot a crater form during the mission, we need to use other methods to attempt to locate the youngest nearby craters. Dating such young craters from orbit would be hard. We can't use crater counts from orbit, as most would show no other craters within them. But a crater with a sharp rim showing little evidence of erosion is likely to be at most a few tens of millions of years old.

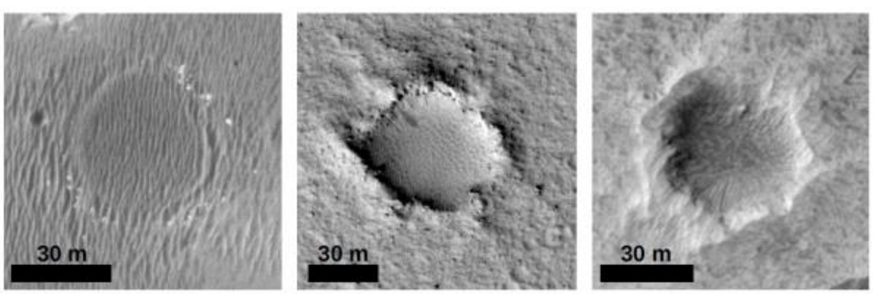


Figure 40: Two styles of degradation of small craters on Mars. The central one is relatively fresh with a sharp crater wall, the one on the left has been filled in and the one on the right has been eroded. (Kite et al, 2017) Images: NASA/JPL/University of Arizona

There are fewer small craters in the HiRISE images than expected, suggesting significant erosion or coverage by wind blown deposits (Shahrzad et al, 2019:2413) so an uneroded uncovered crater may be very young.

Another way to date craters would be through counting small craterlets within them, however sadly numbers of craterlets would be small. Opportunity discovered two small craterlets at 20 cm and 10 cm in diameter but these are rare (NASA, 2005odt).

At one meter size only 1000 craterlets form per square kilometer every 10 million years though this may be undercounted (Hartmann et al, 2017: Figure 2). This makes it only a 5% chance of a one meter diameter craterlet within a 32 meter diameter crater formed in the last 10 million years. The isochrons don't go below 1 meter but if they continue at a similar slope, then we can expect a little under 10,000 at 10 cm size per square kilometer. But there may be far less than that, as many at this size would burn up in the atmosphere and because the craterlets would be easily eroded in dust storms.

Given the large numbers of craters within reach, some of the craters at 16 to 32 meter size might have one or two craterlets within them but this could just be chance and doesn't seem likely to be useful for dating them as older than the other craters. It's also possible that multiple craterlets could be related, due to the break up of a larger meteoroid in the atmosphere. Also since most craters this young would have no craterlets within them this is unlikely to be useful to rule out any cases of much older craters that have an otherwise young appearance.

Recommendation: use of Marscopter and Perseverance to help identify young craters with sharp rims to help sample subsurface organics excavated by meteorites

In future missions, or even this one, the Mars helicopter could perhaps help date floors of young craters within reach of its panoramic camera and its 12+ meters flight elevation (JPL, 2021). The nearest uneroded young crater with the sharpest features is likely to be a good target. Although from the previous section, it's not likely we can use absence craterlets to recognize the youngest craters, if it has many craterlets this would be a contraindication; it is likely older than it looks.

The Marscopter is designed to fly to a height of 5 meters and fly a distance of 300 meters in a single flight (NASA, 2020mhts). It takes 3D high resolution images in flight, using a 13 megapixel colour camera. These are intermediate in resolution between orbital images and the images taken with the rovers at ground level (Golombek et al, 2020).

After the test flights the duration of a single flight was estimated at 600 meters and the height anywhere between 10 meters (Bob Balaram) and 600 to 700 meters (MiMi Aung) and due to the extremely good signal to noise ratio the maximum separation from the rover is at least a kilometer (NASA, 2021mpb). On July 24 2021, it reached an elevation of 12 meters (JPL, 2021)

The Marscopter can see further if it can fly step by step to the top of a local elevation first to add extra height before the launch.

Perseverance can also assist with these age estimates of nearby craters by climbing local elevations and taking high resolution photographs of the landscape below it.

As of May 6, 2022, the marscopter is still functioning though it is facing challenges with the cold of the approaching Martian winter (Agle, 2022)

Exposure of organics through wind erosion - for samples of less degraded past life

Since the delta deposits are of clay materials, they may erode easily. Rapid wind erosion of soft rock could expose ancient organics. Light coloured sedimentary rocks on Mars typically erode at around 100 nm a year, or about a meter every 10 million years, though some areas have slower rates and some faster, up to of the order of 1000 nm a year or one meter every million years (Kite et al, 2017). This could lead to preservation of relatively intact organics. This graph shows possible survival fractions for amino acids in surface samples of soft rocks on Mars currently at a depth of 3 cms:

[Figure needs permission]

> Figure 41: Dashed lines show estimated radiolysis survival for organics currently at 3 cms depth, for organics of atomic mass 117, 200 and 500, while the solid line shows the limit of preservation for isovaline in SiO_2 (Kite et al, 2017).
>
> This graph is based on (Kminek et al, 2006:4). who assume a dose of 200 mGy / yr. Since Curiosity measured a lower 76 mGy / yr (Hassler, 2014). the percentage surviving may well be greater than this

Jezero crater has many wind-formed features. Everywhere is within a few hundred meters of a wind streak or a transverse aeolian feature (miniature sand dune). The wind streaks vary from 35 meters to 3 km and these streaks formed originally within a year (Day et al, 2019:3103).

The delta deposit is 6 km long and around 50 meters deep. However, three kilometers of the deposit has been eroded since formation (Day et al, 2019:3104). Depending on the age of the delta deposit, if it is three billion years old, this averages out at a meter or so of erosion every million years. The rate of erosion has probably fluctuated over time, and it is not known how fast it is eroding today.

Chojnacki et al estimate present day abrasion rates of 0.01 to 0.3 m/Myr for Jezero crater (Chojnacki et al:483). At the lower end it would take 300 million years for the deposit to erode by

3 meters but at the upper end the delta deposit could erode by over three meters in 10 million years.

Based on these erosion figures, it is possible that Perseverance could recover wind eroded material from the delta deposit, with a surface exposure age of only a few tens of millions of years, or even a few million years. If so then there's an opportunity to return relatively pristine past organics, depending on how well preserved they were when buried - and if they are relatively undisturbed by chemical processes since then.

Such recently eroded samples might in principle be so pristine that sterilizing the sample would make a difference to its geological / astrobiological interest - based on a sterilizing dose of over 100 million years worth of X-ray radiation. See Level of sterilization needed similar to ~100 million years of Martian surface ionizing radiation - and would leave present day life and past life still recognizable - if recognizable without sterilization (above)

Recommendation: Extra sample of air and airfall dust to search for Martian life, assess forward contamination issues for terrestrial microbes, dust dangers for astronauts, and to return a random sample of wind-eroded rock from distant parts of Mars

The Decadal review in 2012 recommended one dust sample and two gas samples, along with 4 regolith and 28 rock samples (Board et al., 2012:159). . However the dust and gas samples were dropped from the final design. Grady writes that the sample tubes will almost certainly be covered in dust after 10 years on the Martian surface but it's not scheduled to collect airfall dust (Grady, 2020). It's the same situation for the gas, the only samples will be any of the atmosphere that gets returned in the sample tubes along with the samples.

However, there is a lot of interest in the dust and in the gas. We've seen that dust could carry propagules from distant regions from Mars, see Could Martian life be transported in dust storms or dust devils, and if so, could any of it still be viable when it reaches Perseverance? and the following sections.

One of the biggest knowledge gaps for forward contamination is the potential for transport of terrestrial spores in the dust storms. See: 2015 review: maps can only represent the current incomplete state of knowledge for a specific time – with knowledge gaps on survival of terrestrial life in dust storms and potential for life to survive in habitats hard to detect from orbit - so can't yet be used to identify which areas of Mars are of planetary protection concern in the forwards direction

Also Martian dust contains perchlorates that can be changed through UV radiation into chlorates and chlorites, which are potentially harmful to astronauts. So, a sample of dust will be useful if we send astronauts to Mars in the future, see Dust as one of the greatest inhibitors to nominal operation on the Moon - and likely on Mars too

Finally, a dust sample is a random geological sample of distant parts of Mars that can be eroded by the wind and may be geologically more diverse than the regolith sample Perseverance can access. This is similar to the motivation for SCIM the proposed mission to use aerogel collectors to skim the Martian atmosphere and return micron sized dust particles (Leshing, 2002). Laurie Leshing, one interviewed by Space.com, describes it like this (Tillman, 2014)

> *"Think of it as a microscopic average rock collection from Mars"*

For more about SCIM see Sample return as a valuable technology demo for astrobiology – and proposals to keep the first sample returns simple, a scoop of dirt or skimming the atmosphere to return micron sized dust samples (below)

It would help with all these studies to return a larger and more representative sample of dust and a cleaner dedicated gas sample.

So can anything be done to return more dust?

Our first thought might be that instead of just relying on dust to stick to the outside of the tubes, perhaps the Perseverance rover could leave one of the sample tubes open on the surface for the duration of a Martian dust storm, or several tubes at different stages in the dust storm and also at different times in the Martian year.

Perseverance could leave an open sample tube at one of the sample cache locations while it explores to find more rock samples to add to it (NASA, n.d. WISO).

However, Perseverance's sample collection system seems to be designed with automatic sample capping. It might not be able to leave a sample tube open on the surface (NASA, 2020esgs)

If Perseverance can't do this, the ESA fetch rover could take an extra sample tube or a couple of extra sample tubes, uncapped, and place those on the surface of Mars to collect dust while it fetches the Perseverance samples.

So, how much dust could be captured in a sample tube just through infall?

The deposition rate on Mars is typically 20–45 µm per Earth year (Johnson et al, 2003). The details vary depending on the location on Mars and also vary seasonally (NASA, n.d.monm) with periods of deposition and periods of removal.

For Curiosity's seasonal variations, the dust accumulation rate decreases towards aphelion and the northern summer when the Martian dust devils clean the sensors (through to L_s ~ 300° (NASA, n.d. MSASL)). Dust accumulation increases through to the perihelion and northern winter and dust storm season as dust suspended in the air by the dust storms settles on the sensors (from $L_s \sim 300°$ through to 180°) (Vicente-Retortillo et al, 2018).

For Opportunity, closer to the equator than either Spirit or Curiosity, there were two periods of gradual deposition and removal a year. Meanwhile,Spirit had steady deposition through the colder periods and sudden sharp removal events in the warmer months (Kinch et al, 2015).

Spirit is 14.57°S, Opportunity 1.5°S (NASA, 2004) and Curiosity 4.589°S (NASA, n.d.WiC). Perseverance is 18.45°N (NASA, n.d.WiP)

These three rovers all had times when the dust was cleared by dust devils, but the frequent dust devils would not be able to clear the dust from a sample tube. Indeed, dust devils could deposit dust into a sample tube, so a sample tube would accumulate dust year round.

If it's possible to gather dust at different times in the year, this could help give information about how the dust composition, particle size, and amount of dust deposited varies during the Martian year.

Supposing there is life on Mars and it is wind dispersed, it may do so seasonal. Or perhaps the dust storms themselves could provide a trigger, for instance, the reduced UV levels during dust storms might trigger spore formation, as an optimal time to spread viable spores.

Capturing wind blown dust throughout the year would increase the chance of finding viable spores.

The dust would accumulate in layers in the tube. This could tell us how the size of particles and chemistry of the dust varies, especially at the height of a storm when some of the transported dust may have had little or no exposure to UV. This would be important information to assess dust transport of spores and life. This also increases the chance of finding viable propagules, or spores in the dust.

Another possibility is to send a rotary air pump sampler such as is used to collect microbial samples in terrestrial environments.

[Figure needs permission or another source]

Figure 42: Rotary air sampler used to collect microbial spores in the Wright Valley, McMurdo Dry Valleys, Antarctica, from: Supplementary information for (Archer et al, 2019) Could the ESA fetch rover be equipped with a similar sampler for the Martian dust? If not, it could just use an open sample tube held vertically with a magnet to collect dust.

The tubes or rotary sampler could be placed at a height of just 10 cm above the surface to catch saltating spores traveling in low bounces across the surface. If no spores are found this can begin to provide limits to the amount of life on Mars, at least able to create spores that spread in the dust.

Proposal: magnets could be used to enhance dust collection

Any magnets used in the construction of Perseverance instruments would concentrate the dust and such magnets may provide a good location for a sample tube left temporarily to collect dust during a dust storm, to maximize the amount of dust collected.

Figure 43: MAHLI images of the REMS UV Sensor on sols 36 (left) and 1314 (right) of the MSL mission. The circular patterns are caused by circular magnets around the UV sensors which attract the dust. The magnets help to keep the sensors free of dust.

This shows the result of 1278 days of accumulation of dust, but this includes dust clearing events by dust devils. A vertical uncapped sample tube on Mars would only accumulate and not lose dust so would collect more dust than this.

Vicente-Retortillo et al's measurements of the deposited dust were done by measuring the opacity added by dust accumulated on the sensors themselves.
Source: figure 1 from Vicente-Retortillo et al (2018)

If instead the extra sample tubes are taken to Mars by the ESA Sample Fetch Rover, magnets could be added inside the neck of the sample tubes to attract the dust.

Proposal: to use the sample return capsule as a dust collector – keep it open to the atmosphere before adding the sample tubes

Another alternative would be for the ESA Sample Fetch Rover to leave the entire sample return capsule itself open to the atmosphere for the duration of the surface mission, the capsule that contains the sample tube for the return journey.

This capsule will need to be sealed before placing it in the Mars Ascent Vehicle, and perhaps if it is left open first for a long time, it could retain the accumulated dust and Martian air in the base of the return capsule at the time of sealing. This would be an additional bonus sample with materials from all the dust storms and dust sprites that passed over the container for the duration of the mission.

This might cause problems if it fills with too much dust to leave room for the sample tubes. Perhaps it could be designed with extra depth for the dust and then if necessary, close the container when that depth is reached.

At an expected dust deposition rate of 20–45 μm per Earth year (Johnson et al, 2003), less than 0.05 mm a year, the container wouldn't need to be much larger to allow extra depth for this bonus sample. A few extra mms of depth could allow for this bonus dust sample and allow for off nominal events of more dust than expected – though this proposal would need to be studied carefully to make sure there is no risk of impact on mission objectives.

Proposal: by Jakovsky et al from the 2020 NASA decadal survey to combine a dust sample with a compressed sample of the Martian atmosphere

One of the proposals submitted to the 2020 NASA decadal survey is to could combine a dust sample with a sample of the atmosphere.

Jakovsky et al suggested sampling the Martian atmosphere in a 100 cc container containing 10 liters of the Martian atmosphere compressed 100 fold with a compressor similar to the one mounted on Moxie on Perseverance (Jakovsky et al, 2021).

Alternatively a compressor could even compress the Martian air more than for Moxie, all the way to Earth's atmospheric pressure. This experiment could return enough atmosphere to detect trace amounts of methane and ethane with accuracies of parts per trillion and would also return enough atmosphere for carbon isotope measurements (Jakovsky et al, 2021).

Jakosky et al say that it is not possible to analyze the Martian atmosphere with this sensitivity using gas incidentally collected in Perseverance's sample tubes, because Perseverance doesn't have a getter to remove material outgassed from the walls of the tubes before the sample collection.

So, this proposal adds significantly to the science return for the ESA fetch rover.

Jakosky et al suggest that their experiment could be used to collect dust as well, by running the gas through a filter as it is compressed. The result is a combined dust collection device and atmospheric sample return.

A sample of Martian dust would also help with understanding the atmospheric chemistry.

They propose that large quantities of dust can be returned to Earth for analysis by adding an extra exit valve with a second dust filter to continue collecting dust after the primary gas sample is collected (Jakovsky et al, 2021).

> ***Airborne dust also could be collected with addition of 3 valves and a dust filter [their figure 6]. After gas reservoir is filled and reservoir valves closed, large***

volumes of Mars air would be pumped through filter to collect and trap dust and its valves closed.

This shows how it works (Jakovsky et al, 2021)

a.

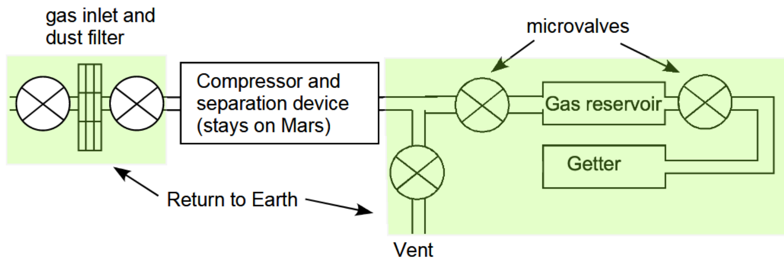


Figure ??

1. To clean the chamber, the outside vent is kept closed, other microvalves kept open, and this vents materials from the walls of the gas reservoir into the getter.

.2. the microvalve to the getter is closed and air from the Martian atmosphere is compressed into the gas reservoir. .
3. finally, the microvalve leading into the gas reservoir is also closed (once it is full) and the vent is opened.

Martian air continues to flow out of the vent – and dust continues to accumulate in the input dust filter

- at this point it works like an air sampler sampling the dust which gets collected in the input dust filter.

(Jakovsky et al, 2021: Fig 6)

This gas / dust sample retrieval experiment could be flown on either the Sample Fetch Rover or the Sample Retrieval lander (Swindle et al, 2021).

Swindle et al also suggest two atmospheric samples a couple of months apart to see how the Krypton / Xenon ratio varies during the year which would help with understanding intriguing anomalies in these gas concentrations in the Martian meteorites. Two samples would also help to measure variations in atmospheric nitrogen isotopes and the isotope ratios for oxygen in CO, CO2, H2O and CO would also help to understand Martian atmospheric photochemistry (Swindle et al, 2021).

Suggestion: In the dust collection phase this dust collector could be switched on for a few minutes several times in each season of the year. It could also be switched on for a short while at the start, in the middle and at the end of a dust storm for the best chance to collect any spores that may be generated seasonally or in response to the storms.

Value to astrobiology of returning the temporary brine layers found by Curiosity at depths of 0 to 15 cms in sand dunes

Perseverance has one regolith bit which it will use to return a single sample of 10 grams of regolith (Boader et al, 2020). (NASA, n.d. WISO) (NASA, 2020tesgs).

For astrobiology, it would be of great interest if it can return additional samples from any sand area, and attempt to collect part of the brine layer detected by Curiosity (Martin-Torres et al, 2015). This could help resolve questions about what Viking detected in the 1970s.

Jezero crater has eleven dune fields, eight of them active, and five migrating with a high sediment flux rate of an average of 11.6 cubic meters per meter per year (table 1 of Chojnacki, 2018).

The most rapidly migrating sand dunes would be of special interest for a sample return, as surface layers of the dunes are superoxygenated but lower layers are reducing, leading to a redox gradient that life can use as a source of energy.

Migrating sand dunes will bring reducing layers to the surface (Fisk et al, 2013). It is a challenge to try to anticipate the best place to look without the necessary ultrasensitive in situ biosignature capabilities, but a search for life might use the regolith bit to sample the sand near the crest of a rapidly migrating sand dune.

Recommendation: modify ESA's sample fetch rover to grab a sample of the near surface temporary brine layers from sand dunes - Perseverance may be able to do this too

Perseverance's samples are sealed with a small plug after collection which gives an opportunity to preserve the conditions present at the time of sampling such as the water content of the sample. (NASA, 2020tesgs).

Perhaps the regolith bit could take these samples? It would be of especial interest if a sample from the sand areas could be taken in the early morning around sunrise, between 4 a.m. and 9 a.m. local time, during the Martian winter through to mid summer, and sealed as soon as possible after collection, so that there is a possibility of gathering a sample of the brine itself at the time of day when brines form in the dune surface layers.

It would also be of interest to gather and seal a sample later in the day to see how much of the water is retained in the soil later in the day by any biofilm.

In this way we could directly test Nilton Renno's hypothesis of biofilms retaining water through to the warmer parts of the day, increasing habitability (Nilton Renno cited in Pires, 2015). Non detection wouldn't refute the hypothesis because of the patchy nature of life in extreme environments but detection would confirm it.

The oxygen content of surface brines is also of interest, since there is a possibility that the brines take up significant amounts of oxygen in Mars surface conditions even at the trace levels present in the Martian atmosphere (Stamenković et al., 2018). See

- Some Martian brines could be oxygen rich permitting aerobes or even primitive sponges or other forms of multicellularity - Stamenković's oxygen-rich briny seeps model (below)

A sealed sample of the Martian brines could also capture any waste gases developed from Martian life in the sample either from outgassing or from microbial activity post collection on the return mission to Earth.

In short, now that Curiosity has discovered the presence of brines in the near subsurface of Mars, it seems an astrobiological priority to find out more about it. We need to know whether it is habitable or not, and whether it has native Martian life or not. This is of astrobiological significance, and answers to those questions may also help us understand potential habitability of other proposed microhabitats.

We might also find other near surface microclimate effects in the sample such as Levin's proposed trapped near surface high humidity layer in the early morning mentioned above (Abe, 2001).

Perseverance is likely to sample the Martian rock varnish. In terrestrial deserts this can be used by cyanobacteria for shelter from UV radiation and may be involved in its formation. It's possible that these samples return viable life if the near surface humidity is higher than expected (Yeager et al, 2019) (Kuhlman et al, 2008).

Although there don't seem to be large salt deposits in the Jezero crater from the preliminary studies, if salt deposits are found it might be of interest to get a sample of those too, to search for microbial life which might take advantage of the enhanced humidity in micropores in the salt (Conley, 2016) (Davies, 2014).

In all these cases then the chance of returning life without sufficiently sensitive detectors to spot the presence of microbes in the dust or salt is low. However if life is ubiquitous then the chance is higher and it also helps to learn more about potential habitats for life for future sample returns and in situ searches.

If Perseverance can't take these samples, perhaps the ESA rover could be adapted like the Viking landers to use its arm to dig a trench into the sand. This could also be useful as an additional sample even if Perseverance does take samples from sand dunes.

Perhaps the arm it uses to pick up the sample tubes could be designed in such a way as to be dual purpose, so that it can also dig into the soil, similarly to the trench dug by Viking.

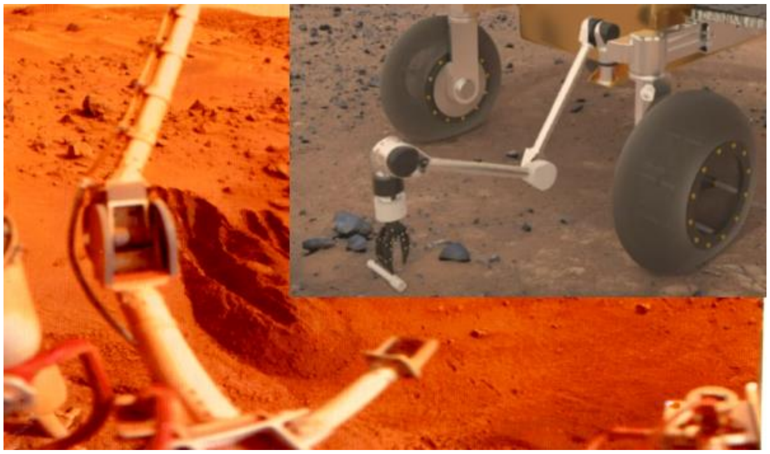


Figure 44: The digging tool, lower center, was used by Viking to scoop up material from the surface soil for the Viking experiments (NASA, 2015).

Inset: Frame at 17 seconds from video of an artist's impression of the ESA fetch rover collecting a sample left on the surface by Perseverance (ESA, 2020)

If this is feasible, then ESA's Sample Fetch Rover could grab a small amount of dirt from the region around the Mars Ascent Vehicle (ESA, 2018), to a depth of five or ten centimeters or so and load it into the return container after adding the samples from Perseverance's cache.

The sample tubes are sealed, so the dirt could just be poured directly into the capsule container on top of them and around them. Another possibility might be to place a horizontal plate on top of the container after inserting the sample tubes, then place a small scoop of dirt in the center of it before adding the enclosing lid.

Figure 45: Concept design for the Orbiting Sample Container - there could be space between the top of the sample tubes and the cover. Perhaps an extra circular plate could be added and then a sample of dirt dug from the nearby soil loaded onto the center of the plate before enclosing the capsule for launch. Analysing this may help resolve questions about the Viking results.

Image combines the NASA graphic (NASA, 2020msros) with an photograph of a small pile of Martian regolith simulant JSC MARS-1A (ZZ2, 2014), and a clipart image of a CD (OpenClipArt, n.d.)

This suggestion to grab some dirt and load it onto the sample container at the end is similar to a suggestion for a minimal sample return mission once made by Chris McKay to just “grab a sample of dirt” (McKay, 2015).

These capabilities are of immediate science value, of some astrobiological interest, and may increase the chance of returning life.

These modifications would be of especial interest if Mars is thought to have a high chance of hosting life

Evidence of past seas with deltas, while modeling suggests habitability of Mars frequently changes in brief episodes of warmer conditions

Mars may have a higher chance of hosting surface or near surface life if there is a way for it to survive on the surface for billions of years. However the constantly changing habitability of Mars is a major challenge for life on Mars.

Although Mars, further from the sun, gets half the sunlight of Earth, its orbit is much more variable than Earth's, through the influence of the other planets. Its axial tilt also varies far more without the stabilizing influence of our Moon.

Currently Mars’s orbit is close to circular and cold all the year round. When its orbit is at its most eccentric, it gets moderately warm every two Earth years when it is closest to the Sun.

Mars would be too cold for seas and lakes and open water surviving long term with a CO_2 atmosphere. Even its salty seas would be solid ice year round if the atmosphere consisted only of CO_2 , even for early Mars at several times Earth’s atmospheric pressure.

However, there is plenty of evidence Mars had liquid water in the early solar system, especially since the discovery of features such as deltas feeding into the ancient oceans.

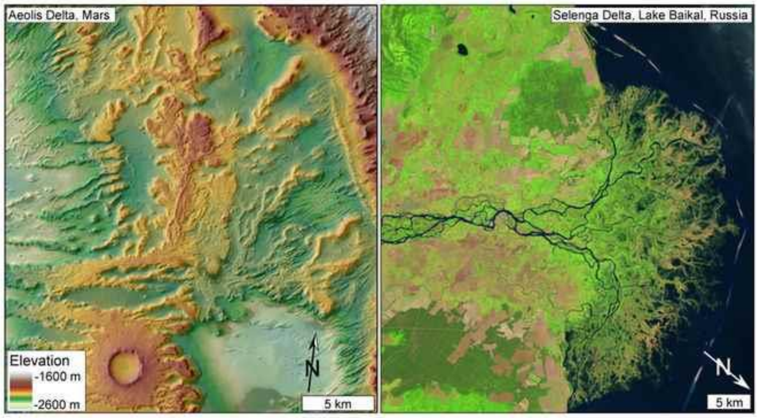


Figure ??

Confirmed ancient delta on Mars, left ,compared with delta on Earth to the right. This is amongst the strongest evidence that Mars had an ocean in the Northern Hemisphere. We can trace a shoreline all the way around the Northern Lowlands, with rivers and deltas flowing into it. How it managed to have an ocean, is still something of a mystery as it would seem to be too far from the sun to be warm enough for this, even with a thick atmosphere.

From: (DiBiase et al, 2013:Fig7)

Later, as Mars lost its thick atmosphere, it continued to be far warmer than expected from a CO_2 only atmosphere. This evidence comes from the lake in Gale Crater. This seems to have been liquid when Mars had at most a few tens of millibars of carbon dioxide – or Curiosity would have spotted much more by way of carbonates (JPL, 2017ncr).

That's even more of a challenge to explain, especially since Gale crater doesn't show any sign of features you'd expect from an ice covered lake such as ice wedges, polygonal features in the landscape which we'd be able to detect.

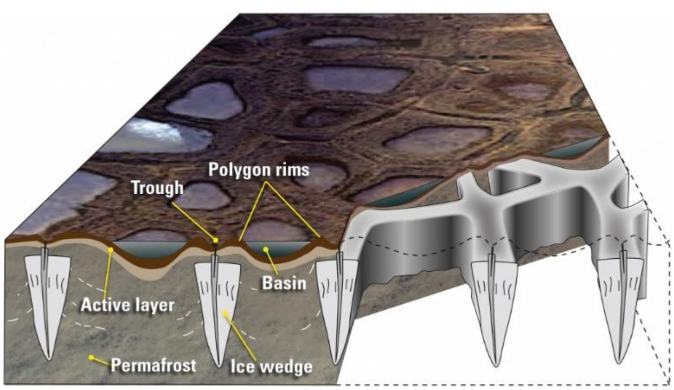


Figure ?? Ice wedges, which form in thawing permafrost and should penetrate the lake bed of a shallow frozen lake on Mars as it thaws and freezes (USFWS, n.d.)

It seems to have been not only liquid, but warm enough to be ice free most of the time. How could that be?

Carl Sagan suggested a mix of greenhouse gases, including hydrogen or ammonia as a way to warm up early Mars or Earth in a letter to Nature in 1977 (Sagan, 1977). However both of those suggestions have drawbacks. Hydrogen is lost rapidly. Ammonia gets decomposed by UV light, and we don't know of a way that Mars could have made large enough quantities of ammonia to keep it warm.

Another solution is sulfur dioxide, another greenhouse gas produced by volcanoes. A study of the sulfur content of our Mars meteorites suggests that early Mars might sometimes have had enough sulfur dioxide to keep it warm (Franz et al, 2014).

Mars has had many episodes of volcanic activity in the past. Though it would normally be far too cold for liquid water, perhaps it warmed up from time to time after those episodes.

Another greenhouse gas Mars could produce from volcanoes is hydrogen sulfide, but this is much less effective as a greenhouse gas, with a third of the temperature change for the same partial pressure. Also, sulfur dioxide's effect is amplified by water vapour, especially in a dense atmosphere, while water vapour reduces the greenhouse effect for hydrogen sulfide (Johnson et al, 2008:table 3)

Another proposal is a thick carbon dioxide atmosphere mixed with a small amount of both hydrogen and methane. Collisions of carbon dioxide with the methane and hydrogen jostle the molecules, temporarily changing their state in a way that makes them more absorbing of some frequencies of light. The resulting mixture has a much greater warming effect than any of these three gases separately (Jacob, 1999:7.3.1)

Most greenhouse gases like sulfur dioxide and water vapour have molecules which are asymmetrical. This lets them interact with electromagnetic radiation through a permanent "dipole moment", a charge separation as a result of their asymmetry, in just the right way to trap photons in the far infrared (Jacob, 1999:7.3.1).

Carbon dioxide is symmetrical (carbon atom in the middle and oxygen atoms to either side in a straight line), but it can also bend and stretch in a way that makes it sometimes asymmetrical which leads to the charge separation needed to absorb photons in the far infrared, ideal for trapping heat (Jacob, 1999:7.3.1).

Hydrogen, and nitrogen consist of only two identical atoms joined together by a single bond and so, can't bend or stretch to become asymmetrical, not in a gas consisting all of the same type of molecule (Jacob, 1999:7.3.1).

However, when a heavier molecule such as nitrogen hits a hydrogen molecule it distorts it momentarily in a way that lets it absorb light over a broad part of the spectrum, so it can absorb heat also, more easily. It does this by giving it a "dipole moment", an uneven charge distribution. The hydrogen in Titan's atmosphere keeps it warmer than it would be otherwise through this process. Titan is especially interesting because it has both a "greenhouse effect" because of the hydrogen mixed with nitrogen, and an "anti greenhouse effect" - because of its smog layer which reflects heat away (McKay et al., 1991).

Similarly, when two nitrogen atoms collide and stick together momentarily to make a temporary "super molecule" which can be asymmetrical and absorb light (Karman et al, 2015). This nitrogen collisions processes has a significant warming effect in the far infrared for Earth, Titan and early Mars.

Methane on its own is a slightly "anti-greenhouse" gas because it absorbs incoming light in the near infrared before it reaches the surface (Wordsworth et al, 2017:section 3) while it is transparent in the far infrared so lets the heat out (Wordsworth et al, 2017). However collisions with CO_2 change its absorption peak to the window region of 250 to 500 waves per centimeter which turns it from an anti-greenhouse gas to a greenhouse gas.

Wordsworth et al showed that when you add in these collision effects to a CO_2 atmosphere with a small amount of hydrogen and methane, the greenhouse effect can be strong enough for liquid water on early Mars (Wordsworth et al, 2017).

This graph shows how the collisions help fill in the gap in the carbon dioxide absorption spectrum.

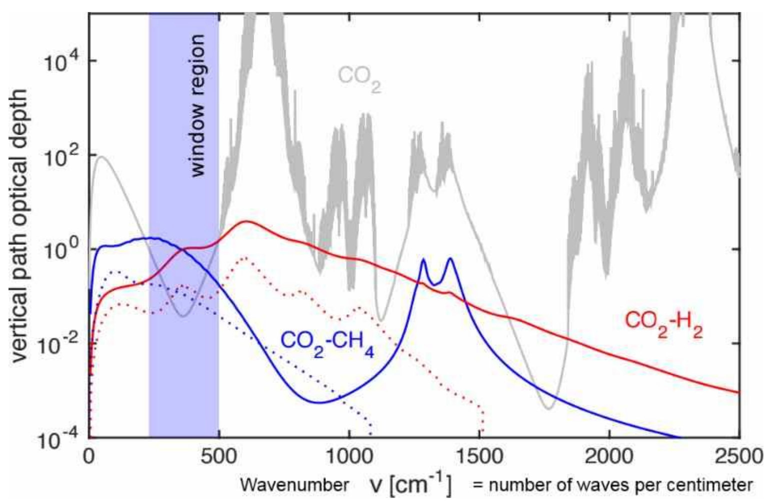


Figure ??

Here the grey curve shows how carbon dioxide traps sunlight. It's got a window in the the infrared, which lets heat out and cools the planet. The red and blue lines show the optical depth for collisions of carbon dioxide with methane and hydrogen which are both strong in the gap. The dotted lines show the effects of collisions of methane and hydrogen with nitrogen. Visible light extends from around wavenumbers 14,000 to 25,000. So this figure shows a region in the far infrared. (Wordsworth et al, 2017:figure 1)

The authors of the paper found that adding 3.5% of hydrogen and 3.5% of methane (molar concentration) to a carbon dioxide atmosphere at 1.5 atmosphere raises the global average surface temperature by a rather surprising 43 °C from 230°C to 273°C.

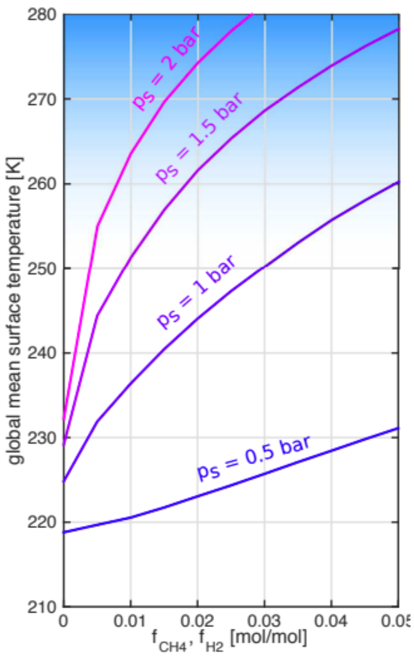


Figure ?? The effect of adding in equal amounts of methane and hydrogen to a CO_2 atmosphere for early Mars at 0.5 bar, 1 bar, 1.5 bar and 2 bar for the total atmospheric pressure (Earth's atmosphere = 1 bar).

From (Wordsworth et al, 2017:figure 2)

That's enough to reach a temperature of zero degrees centigrade, averaged over the Mars surface. Since that's an average of zero, many regions will have temperatures above zero.

This is more than enough to permit liquid water in the form of lakes and seas, given local variations in climate depending on latitude and altitude and other factors.

It's an interesting proposal, but there are quite a few problems with this model which they cover in the discussion section (Wordsworth et al, 2017).

Here is a summary of potential problems Wordsworth et al. found with their own model.

- **Their model can't work for present day Mars as its surface is highly oxidizing today.** The methane and hydrogen would soon be removed from the atmosphere.
- **It could work for the early Mars surface, but its surface would need to be reducing, rather than oxidizing.** (A reducing atmosphere is one with methane or hydrogen etc which removes oxygen and reactive oxidized materials).

Titan as an example of a moon with a reducing atmosphere, with high levels of methane, in a nitrogen atmosphere (Wordsworth et al, 2017)

However, even with an early Mars compatible with their model, with a reducing surface, and reducing atmosphere, they still need:

- **a continuous source of hydrogen to keep the atmosphere hydrogen rich**.

That then is their puzzle, where does the hydrogen come from in a planet with a CO_2 atmosphere?

- **Could the Martian mantle be reducing? This doesn't really work.** Volcanoes normally produce carbon dioxide as the main gas, both on present day Earth and on Mars. However, that depends on whether the mantle is oxidising or reducing. One way the early Mars could have a hydrogen rich atmosphere is if the mantle is also reducing, so that volcanoes produced hydrogen instead of carbon dioxide (Wordsworth et al, 2017)

However, hydrogen is not warming by itself. It needs the carbon dioxide in the atmosphere to collide with.

A reducing mantle solves the hydrogen problem, but then, how does the carbon dioxide get into the atmosphere, to collide with the hydrogen? It is hard to get volcanoes that produce both carbon dioxide and hydrogen in large quantities at the same time.

If the mantle was reducing enough to outgas hydrogen it would tend to retain carbon in the melt, and so wouldn't produce carbon dioxide in any quantity. How can you get enough hydrogen into a carbon dioxide rich atmosphere to act as a greenhouse gas, with these high percentages of 3.5% each of hydrogen and methane (molar concentrations)?

- **Hydrogen could be added to the atmosphere is through serpentization**

Even with a non reducing mantle, with volcanoes producing carbon dioxide as they do on Earth, a planet can still produce hydrogen by the reaction of the rock olivine with water to produce hydrogen. This happens on Earth locally in hydrothermal vents (Wordsworth et al, 2017).

On Earth this happens only over small parts of its surface. However, if 5% of the Mars surface was rich enough in olivine for serpentization it might create enough hydrogen to keep the surface warm enough for liquid water, for as long as it stayed like that. That would work, but that's a large amount of serpentization.

- **Hydrogen and methane could be created by huge meteorite impacts**

The hydrogen and methane could also be created during huge meteorite impacts, through the heating of the atmosphere and reactions caused by the impact itself.

Of all their ideas about how it could happen, perhaps this impact generated hydrogen has most in its favour. Mars had numerous really huge impacts in the early solar system at just the same time that it had its oceans and lakes.

If this is right, the picture is one of episodes of warmth after and during a time of massive impacts, or high levels of serpentization, rather than a continuously warm climate in early Mars. This is similar to the sulfur dioxide and the volcanic eruptions idea.

So, in short, either sulfur dioxide or a small amount of hydrogen and methane could warm up early Mars enough for liquid water and explain the deltas and Curiosity's discoveries about the lake in Gale crater. But we don't have proof yet that either of these things happened. If Mars did have strong greenhouse gases like that, in both cases the effects are likely to have been temporary.

These greenhouse gases might have kept Mars warm enough for liquid water for short periods of time, perhaps after volcanic activity (for the sulfur dioxide) or large meteorite impacts or times of widespread serpentization (for the hydrogen and methane), or maybe both were factors (Wordsworth et al, 2017).

Another idea is that perhaps

- **Mars had only a "part time liquid" sea in every two year orbital cycle, when Mars was closest to the Sun**.

This would let Mars have liquid seas mainly when its orbit was at its most eccentric. The details of its climate would also depend on its tilt, which would change which hemisphere gets warmest when Mars is close to the Sun, and by how much. The tilt varies a lot - Earth's hardly at all. Earth's orbit stays close to circular, for billions of years, while Mars' orbit constantly changes in eccentricity too.

I will summarize the "part time liquid" suggestion based on a summary in a proposal for research by Kite et al from 2014 (Kite et al, 2014)..

We know (high confidence) that Mars supported widespread areas of liquid water in lakes and seas at least episodically. What we don't know are the details of what caused these liquid water conditions.

The early seas always formed in the northern hemisphere, because most of the low lying land is there. So, the best times for liquid water seas might be when Mars is closest to the sun during its northern summer. Mars' axis precesses, just like Earth's axis, sometimes with the northern hemisphere tilted towards the sun when it is closest to the sun and sometimes with the southern hemisphere tilted towards the sun, so the northern oceans would be liquid only at times when the northern hemisphere is tilted to the sun.

The tilt of its axis varies greatly also, sometimes almost vertical, sometimes tilted so far that it is coldest at its equator instead of its poles.

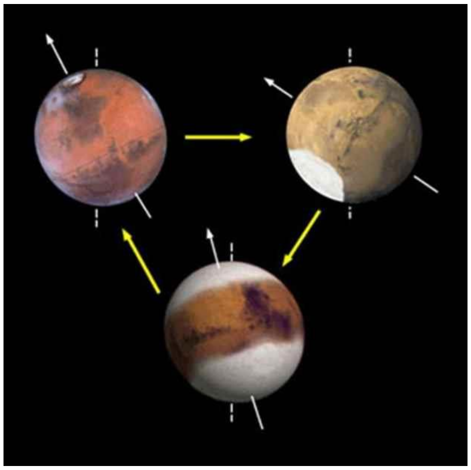


Figure ??

Variations in the tilt of Mars' axis (NASA, 2011cit). At present it is tilted by 25 degrees, similar to Earth. But with no stabilizing Moon, its tilt varies much more than for Earth. Sometimes it tilts so far that its equator is colder than its poles with ice sheets at the equator, as shown at top right. Other times it is almost vertical. When it is almost vertical, ice migrates to its poles creating large ice sheets that trap it there,

When Mars’ axis is almost vertical with the larger ice sheets, it probably never gets warm enough for the ice to melt.

To find out when Mars is most habitable, Kite et al. look at how the tilt of its axis varies. The tilt of the axis of Mars is chaotic in the mathematical sense of "chaos theory". This means you can't predict it exactly over long timescales. This also means we can't retrodict - work out what it must have been in the past based only what we know about Mars' orbit and spin axis in the present.

Perhaps we may get ground data to sort out the past history, but meanwhile, we have no way to retrodict precisely. Instead, we have to try out different possible past histories and compare possibilities to see what sorts of things could have happened in Mars' past.

At present, Mars' axis is tilted by 25 degrees. When the tilt is at least 40 degrees it may get warm enough for water to stay liquid. Early Mars with a thicker atmosphere could have had liquid seas at those times (Kite et al, 2014)..

[Figure needs permission]

Figure ?? Three different random runs showing possible past histories for the chaotic changes of Mars’ axial tilt. (Kite et al, 2014).

They also needed to take account of the eccentricity of its orbit, as it needs to be reasonably eccentric to have liquid water. When they took account the eccentricity of its orbit as well, they got this, showing different runs, with different possible past histories.

[Figure needs permission]

Figure ??

These show three equally likely possible pasts for Mars. The blue peaks show availability of liquid water. The black line shows the atmospheric pressure, and the red line shows the variation in the tilt. (Kite et al, 2014).

During the times shown with liquid water in these diagrams, Mars doesn't have liquid water on its surface all year round. It would still be frozen with no liquid water, and so largely dry, every two years, when furthest from the sun.

They count Mars as continuously habitable if it has liquid water for at least part of every Mars year (two Earth years). In their simulations, the longest continuous reasonably habitable period was 60 thousand Earth years.

Evidence of temporarily more habitable Mars backs up the modelling including evidence from the Zharong rover of substantial amounts of water in Utopia Planitia about 700 million years ago – would life survive in a planet with these frequent changes of habitability or does it go extinct easily, and if so does it re-evolve?

Study of sediments on present day Mars back up these conclusions of temporarily more habitable Mars.

Figure ??

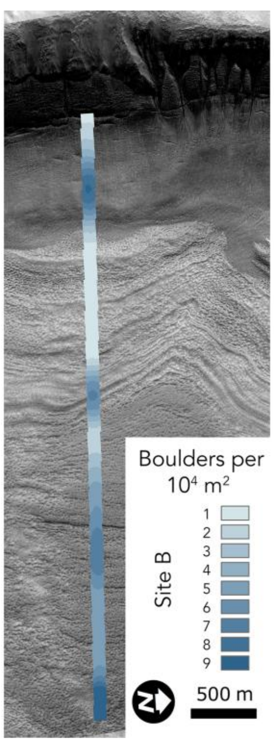
Sedimentary layers in in an unnamed crater in Arabia Terra, Mars which formed due to variation the tilt of Mars' axis over a 100,000 year cycle (Lewis et al, 2008)

Caltech researchers studying these layers in a 3D stereographic projection found evidence of variation in climate with each layer formed over a period of about 100,000 years when conditions were favourable for forming them. Though they can't say in detail how they formed, there's clear evidence that they formed due to variation in the climate of Mars which would also correspond to variations in habitability (Lewis et al, 2008).

This suggests Mars could have changed in habitability frequently, sometimes more habitable, and sometimes less habitable, in two year periods during the Martian year when it was closer or further from the sun, and also over longer timescales because of varying tilt (every 150,000 years or so) and varying orbital eccentricity (over tens of millions of years).

Mars might have been almost completely dry for most of the time, alternating with periods of a few tens of thousands of years when it had liquid water every two years. Impacts also might have made a big difference to habitability, both by creating liquid water and also in the early solar system with the larger impacts, by destroying life. Especially in the very early solar system, when Mars was most habitable, it would have had many large impacts.

Mars is still changing in habitability frequently. An analysis of boulders at the terminal end of glaciers, which survive, buried by dust, found between 2 and 22 boulder bands per glacier with a median of 6. Based on this they estimated that Mars had between 6 and 20 ice ages in the last 300 to 800 million years (Levy et al, 2021). They concluded that the glaciations are triggered by orbital forcing rather than the changes in the tilt of Mars's axis (Levy et al, 2021).



Example of variation in boulder count on the debris apron of a Martian glacier. These may correspond to distinct ice ages on Mars with between 6 and 20 ice ages in a time period of 300 to 800 million years. Extract from Figure 1 of (Levy et al, 2021)

The Chinese Zharong rover landed in the Utopia Planitia region of Mars, the same low lying region of the northern hemisphere explored by Viking 2. The site is flat with no evidence of channels for running water and they found evidence of layers of duricrust, flat plate like layers of hydrated minerals, thick enough to form cliffs. The duricrust is too thick to be explained by water diffusion from the atmosphere, requiring substantial amounts of water (Liu et al, 2022)..

Their conclusion is that the region of Utopia Planitia explored by the rover had substantial layers of briny subsurface liquid during the modern Amazonian period. The region explored by Zhurong could be as young as 700 million years old by crater counts (Liu et al, 2022).

So, could Martian life survive these frequent changes of habitability?

Some life might be able to survive through all the changes, surviving through dormancy or below the ground. That could perhaps lead to Mars having life as evolved as on Earth. The current paper suggests it could even lead to life of greater genomic complexity on Mars than terrestrial life.

- Scenario: evolution on Mars evolves faster than on Earth because of an oxygen rich atmosphere and frequent freeze / thaws of oceans, leading to life of the same genomic complexity as Earth or even greater, and with multicellularity evolving early

Or in another scenario, life frequently goes extinct on Mars and then evolves again from scratch. In this scenario potential habitats might be uninhabited, or may have newly evolved early life.

- Possibility of early discovery of extraterrestrial microbes of no risk to Earth such as pre-Darwinian life as suggested by Weiss – if microbial challenge experiments show they are quickly destroyed by pervasive terrestrial microbes

If parts of Mars are uninhabited, there may still be prebiotic biology of great interest for understanding the origins of life.

- Examples of what we might find on a pre-biotic uncontaminated Mars - microhabitats with autopoetic cells, Ostwald crystals breaking the mirror symmetry of organics, or naked genes, adsorbed on mineral particles with impenetrable membrane caps, but not yet quite life

Suggestion of a self perpetuating “Swansong Gaia” maintaining conditions slightly above minimal habitability for billions of years - as a way for early life to continue through to present day Mars

From the last section it’s clear that Mars frequently varies hugely in habitability. At present it is barely habitable and may have surface microhabitats, as we saw. But there seems no reason to suppose that this is the least habitable it gets.

When it’s at its least habitable – wouldn’t it be at times sterile on the surface, perhaps for hundreds of millions or billions of years?

Jack O’Malley-James introduced the idea of a swansong biosphere (O'Malley-James et al, 2013) (O'Malley-James, 2014) (O'Malley-James et al, 2014). This is what is left of a once thriving biosphere as a planet eventually becomes either too hot or too cold, or in other ways unsuitable for life.

Mars may once have had a swansong biosphere. However, even if at times Mars was very habitable, perhaps in areas like Utopia Planitia as recently as 700 million years (Liu et al, 2022)., how did life continue to the present on a barely habitable planet, which may well sometimes have been totally uninhabitable on the surface? Cockell’s “trajectory 5” seems plausible, where originally Mars was a mix of inhabited and uninhabited habitats and uninhabitable environments (Cockell, 2014).

> *“but as hydrological conditions deteriorated and geochemical turnover became less efficient, life was eventually constrained to such small pockets of existence that it became functionally extinct, and eventually a total extinction occurred. At this point Mars transitioned into a planet harboring only uninhabitable and uninhabited habitats. The extinction event would not have precluded new habitable places becoming available, for example, from obliquity-driven liquid water formation or in impact-induced hydrothermal systems; but a lack of connectivity and sufficient water flow prevented their colonization from the last remaining vestiges of life until eventually, when life became extinct, there was no life to occupy uninhabited habitats that persist, or are transiently produced, to this day.*

Once CO_2 emissions on Mars are so low that the planet is no longer easily habitable, what stops it tipping all the way to sterile, at least briefly?. Why would the planet remain at the point where it is almost totally uninhabitable but not sterile, for billions of years?

One solution is that life can survive at least brief periods of uninhabitable conditions through dormancy. We saw that some microbes of radiodurans could survive dormancy for 2.8 million years on the surface and a hypothetical more radioresistant microbe could survive for 42 million years. See:

- Level of sterilization needed similar to ~100 million years of Martian surface ionizing radiation - and would leave present day life and past life still recognizable - if recognizable without sterilization

This could be enough to survive through shorter periods of uninhabitable surface conditions.

Below the surface life could survive significantly longer and if Mars has caves or subsurface hydrothermal vents which remained habitable over long periods of time life could continue there indefinitely, or in the deep hydrosphere below the cryosphere, and then return to the surface during periods of greater habitability. In that situation the search for life on Mars may be a long one. There may be life in some surface habitats, with many other surface habitats that life hasn't had time to spread to, and most life on Mars remaining deep below the surface.

However, what if the surface never gets totally sterile? Could it be that the present day conditions are as uninhabitable as it ever gets, or that it only briefly gets less habitable than this?

Swansong Gaia hypothesis – that Mars would have far more CO_2 without life – photosynthetic life itself keeps Mars barely habitable by growing and taking CO_2 out of the atmosphere as Mars gets more habitable

The current paper's Swansong Gaia hypothesis is that Mars produces more CO_2 than we would expect from CO_2 levels in the atmosphere in the past and present - and that it was life itself that made Mars almost uninhabitable. Without life Mars might have continued with lakes, rivers, seas even through to the present. But over a very wide range of emission scenarios for Martian volcanoes, life is able to remove nearly all the CO_2 from the atmosphere to keep Mars just short of uninhabitable.

On Earth photosynthetic life (including cyanobacteria, and forams which photosynthesize in symbiosis with unicellular algae) creates a carbon sink which helps keep the planet from getting too warm (Richardson, 2019).

For Mars, cooling down is the opposite of what is needed for life. Photosynthetic life makes the planet less habitable as it removes carbon dioxide from the atmosphere. Martian secondary consumers of the photosynthetic life might return some of the CO_2 to the atmosphere, but not all of it. Some organics and carbonates are likely to accumulate, as they do on Earth, however, on Mars there are no tectonic processes to return them to the atmosphere.

As the levels of greenhouse gases increase in the atmosphere, photosynthetic life would also increase but with a delayed effect, given the time it takes to spread throughout the planet. During the warming phase, life might also add methane and hydrogen, increasing the warming effect by the collision processes mentioned in the section:

- Evidence of past seas with deltas, while modeling suggests habitability of Mars frequently changes in brief episodes of warmer conditions (above)

Eventually however, the spreading photosynthetic life would start to take more and more CO_2 out of the atmosphere as it becomes more abundant. When the volcanoes produce high levels of CO_2 and SO_2 for an extended period of time, this might be enough to keep it habitable, and make up for the CO_2 removed by life. However once they slow down, with no tectonic processes able to return the CO_2 to the atmosphere, eventually life would exhaust the extra pulse of CO_2 from the volcanoes.

As levels fall, the feedback in the other direction to stop removing the CO_2 would be almost instant, that as it gets close to uninhabitable the photosynthetic removals of CO_2 would reduce to a minimum immediately, so that the surface never becomes totally uninhabitable. Or it might become uninhabitable rarely during times of exceptionally low emissions of CO_2 and SO_2 from the volcanoes.

Superimposed on all this are the fluctuations due to the variations of axial tilt of 100,000 years, which could lead to periods of uninhabitability lasting for tens of thousands of years, but life could survive those easily through dormancy. But on longer timescales there would never be times when the surface is uninhabitable for long enough to make surface life extinct everywhere.

The current Martian atmosphere average pressure is remarkably close to the triple point for water of 6.1 millibars. In places it is below the triple point, in Hellas basin by one model it is 12.4 millibars and would boil at 10°C (Schulze-Makuch et al, 2010b). Perhaps this is not a coincidence. Much of the CO_2 is supplied through volcanic CO_2 outgassing, at a gradually diminishing rate, and the amount of outgassing during the more recent cold dry Hesperian period is thought to have been modest. CO_2 removal may be greater than the emissions when there is abundant liquid water on Mars, and this could generate a feedback process that keeps the atmosphere close to the triple point.

There are two main abiotic processes involved in carbon dioxide loss from the Martian atmosphere, loss to interplanetary space through carbon dioxide sputtering in the upper atmosphere, and carbonate formation (Hu et al, 2015).

The rate of loss to interplanetary space through carbon dioxide sputtering would not be much affected by the presence of liquid water.

However, the rate of loss through carbonate formation could be sensitive to the presence of liquid water. If the atmosphere increases above the triple point for water as a result of volcanic outgassing, this leads to liquid water forming on the surface of Mars, either as open springs, rivers and lakes, or as transitory pockets of water or moisture. This increases the rate of carbonate formation through carbon dioxide dissolving in the water and this leads to the atmosphere thinning back to close to the triple point for water of 6.1 millibars average.

This process can occur abiotically. Abiotic photosynthesis could lead to conditions close to the triple point of water, a suggestion already in the literature (Kahn 1985) (Nolan, 2008 page 137) (Haberle et al, 2001).

From thermal infrared spectra, the Martian dust contains 2-5% of carbonates by weight which is enough to sequester several bars of CO_2 atmosphere (Bandfield et al., 2003) (Niles et al., 2013: section 3.1), this is in addition to the carbonates in surface rocks (Niles et al., 2013: sections 3.2, 4), and detected in the subsurface through study of Martian meteorites (Niles et al., 2013: section 2). The chemical composition of the carbonates in the dust may help reveal when it was formed, with Mg and Ca carbonates and oxides common in the present day dry and thin atmosphere, and smectite clays and Ca carbonates if the weathering occurred in wetter conditions with a thicker $CO_2$3 atmosphere (Bandfield et al., 2003).

All of this can be explained by abiotic processes of carbon sequestration such as abiotic photosynthesis, which would also create a feedback cycle that sequesters carbon whenever water is abundant enough. This may be enough by itself to keep the surface of Mars barely habitable for life while avoiding any state where it is totally uninhabitable, through this mineralization carbon buffering process.

The new proposal here is that if Mars does have photosynthetic life it would speed up this natural process. In this proposal, life would set up feedback cycles that limit its own growth. Biotic photosynthesis would work similarly to abiotic photosynthesis, but would be a stronger feedback, and act faster to restore the atmosphere to its barely habitable state after a pulse of volcanic activity or impact of a large comet. It could also keep the surface barely habitable over a wider range of CO_2 supply levels to the atmosphere.

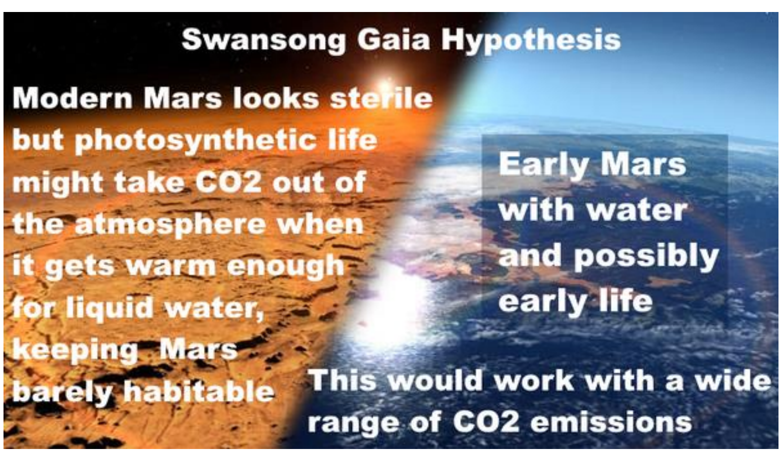


Figure 46: Swansong Gaia hypothesis. Modern Mars looks sterile, but photosynthetic life might take CO_2 out of the atmosphere when it gets warm enough for liquid water, keeping Mars barely habitable. This would work with a wide range of CO_2 emission scenarios

Image credits: NASA's Goddard Space Flight Center from: (Steigerwald, 2019)

This is how it would work:

- Initially Mars has a thick atmosphere with a sea, rivers, surface water and warm conditions to encourage photosynthetic life. This either evolves on Mars, or is seeded from Earth
- Biotic and abiotic photosynthesis removes carbon dioxide from its atmosphere. Cyanobacteria precipitate carbonates and so speed up the abiotic process of carbon sequestration through direct formation of carbonates through extracellular and intracellular cyanobacterial calcification (Benzerara et al). The carbonates, together with clays and other sediments would also trap organics from life which is another way to take CO_2 out of the atmosphere. Without plate tectonics, Mars can't return this to the atmosphere again
- The sequestered carbon dioxide thins the Mars atmosphere reducing the greenhouse effect.
- This makes Mars less habitable for photosynthetic life.

This feedback loop continues until the CO_2 removed is in equilibrium with the CO_2 supplied to the atmosphere. At this point, Mars is so cold and dry that its photosynthetic life has minimal effect on its atmosphere.

Then in the other direction, as Mars warms up slightly due to a new pulse of CO_2 introduced to a planet with minimal life on it:

- Life starts to spread and generates hydrogen and methane which adds to habitability by enhancing the CO_2 greenhouse effect
- In the more habitable Mars life briefly becomes abundant and starts to take CO_2 out of the atmosphere
- After a delay life then makes Mars gradually uninhabitable again.

Mars does have continuous introduction of some CO_2 into the atmosphere from volcanoes on a geological timescale, and it has other abiogenic sources of carbon dioxide, for instance from comets, so it would not lose all the CO_2. Once the amount of life present is very low, CO_2 builds up again. This would lead to an equilibrium, a homeostasis, where there is just enough life to remove nearly all the CO_2 from the atmosphere as it is introduced by comets, volcanic eruptions, etc.

Mars is still geologically active with numerous young fissure fed flood lavas on the Elysium Planitia from 500 to 2.5 million years ago. In a preprint, Horvath et al suggest evidence for a possibly volcanic feature with a possible age range of only 53 to 210 thousand years. They suggest that the Cerberus Fossae region may still be active today (Horvarth et al).

In addition, measurements of carbonates in young Martian meteorites suggests that carbonate formation is ongoing on Mars, with evidence of carbonate formation at 3.9 billion years ago (ALH84001), ~600 million years ago (Nakhla) and < 200 million years ago (EETA 79001) (Niles et al, 2010). These rocks are from at least three meters below the surface. There has to be an input of CO_2 into the system to form these carbonates.

The carbon 13 ratios ($\delta^{13}C$ ratios) in the meteorites are highly variable. This is an evolving field and the reason is not known for sure yet, but it may be because of changes in the isotopic composition of the atmosphere perhaps through atmospheric loss of carbon 12. The current ratio is 46%±4% as measured by Curiosity (Webster et al, 2013) and the early Martian atmosphere may have had a composition of 10% to 20% (Shaheen et al., 2015). It was previously thought to be the other way around, low later with Phoenix measuring -2.5%±4.3% for the current concentration (Niles et al., 2013: section 2.2). But the Phoenix observation was found to be mistaken (Shaheen et al., 2015).

On Earth, similar variability is due to biogenic fractionation and Jull et al did suggest this as one of several hypothetical reasons for variation in this content in the Martian meteorites (Jull et al, 1995). If there is life on Mars it would be likely to cause biogenic fractionation, but the potential for evolution of the ratios in the atmosphere and other geological sources of variation will make it hard to disentangle any biogenic signature component in variations in the carbon isotope ratios.

If this biological feedback loop is present, we would predict the current situation on Mars to have enough CO_2 to be barely habitable, but not to have lost so much as to have no life at all. It would occur in such trace amounts that its effect on the atmosphere is only barely detectable, if at all.

The result is that Mars would look pretty much the same as Mars does to us now, with life not easily detectable from orbit. There is enough oxygen in the atmosphere to mask any seasonal fluctuations in oxygen produced by life in marginal habitats.

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When there is enough CO_2 for open water on Mars, the strength of this feedback effect could be amplified further by organisms similar to terrestrial forams in the early lakes and oceans, or other similar organisms.

On Earth forams evolved calcareous tests (shells) less than half a billion years ago during the Cambrian explosion (Fig 2.1 of Boudaugher-Fadel, 2018:46). However the Martian water was oxygen rich long before we have evidence of abundant oxygen on Earth.

So, it's possible Mars evolved microorganisms with carbonate shells at an earlier stage of evolution. Planktic foraminifera produce as much as half the terrestrial particular carbonate flux to the ocean floor at a rate of around 2.9 gigatons per year (Jacob et al, 2017). So this process might cause a similar increase in the carbonate flux on Mars and be a significant increase over the abiotic processes.

Forams incorporate oxygen from the water to make their shells, rather than from the atmosphere. However, Martian lakes and seas could have oxygen rich surface layers, similarly to Gale crater lake (Hurowitz, 2017) (Doyle, 2017) (Lanza et al, 2014) (NASA, 2017).

Terrestrial forams can be single cell secondary consumers, or kleptoplasts, ingesting chloroplasts from green algae to photosynthesize (Serôdio et al, 2014). The Martian analogues of forams could be either of those, or they might be themselves photosynthetic.

The feedback process involving forams or similar creatures would work like this:

- Some of the dissolved carbon dioxide in the water gets incorporated in the shells of forams accelerating sequestration of carbonates in lakes and oceans
- This cools down the planet and makes the lakes and oceans less habitable until they freeze over and the feedback stops.
- At times when the Mars orbit has high eccentricity some of the ice would still melt when it is closest to the sun. This would lead to a situation where the lakes are ice covered for most of the year but have liquid water for a short while in summer, with the duration of ice free conditions based on an equilibrium between sequestration by the forams with carbonate shells, and the volcanic emissions of CO_2.

Once photosynthetic life is no longer active in large numbers, and the forams no longer form shells, in large numbers, Mars is free to warm up, and its atmosphere thicken, due to changing eccentricity, or a period of increased volcanic emissions of greenhouse gases, but as it does so, the spread of life across the planet then takes more carbon dioxide from its atmosphere and cools it down again.

The reason there is any CO_2 left may be because of the continuing low levels of volcanic emissions on timescales of millions of years. Another source of CO_2 would be the infall of organics from comets and meteorites (Frantseva et al, 2018) (Goetz et al, 2016:247), These would combine with superoxygenated surface layers, and ionizing radiation that dissociates surface organics back to gases such as methane and carbon dioxide and water vapour. Some CO_2 would also be delivered in comets.

In this way, this "Swansong Gaia" feedback loop continually keeps Mars only marginally habitable. Whenever it warms up and gets a bit more habitable this "Swansong Gaia" feedback loop would kick in making it less habitable again.

Interactions of nitrogen cycle with Swansong Gaia - if life returns more nitrogen to the atmosphere when Mars is wetter, the Swansong Gaia cycle is reinforced

So far the Swansong Gaia hypothesis is based on the carbon cycle on Mars. But Mars would have a nitrogen cycle too, and nitrogen is essential for life. So how would its nitrogen cycle interact with the Swansong Gaia carbon cycle? We look at different scenarios depending on whether Martian life has nitrogen fixation, and denitrification which returns nitrogen to the atmosphere.

For this to work through to the present, then Martian live needs to be able to do nitrogen fixation at low nitrogen levels – otherwise it would only apply earlier on when there was more nitrogen in the atmosphere.

For the possibilities of nitrogen fixation in the present day Martian atmosphere see:

- Sources of nitrogen on Mars as a potential limiting factor – potential for Martian life to fix nitrogen at 0.2 mbar – and "follow the nitrogen"

Nitrogen fixation scenario 1: Martian life never developed nitrogen fixation – weaker Swansong Gaia effect

In this scenario, life is limited to habitats and microhabitats with nitrate deposits or abiotic nitrogen. For this scenario see Possibility that past life in Jezero crater or even modern life never developed nitrogen fixation – or if it did, that nitrogen fixation was never taken up by microbes in oxygen rich surface layers

In this scenario, the Swansong Gaia effect that we looked at in the previous section would still exist but be weaker. As the planet warms there would be large amounts of water but most of this water would be inaccessible to life so then as photosynthetic life spread across the oceans and lakes, it would take less CO_2 from the atmosphere than if it was capable of nitrogen fixation

There would still be a Swansong Gaia feedback. Life would be more abundant when the planet warms in habitats with sufficient access to nitrates, but it would be a weaker feedback than if life has nitrogen fixation and photosynthetic life can spread throughout the planet

Nitrogen fixation scenario 2: Martian life has nitrogen fixation and also denitrification to return nitrogen to the atmosphere, similarly to life on Earth – strong Swansong Gaia effect

If life on Mars does have nitrogen fixation, what happens will depend on whether Martian life is also capable of denitrification, biological pathways that can return the nitrogen from nitrates and ammonium back to the atmosphere.

Earth's atmosphere is maintained at its high levels by denitrification. Capone et al. say that on a planet with oceans and continents, since nitrates are so readily soluble in water, without denitrification, nitrogen on the land would be substantially depleted, nitrogen would end up in the ocean and terrestrial life would be impossible (Capone et al., 2006).

If life on Mars is as on Earth and returns nitrogen to the atmosphere through denitrification, then during a warming spell after a pulse of CO_2, life would use denitrification of the nitrate deposits to produce the nitrogen needed for photosynthetic life to spread through the planet and to continue to remove the CO_2 from the atmosphere for as long as it is produced.

In this scenario, as the volcanoes move to a new phase with less CO_2 produced, the CO_2 levels go down, the low atmospheric pressure and the colder and drier conditions lead to life producing less nitrogen.

Since it's nitrogen rather than water that limits life, In this scenario, it's not impossible that Mars still has widespread liquid water. There could be nitrogen fixing photosynthetic life on Mars in low concentrations throughout the planet and there would be habitats that aren't uninhabited, but have very low concentrations of life because of the limitations set by the low nitrogen levels in the atmosphere.

This is a scenario with many potential habitats but with life at low concentrations and hard to spot except in habitats with high levels of nitrogen already fixed, for instance as nitrates or organics from life.

Nitrogen fixation scenario 3: Martian life has nitrogen fixation but no denitrification –Swansong Gaia effect varies in effect depending on deliveries of nitrogen by comets

Now let's look at another scenario, where Mars developed nitrogen fixation but not denitrification.

In this scenario, there is no way for life to return nitrogen to the atmosphere. Nitrogen builds up at times after large comet impacts or multiple comet impacts which deliver nitrogen to the planet.

If enough nitrogen has built up, then after a warming pulse of CO_2, photosynthetic life spreads through the planet but it quickly uses up all of the easily accessible nitrogen in the atmosphere.

This gets cycled through the biosphere for a while but eventually is removed from the biosphere as buried nitrates and organics.

In this scenario, after an increase in CO_2 emissions by volcanoes, CO_2 levels might rise first, then fall due to photosynthetic life, but rise again as CO_2 continues to get added to the atmosphere and life can no longer remove it because it is limited by the reduced amount of nitrogen in the atmosphere.

During times of less CO_2 emissions, life wouldn't use much nitrogen so the nitrogen could build up and be ready for life to use at the start of the next CO_2 pulse.

In this scenario, the Swansong Gaia feedbacks vary. When there is a lot of nitrogen in the atmosphere, the feedbacks are stronger, and when there is a shortage of nitrogen the feedbacks are weaker and the climate is more variable.

In a closely related scenario, Mars has nitrogen fixation but levels of nitrogen fixation always use more nitrogen than is produced by denitrification and other sources such as comets.

Nitrogen fixation scenario 4: Martian life behaves like the life in terrestrial hyperarid deserts – nitrogen fixation and denitrification but denitrification stops in the driest conditions - strongest Swansong Gaia effect

Finally, if Mars life behaves like life in Mars analogue deserts a more complex picture of denitrification / nitrogen fixation arises (Shen et al, 2021) which we'll see actually reinforces the Swansong Gaia effect.

In the driest conditions the research of Shen et al suggests there is no biotic nitrogen fixation and no denitrification, just nitrate assimilation with the nitrates fixed abiotically from the atmosphere. They deduce this based on the isotope ratios for nitrogen and oxygen.

In wetter sites they detected more complex pathways and denitrification dominates. They found that some denitrification does occur in some hyperarid sites (Shen et al, 2021).

They suggest (Shen et al, 2021).

> *"These results suggest that N cycling on the more recent dry Mars might be dominated by nitrate assimilation that cycles atmospheric nitrate and exchanges water O during intermittent wetting, resulting stable isotope biosignatures could shift away from martian atmospheric nitrate endmember.*
>
> *"Early wetter Mars could nurture putative life that metabolized nitrate with traceable paleoenvironmental isotopic markers similar to microbial denitrification and nitrification stored in deep subsurface."*

This suggests a scenario where as Mars becomes more habitable after a warming pulse, life through denitrification makes the biosphere more habitable for nitrogen fixing photosynthetic life, which would strengthen the Swansong Gaia effect, with the CO_2 removed more rapidly the thicker the atmosphere.

As the CO_2 is removed and the planet becomes drier, the balance between denitrification and nitrification would shift in the other direction. Over much of Mars, where nitrates are less available, life would be limited by the nitrogen fixation before it reaches the point where it is limited by the availability of liquid water. Once CO_2 levels get low enough so that denitrification stops, nitrogen levels in the atmosphere fall, and photosynthetic life is reduced. With less photosynthesis to remove it, the CO_2 from volcanoes would build up again until denitrification could produce enough nitrogen for photosynthetic life to flourish enough to take the CO_2 out of the atmosphere.

This would be an extra feedback that would tend to keep the planet at a warming level high enough to keep nitrogen fixation and denitrification in balance, and at a level where some nitrogen fixating photosynthetic life is possible throughout the planet.

In this scenario, Mars is likely to have enough water availability to be more like the wetter parts of terrestrial Mars analogue hyper arid deserts where denitrification begins to dominate over nitrification. Perhaps biofilms in the brines found by Curiosity could be wet enough for denitrification to keep the nitrogen levels in the atmosphere from falling too low, or it might be due to life in the deep subsurface, or in the layers of fresh water in the polar ice that form due to the solid state greenhouse effect, or the flow like features, or in Martian caves or the lakes below glaciers. See:

- Proposed surface microhabitats on Mars that could achieve higher densities of life and be a source for propagules in the dust – including brines that form rapidly when ice overlays salt at high latitudes, caves that vent to the surface, fumaroles, and fresh water melting around heated grains of dust trapped in ice layers through the solid state greenhouse effect

There might be another way to achieve this balance. The nitrogen could be delivered by comets, possibly in larger quantities than previously thought, in the form of ammonium salts, and the same balance would be reached of just enough nitrogen for some nitrogen fixation throughout the planet but instead of denitrification, it's the nitrogen delivered from comets that helps to sustain the levels of nitrogen needed to balance the nitrogen lost through nitrogen fixation (Poch et al, 2020).

There could be other more complex scenarios than these with sometimes denitrification dominating and sometimes nitrogen fixation dominating, sometimes Swansong Gaia reinforced and sometimes weakened.

Warming from methanogens limited by Swansong Gaia feedback from photosynthesis which produces oxygen which turns much of the methane to CO_2 and also fixes the CO_2

This might also explain the trace levels of methane too, with another parallel methane cycle. As the methanogens produce more methane, this warms the planet, leads to more of the photosynthetic life and so leads to CO_2 removed which acts to cool the planet to counteract the warming of the methane.

In addition photosynthesis would lead to high levels of oxygen in surface water and in the atmosphere, which would turn much of the methane to CO_2 reducing its warming impact.

Methanogens could potentially make the planet more habitable if they were the only lifeform there. However, they would never be able to build up to large numbers in a sustained way, because whenever they warm the planet they stimulate so much photosynthetic life that they automatically, though indirectly, self limit themselves.

However, the methanogens might be able to spread rapidly in some initial pulse, for instance if most of the liquid water is below the ground at that point, out of reach of sunlight for photosynthesis.

That could give a period of warmer conditions and for lakes to form before the photosynthetic life spreads through the newly warmed planet.

The Swansong Gaia could be a result of a mix of methanogens that warm the planet and photosynthetic life that cools it. The methane is too reactive to remain long in such an oxygen rich biosphere, and so is unable to significantly counteract the effect of the removal of CO_2 by the photosynthetic life forms.

Methanogens in the deep subsurface could be self limiting in another way, which could limit them even in absence of photosynthesis.

Self limiting consortiums of methanogens, methanotrophs, and Fe(III)-reducing bacteria converting underground aquifers to calcite, and so maintaining a subsurface barely habitable Swansong Gaia hydrology

As with the terrestrial Gaia there may be not just one cycle, but several interlocking and reinforcing cycles. There are many biological pathways that bacteria can use to form calcite (cement) for instance, with some of them used in self healing concrete (Rummel et al, 2017)

(Dhami et al, 2013) One such method may be of special interest to Mars. A consortium of methane oxidising and sulfate reducing bacteria can convert underground aquifers to calcite through anaerobic oxidation of methane (Rummel et al, 2017) (Drake et al, 2015). Perhaps these conditions may occur in the Martian subsurface, for instance at the sources of the methane plumes if these originate in geothermally heated underground aquifers.

The methanotrophs naturally form a layer above the methanogens to catch the methane. Since they form calcite, this could block it off and prevent it reaching the surface. From time to time some of it would break through and this could explain the methane spikes.

In this case the methanotrophs render their subsurface habitat less habitable by converting some of the water to calcite and blocking off their own supply of methane. If this happens near the surface, the warmer conditions may act as a feedback to block off the methane more and so reduce the warming effect on the atmosphere.

By forming calcite the methanotrophs also take CO_2 from the atmosphere. If Mars never developed photosynthesis, this could also be an independent cycle involving just the methanogens and methanotrophs, that keeps the subsurface almost but not completely uninhabitable for the methanotrophs while at the same time limiting the release of methane from the methanogens to a slow trickle with almost no warming effect.

Another cycle might involve siderite (iron carbonate), which is produced in prodigious amounts by some Fe(III)-reducing bacteria (Onstott et al, 2019), which is a proposed metabolism for subsurface life on Mars (Parnell et al, 2016) (Onstott et al, 2019), these could also form carbonates with other metals such as copper (Onstott et al, 2019). As the methanogens warm the planet, more water would be available in subsurface layers suitable for Fe (III) reduction and the formation of siderite would help to cool it down again.

This is not meant to be an exhaustive list of cycles that could help perpetuate a Swansong Gaia. The idea here is to demonstrate the basic principle of a Swansong Gaia by suggesting various ways that life could maintain a planet in a barely habitable state for billions of years. Mars may also surprise us with something unexpected that has the same end effect of keeping it only barely habitable in a Swansong Gaia.

Could seasonal oxygen be a possible signal of photosynthesis maintaining a Swansong Gaia homeostasis on Mars?

Could the seasonal oxygen observed by Curiosity be the result of low levels of oxygenic photosynthesis, the main process driving this hypothetical swansong Gaia?

The oxygen levels rose in spring and summer to levels 30% above those explainable by chemistry alone and dropped back to normal levels in the fall. Methane similarly rises by more than 60% of expected levels in summer, and also spikes randomly and unpredictably (Shekhtman, 2019). The authors write (Trainer et al, 2019:3021):

> *Though Mars has the potential to generate significant O_2 release due to abundances of oxidants in/at its surface, the mechanisms by which O_2 could be quickly generated and then quickly destroyed are completely unknown. As with all surprising results, we hope that continued in situ, experimental, and theoretical results may shed light on this intriguing observation.*

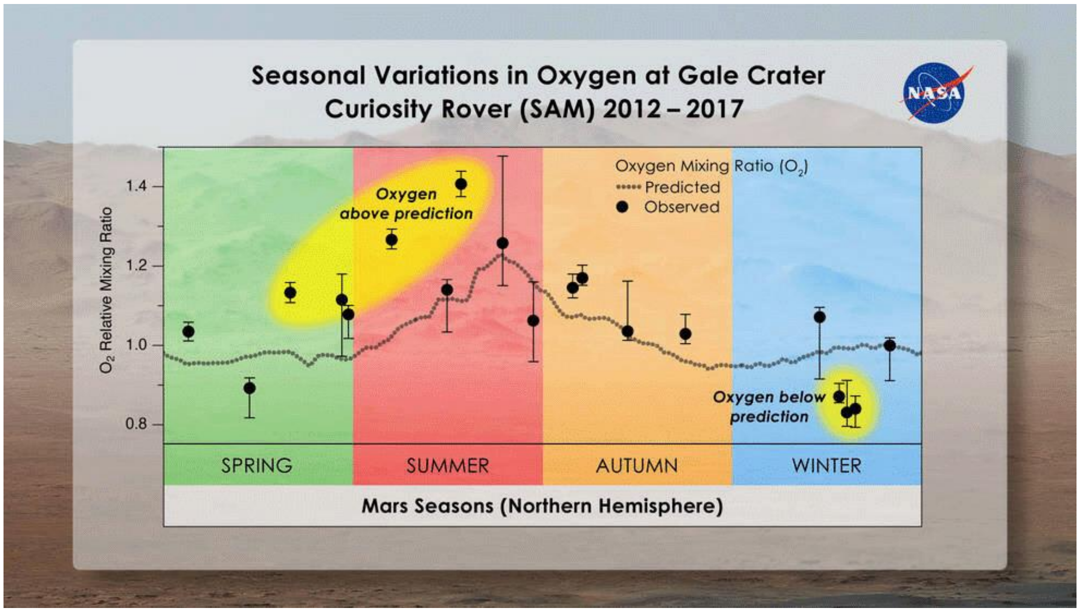


Figure 47: excess of oxygen in spring to summer and deficit in winter over the expected seasonal variation as measured by Curiosity.

Credits: Melissa Trainer/Dan Gallagher/NASA Goddard (Shekhtman, 2019)

There is a weak correlation suggesting less oxygen is generated when there is more dust in the air. This is something one would expect from photosynthesis.

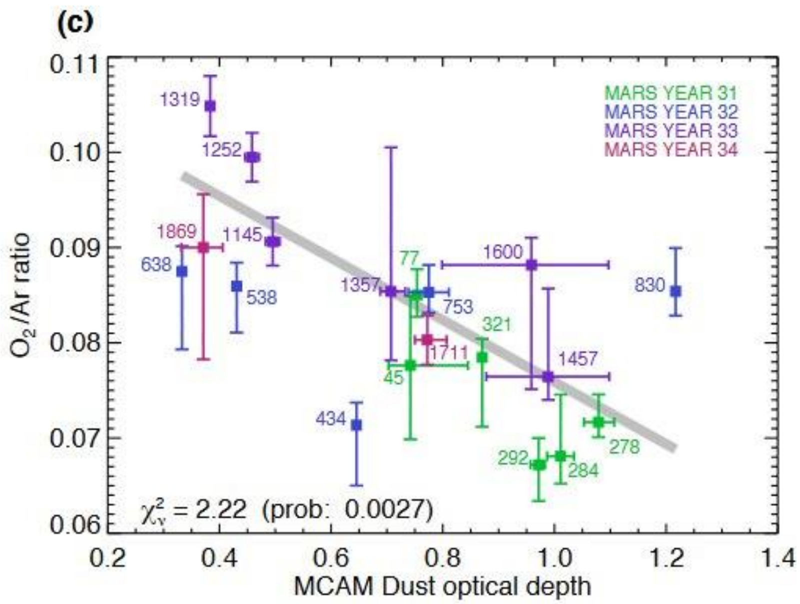


Figure 48: Weak inverse correlation of oxygen to argon ratio with dust optical depth, less oxygen is produced when the atmosphere lets less light through, Figure 59 of Supporting information for (Trainer et al, 2019)

Optical depth of 0.3 means 74% of the light is let through. Optical depth of 1.1 means 33% of the light is let through.

They didn't find any correlation of the dust with seasonal and interannual pressure variation, or temperature variation.

If the oxygen excess is all due to photosynthesis, we need to explain a change of around 400 ppm which corresponds to 10^{20} molecules cm^2 (page 3017 of Trainer et al, 2019) or about 0.006 moles of oxygen per square cm or about 26.6 grams of oxygen per square meter

Cockell studied a similar situation on an Earth analogue exoplanet with photosynthetic life growing inside the rocks, and estimated an output of 2.2 g $O_2/m^2/y$ for terrestrial cryptoendoliths (Cockel et al, 2009), or less than a tenth of the 26.6 g $O_2/m^2/y$ found by Curiosity. Mars is not quite an Earth analogue, it has half the solar flux of Earth because it is further from the sun, however it has less of the light blocked out by the atmosphere which compensates enough to make photosynthesis on Mars roughly comparable.

However life on Mars could be more productive than this. In the same paper (Cockel et al, 2009), table 1, the figure for mats living beneath soil surfaces in Yellowstone National Park is 55 g $O_2/m^2/y$. This figure is for a small patch of acidic gravel near to one of the Yellowstone geysers. This habitat was suggested as a model ecosystem for Mars in a 1995 paper (Rothschild, 1995 summarized in Cockel et al, 2009 table 1).

Desert crusts with a euphotic zone for photosynthesis only a few mm thick can be much more productive. Garcia-Pichel et al recorded 950-2640 g O_2/m²/y in a desert crust in Utah, and other results for desert crusts are similar (Garcia-Pichel et al, 1996, summarized in Cockel et al, 2009 table 1).

Another possibility is a grit crust. This is a mix of lichens, fungi, algae and cyanobacteria. They coat pebbles and glue small rocks to each other. The optimal level of water for photosynthesis was 0.25 mm per day (Jung et al, 2020, summarized in Lee, 2020). Perhaps lichens and cyanobacteria could form such a crust using the brines detected by Curiosity? I couldn't find figures for oxygen production from a grit crust.

Rothschild found a much higher figure in an intertidal cryptic microbial mat of 130,000 g O_2/m²/y (Rothschild et al, 2002). If Martian life was as productive as that mat it would be able to produce the measured signal using much less than a thousandth of the light flux.

We see that 26.6 g O_2/m²/y is too high a figure to explain as life inside the rocks themselves in Martian conditions. It could be due to microbial mats below the surface but more easily explained as a more productive desert crust.

Such a fast metabolism would be a challenge in the very cold brine layers. Perhaps if a biofilm can retain the water through to the much warmer daytime temperatures, it can make such figures plausible, and if life is abundant on Mars, present at both Viking lander sites for instance.

It's also possible that Martian life is better at photosynthesis than terrestrial life. In the best scenarios in labs and ideal conditions, terrestrial life achieves only 3% efficiency due to slowness of the Calvin cycle and the large antenna size which has evolved to be most efficient at collecting light at low light levels. In addition, most terrestrial photosynthesis rejects 50% of the red light.

Martian life could have evolved a faster form of photosynthesis than the Calvin cycle, and a variable antenna size to cope with dust storm conditions. It might also capture nearly the full spectrum of sunlight (like seaweeds). In total, Martian life could potentially achieve roughly an order of magnitude increase in efficiency compared to terrestrial photosynthesis. For discussion of this, see Martian microbes better adapted to terrestrial conditions than terrestrial life, example of more efficient photosynthesis (below).

Martian life could also be adapted to the colder conditions on Mars with a faster metabolism, using chaotropic agents such as the perchlorates which can speed up reactions at low temperatures.

Trainer who is the lead author of the paper on the Curiosity oxygen results, interviewed by Scientific American, put it like this: (Andrew, 2019):

“People in the community like to say that it will be the explanation of last resort, because that would be so monumental. There are abiotic mechanisms aplenty, both known and unknown, to rule out first before leaping to any more sensational claims.”

It would be a surprise to see the signal of a Swansong Gaia so easily as this. Martian life might only inhabit the RSLs and other limited habitats, or be limited by the presence of nitrates, or it might not have developed photosynthesis. If it does occur in these brines it might have a very slow metabolism.

Oxygenic photosynthetic life on Mars could be expected to generate a signal like this, but perhaps a weaker one, easily masked by the naturally occurring oxygen in the atmosphere. This level of oxygen would require a highly productive cryptic near surface microbial mat or desert crust or similar. However, it seems not impossible that biology explains all or most of the signal.

Swansong Gaia maintains a homeostasis, though at a much lower level of habitability than the original Gaia hypothesis – not the same as Kleidon’s “anti Gaia” which makes a planet rapidly uninhabitable

Lovelock’s Gaia hypothesis in its strongest form suggests that life through homeostasis maintains a disequilibrium that makes a planet more habitable for itself, changing conditions so that the probability of growth of the entire biosphere is maximised (Margulis et al, 1974). It does this by controlling various parameters including surface temperatures, atmospheric composition. He compares this with the equilibrium or close to equilibrium states of the Mars and Venus atmospheres and suggests these are representative of lifeless planets (Lovelock et al, 1974). This is proposed as a “life detection” method for alien biospheres (Lovelock, 1975)

It might be that life was too sparse or existed below the planetary surface, in either case neither it nor its effects might be visible. There are reasons for believing, however, that once life is initiated on a planet it can only persist if it is able to control the planetary environment. This ability may be an important evolutionary step in the early stages of life.
…
If it is a property of biospheres to optimize their use of raw material and free energy to control the planetary surface conditions at those most favourable for survival then this form of biosphere or an alien chemical one should be recognizable. Except by a purposeful act of camouflage any life system will reveal its presence through the chemical disequilibria caused by its contrivances.
(Lovelock, 1975)

In our “Swansong Gaia” for Mars, life does control its environment through homeostasis. However, life keeps it constantly at a barely habitable state, not letting the CO_2 levels go so low as to be totally uninhabitable, yet not so high as to permit life to spread widely. In this way it is a controlled environment but one that keeps Mars barely habitable.

Not only that, the proposal is that life could keep Mars in a less habitable state than it would be without life. Without life, less carbonate would form and the abiotic processes may not be enough to keep it as cool as it is now. The CO_2 would build up and an abiotic Mars might have streams and lakes, with the thickness of the atmosphere varying more than it does today, in a less stable environment, but one more habitable for life.

Kleidon coined the term “anti Gaia” for life that makes a planet less habitable for itself with no homeostasis, ending up with an abiotic planet (Kleidon, 2002). However in the process described here, life does have homeostasis, and so a new term was needed, “Swansong Gaia” seemed appropriate. It is this homeostasis that lets the planet remain barely habitable but still with life on it, for billions of years. This is relevant to the search for life, as it would suggest that Mars has been continuously barely habitable, since it formed.

The habitability wouldn’t be constant, any more than it is for Earth with the ice ages, or possible snowball Earth. It would fluctuate, sometimes more habitable but never becoming so uninhabitable as to lose life altogether.

If this is true, it increases the potential for finding present day life on Mars, and reduces the potential for newly evolved life, though that might still be possible. With this hypothesis we would expect to find that some life has been continuously present on Mars since it first evolved despite the harsh conditions.

This could explain why life is so hard to detect on Mars. It would still be there, though at very low concentrations that would require dedicated in situ life detection to find.

This “Swansong biosphere” end state could be the natural end state of all Mars-like planets with life - or it could be an accident of history. It could be that if photosynthetic life or phototrophs never developed, and only methanogens, Mars would be more habitable today.

This suggestion could increase the potential for present day life on Mars. What we see on Mars is exactly what we would expect to see if life has continued on Mars through to the present day in the homeostasis of a “Swansong Gaia”.

Potential limits on the biomass of a Swansong Gaia on Mars using the amounts of free CO and H_2 in the atmosphere

Sholes et al have suggested a limit on the amount of life there could be on Mars based on the CO and H_2 in the atmosphere. The CO is produced photochemically from the carbon dioxide, and so they can work out how much there should be and compare it with the amount there would be if life was able to take advantage of it.

They argue that these sources of energy are easily available to Martian microbes using ancient metabolic pathways of terrestrial life. They argue that such pathways involving simple reactions should evolve easily on Mars. On the assumption that microbes on Mars would use these gases they work out a maximum Martian biomass of 2×10^{11} kg or 10^{27} cells (Sholes et al, 2019). The calculation is based on a minimal basal power requirement for life of 3×10^{-23} kJ $s^{-1}cell^{-1}$. However the minimal amount to avoid racemization of cells is 10^{-24} kJ $s^{-1}cell^{-1}$ so in principle 30 times more cells would be possible.

Note that this use of CO as an antibiosignature has been challenged. Life could generate CO indirectly. The CO could be produced by photochemical processing of methane into CO in the atmosphere, with the methane perhaps produced by methanogens originally, in a model motivated by an Archaean Earth (Schwieterman et al, 2019:3)

However, if we can use the Sholes et al estimate, it works out at a limit on biomass of around 1.4 gram per square meter of the Martian surface of 144.8 million square kilometers or an average of 700 million cells per square centimeter for life that subsists off CO and H_2 in the atmosphere.

Their figure of around 1.4 g/m² of biomass or 7×10^8 cells / cm² is not unreasonable as an approximate limit to the amount of surface life on Mars, especially since the biomass would also be localized (very little probably in the southern uplands) and patchy.

Terrestrial cell counts at the cold-arid limit of life are often of the order of about 1000 cells / gm (Goordial et al, 2016) which is well below 7×10^8 cells per cm². Even a global habitable layer to a depth of 20 centimeters with ten times this cell density would contribute 2×10^5 cells, 0.1% of their limit and would not contribute enough biomass to have any noticeable effects on the CO globally.

Curiosity was able to monitor CO, but the measurements were hard to do. The signal is 15% ionized CO and 85% CO_2 fragmenting into CO^+. It behaves much like a passive tracer gas in the atmosphere, tracking the signal from argon. They spotted a significant increase after sol 1000. This may be suspect and involve either contamination or instrument errors (Trainer et al, 2019:3013). However, it is possible that future investigations do find signatures of life that uses the CO, if this varies seasonally.

Testing the “Swansong Gaia” hypothesis through looking for evidence of cycles on Mars that maintain this homeostasis

If the hypothesis is correct then our missions to Mars should detect life actively removing CO_2, such as photosynthetic life on Mars but in low quantities so that the life is only barely detectable.

The other side of this is greater knowledge of the CO_2 emissions from volcanoes and modelling of the abiotic processes that remove CO_2 from the atmosphere, and proof that the atmosphere would be thicker without the presence of biology, again through modelling.

Recent carbonate deposits could be studied to determine if they were the result of biology or abiotic processes, and the percentage that is due to biology versus abiotic processes could be fed in to inform modelling to deduce how much of the homeostasis is due to biology and how much to abiotic processes. This would include analysing the carbonates in the dust to narrow down the estimates of how much CO_2 is sequestered in them, and to determine how much is due to life processes.

This could determine relative contributions of the abiotic explanation of homeostasis at 6 millibars and biology and find out whether or not biology is a significant factor here.

We could also study the past deposits on Mars. At times when the atmosphere is thicker we should find more evidence of life, and again could calculate that this was sufficient to significantly accelerate the reduction of CO_2 to current levels.

So, the method of confirmation would be similar to the Gaia hypothesis, we would need to find evidence sufficient to show that Martian life is involved in a self maintaining homeostasis that constantly restores the atmospheric pressure to 6 millibars whenever it significantly departs above it.

Recommendation to return a sample for teleoperated ‘in situ’ study to above Geosynchronous Equatorial Orbit (GEO) in the Laplace plane, where particles in a ring system would orbit

If there is a significant possibility of present day life in the samples, or of well preserved past life that would be degraded by the sterilization procedures, we can return the sample to a location with no contact with Earth’s biosphere for telerobotic study.

With the current plans, the Earth Entry Vehicle for the Mars samples would be designed to re-enter without a parachute since after the experience of the Genesis probe they feel they can’t rely on a parachute to protect the capsule (Andrews, 2020). Returning the sample to orbit removes the need for the aeroshell.

The current paper recommends returning the samples to an orbit within the Laplace plane (where Earth's ring particles would orbit if it had a ring system) above GEO

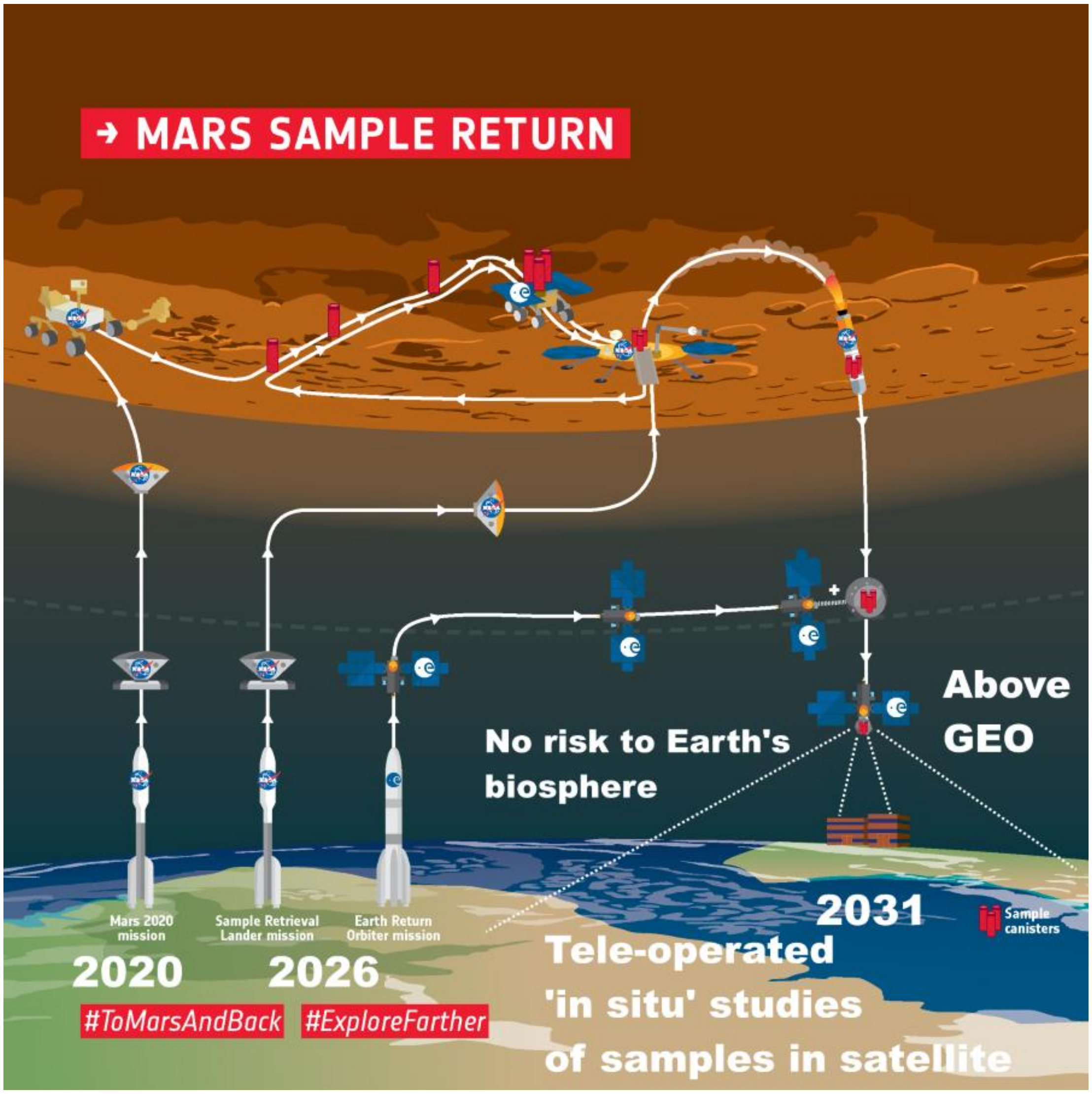


Figure 49: Text added to ESA graphic (Oldenburg, 2019) showing concept of tele-operated in situ study above GEO.

Why we can’t return the sample to the ISS, the Earth-Moon L1 position or to the Moon – ISS doesn’t break chain of contact – Earth Moon L1 is gravitationally unstable - and a return to the Moon isn’t currently permitted under COSPAR

The usual suggestion is to return it to the ISS or the Moon but there are issues with both these locations.

However there are problems with a sample return to low Earth orbit or the Moon.

- **Satellites in a low Earth orbit need active control** to prevent re-entry to Earth’s atmosphere.

  The 1980s Anteus mission to return Martian samples to LEO (Devincenzi et al, 1981) would have relied on an optional emergency boost of the laboratory to 8000 km in case of any breach of containment, however this doesn’t provide passive protection of Earth’s biosphere.

- **If the samples are returned to the ISS, then the ISS itself becomes part of the chain of contact with Mars.** The idea of a chain of contact is that microbes can be transferred from one surface to another. So nothing that contacts Earth’s biosphere unsterilized should touch a surface that has been exposed to the Martian environment. Also - microbes can be transferred many times - so in turn if something does touch a surface that could be contaminated with Martian life, that in turn counts as contaminated and so on.

  Eventually the astronauts return to Earth. Their spacecraft that they use to return to Earth is part of the chain of contact with the ISS and so also, part of the chain of contact with Mars. Also Martian life could be returned to Earth on their clothes on their skin or indeed internally as we saw in Complexities of quarantine for technicians accidentally exposed to sample materials

- **The Earth-Moon L1 position in gravitational equilibrium between the Moon and Earth is otherwise a good location but is gravitationally unstable**, and needs active station keeping to keep the sample close to that location.

- **Low orbits around the Moon are also unstable.** There are “frozen orbits” where it could remain for a long time but not as stable as above GEO.

- **The Moon may be useful for sample curation in the future** (Schrunk et. al, 2007:145), **however with no infrastructure currently on the Moon, it would need a more complex mission.**

  Also, humans are likely to return to the Moon soon, so the Moon has planetary

protection like Earth. The COSPAR guidelines for category 5 (sample return) missions currently say that (COSPAR, 2011) (Debus, 2004)

> *"(The Moon must be protected from back contamination to retain freedom from planetary protection requirements on Earth-Moon travel)".*

- **If we return Mars samples to the Moon, a crash landing risks contaminating the lunar environment** and causing issues with astronauts, explorers and tourists exploring the Moon

- **If the idea is to use a facility on the Moon to let humans handle the samples - the same issues of quarantine apply as for a facility on Earth** - the issues of a symptomless superspreader, or mirror life becoming part of the human microbiome etc, since at some point the humans would return to Earth.

  See: Complexities of quarantine for technicians accidentally exposed to sample materials

High orbits such as semi-synchronous orbits may work well if the sample is kept well out of the way of existing satellites

Another option might be a high orbit, such as a semi-synchronous orbit, if the sample is kept well out of the way of any existing satellites in similar orbits.

However an orbit above GEO seems close to optimal for preventing back contamination risk.

Advantages of GEO – nearly as far from Earth as from the Moon in terms of delta v – but much less latency for telerobotics and easier of access for payloads than the Moon

GEO is nearly as far from both the Earth and Moon in terms of delta v as any satellite orbit close to Earth. To move from GEO to an Earth atmosphere skimming Geostationary Transfer Orbit requires a delta v of over 1.4 km / sec (Gülgönül et al, 2018:1078), and the similar delta v from GEO to a Moon skimming Lunar Transfer Orbit is.over 1.2 km/sec (Salvatore et al, 2000:65).

Orbits near GEO are also stable. Long term stability analysis of orbital dynamics in GEO finds no long term possibilities for Earth re-entry under the influence of the oblateness and triaxiality of Earth or three body interactions involving the Moon, Earth and Sun (Colombo et al, 2017).

We already send large Earth observation satellites to GEO, so we should be able to send large payloads of new instruments, if needs be, to examine the samples telerobotically. There will be enough payload capacity to send much larger instruments to above GEO to study the samples than the instruments proposed by astrobiologists for in situ studies on Mars.

Essentially the idea here is to let scientists on Earth use telepresence with binocular vision and haptic feedback to study the samples in the satellite, much as astronauts in orbit plan to tele-operate rovers to study surface rocks on Mars in future orbital missions (Valinia, 2012) (Oleson et al, 2013) (Hopkins et al, 2011) (Kwong et al 2011) (Cichan et al, 2017).

See Modern miniaturized instruments designed to detect life in situ on Mars - could also be used to examine returned samples in an orbital telerobotic laboratory

If any instruments or experiments need terrestrial gravity, this could be supplied as artificial gravity using a small centrifuge in the receiving facility. Also, unlike a terrestrial receiving facility, the orbital "lab" could supply Martian gravity for experiments, which is not possible on Earth.

The concept is similar to the LAS design for a Mars Sample Receiving Facility from 2009, which relies on telerobots to do almost all the sample handling (Beaty et al, 2009:75). The difference is that the telerobots are in orbit.

Telerobotics is a rapidly evolving technology, and is already mature enough for this to be a feasible way to study the samples. 2031 is the earliest date to return the samples. By then, telerobotics will have developed for another decade.

This technology will soon be used by astronauts in orbit around the Moon to tele-operate surface equipment and rovers, and will eventually be used in Mars orbit in the same way. This is under active development as part of the joint NASA / ESA / DLR / ROSCOSMOS Meteron project (ESA, n.d.MET).

Easier to avoid satellites in GEO because of low relative velocity

Any Mars sample return needs to avoid orbits close to existing satellites because by the 2030s those satellites may be visited by astronauts for in orbit servicing, or might be recovered for recycling.

We also need to be sure that the sample receiving satellite can’t be breached by debris from an explosion in another satellite, and we also have to consider debris from any potential collision between a visiting robotic spacecraft and the sample receiving satellite.

It’s easier to avoid satellites in GEO, as they have low relative velocities to each other, all orbiting in the same direction around Earth in geostationary orbits, and at the end of their life are retired to a graveyard orbit a few hundred kilometers higher.

The samples could be returned, say, to 50,000 kilometers above Earth's surface. This is far enough from the satellites in GEO at 35,786 km above Earth's surface (ESA, n.d.GEO) to bring no risk of back contamination, even if in the 2030s, satellites in GEO are serviced by astronauts in situ. It is also several thousand kilometers above the graveyard orbit which starts 300 km above GEO (ESA, n.d.SDM).

The latency for round trip telerobotic communications to the satellite would vary between 0.33 and 0.41 light seconds compared to around 2.58 light seconds for the Moon.

An orbit within the Laplace plane above GEO contains debris in event of an off nominal explosion or other events

This article recommends an orbit in the Laplace plane, in between the ecliptic and the equatorial plane. This is a frozen orbit and any debris from a satellite in this plane will remain close to it, similarly to the motions of the ring particles of Saturn.

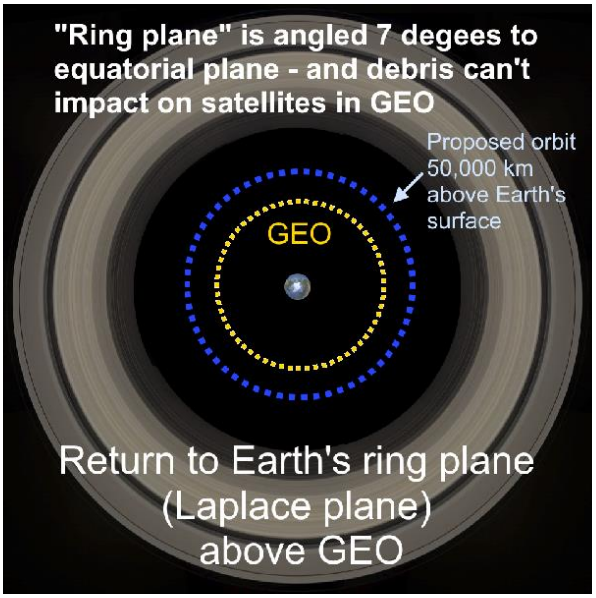


Figure 50: Earth's "ring plane" is inclined at 7 degrees to the equatorial plane. Saturn's rings superimposed to scale

(Rosengren et al, 2014), Cassini photograph of Saturn from above stretched and mirrored to approximate a circle (APOD, 2013), and Earth from above the North Pole (NASA, 2019aaasi)

Despite the name, this region is not exactly planar. The "Laplace plane" approximates the equatorial plane, at 0° inclination, close to Earth and the plane of the ecliptic at 23.44° inclination when far from Earth. The Laplace plane in the region above GEO is inclined at approximately 7.2° from the equatorial plane.

The advantage of the Laplace plane is that it is the natural place where light ring particles orbit, as for Saturn's rings. Even high area to mass ratio objects (HAMR) shed by the satellite such as thermal insulation will remain close to this orbit under perturbations by solar radiation pressure (Rosengren et al, 2013:11). It turns out that they are protected from eccentricity changes that could bring them into GEO space.

Satellites often lose fragments of the insulating material that surrounds them. This insulation would need to be designed to reduce the risk of shedding, however the Laplace plane will provide additional protection from dispersed sample materials, not only for shedding, but also in the remote case of an off nominal event such as an explosion in the facility or a collision into the facility by a spaceship sent to bring equipment to study the samples telerobotically or to receive materials to sterilize and return to Earth.

The inclination of the Laplace plane actually depends on the area to mass ratio of the particles, increasing to 18 degrees for high area to mass ratio particles..

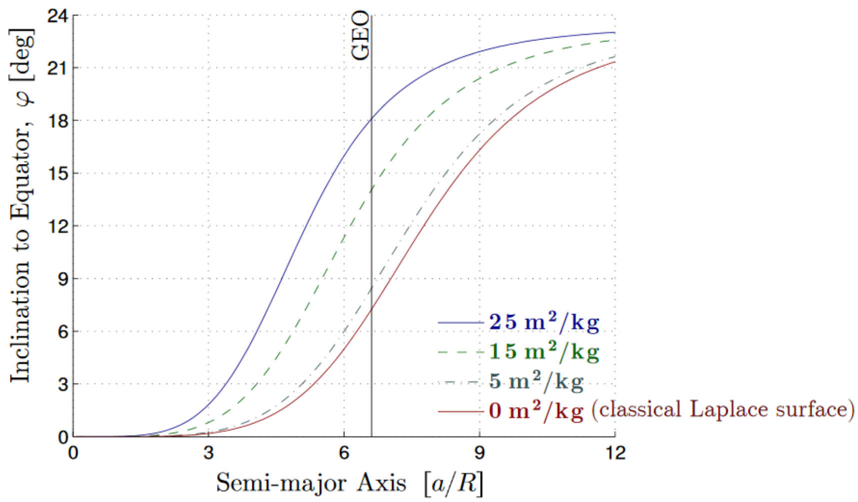


Figure 51: How the inclination of the Laplace plane depends on the area to mass ratio of the shed HAMR particles (from figure 1 of Rosengren et al, 2013)

However if the particles are released from the classical Laplace plane, even high area to mass ratio particles will be trapped in inclination and angle of the ascending mode phase space far from the Geostationary orbit at zero inclination.

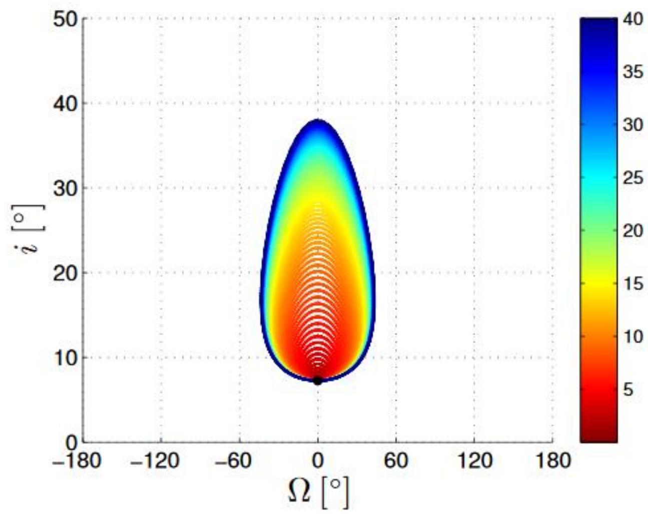


Figure 52: Evolution of shed HAMR particles released from the classical laplace plane shown by the black dot. Vertical axis shows inclination (zero for geostationary orbit) and horizontal axis shows the angle of the ascending node (direction from Earth to the point where the particle crosses Earth's equatorial plane)

The colour is for a parameter Λ which is high for HAMR particles, the "Solar Radiation Pressure angle" in degrees. So blue indicates high area to mass ratio particles. (from figure 2b of Rosengren et al, 2013)

The evolution of eccentricity is also acceptable with no risk of the objects hitting Earth's atmosphere.

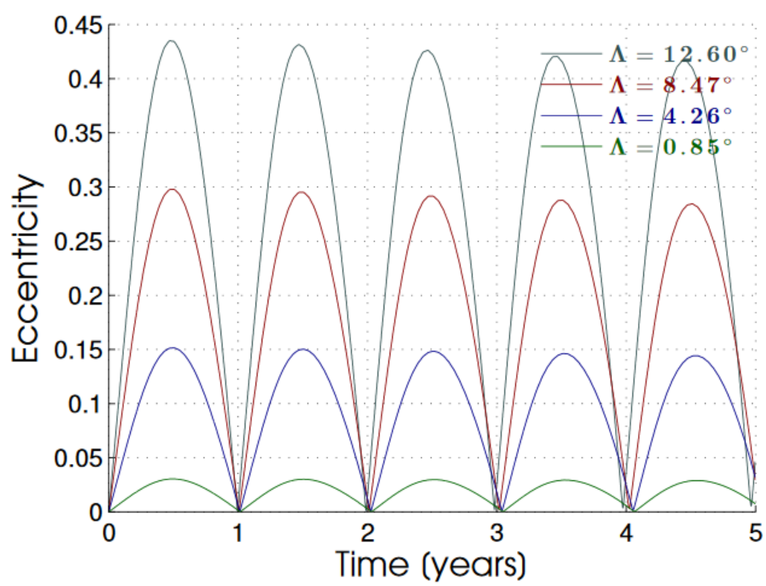


Figure 53: Evolution of eccentricity of shed HAMR particles released from the classical laplace plane. Λ as before is the “Solar Radiation Pressure angle” in degrees which is high for HAMR particles
(from figure 2b of Rosengren et al, 2013)

The Laplace plane is easy of access via low energy transfer of an Earth Return Vehicle from Mars to above GEO using either a Distant Retrograde orbit or LL2 halo orbit as intermediary

The current plan is for the ESA Earth Return Orbiter to remain in orbit around Mars, and transfer the sample to an Earth Entry Vehicle which returns to Earth (Huesing et al, 2019).

If the sample is returned to above GEO then no aeroshell is needed and instead the orbiter itself, or an Earth Return Vehicle returns to above GEO. There is also no need to break the chain of containment since it won’t return to Earth, so the mission concept is simpler.

The Earth Return Vehicle can get to an orbit above GEO from Mars initially using “ballistic capture”, also known as “weak stability boundary transfers” (Topputo et al, 2015), the low delta v, fuel efficient, non linear three or four body transfer orbits pioneered by Belbruno and Millerand, and first used for the Japanese Hiten mission in 1990 (Belbruno, 2018) (Dutt, 2018).

The ESA Earth Return Orbiter will use continuous low thrust transfer (Huesing et al, 2019) which is ideal for such an orbit. Since the ERO is needed for communications with Perseverance, the orbiter would stay in Mars orbit. We propose that the Earth Return Vehicle, or the EEV, uses the same technology.

The ERO will orbit at a height of 400 km above the Mars surface. From any orbit above 100 km, there is less delta v for ballistic transfer from Mars than direct transfer. This is also perfect for a continuous thrust. It would start by spiralling outwards to a high orbit several million kilometers from Mars, and from there, if the right moment is chosen to depart from that distance orbit around Mars, the delta v to Earth is greatly reduced (Topputo et al, 2015)

One way for the ERV to get captured to above GEO, starting from a return orbit from Mars is via a distant retrograde orbit (DRO) around the Moon as an intermediary orbit (Lock et al, 2014). A DRO is essentially an Earth centered prograde orbit with the same orbital period as the Moon with the satellite alternating between outside of the Moon and inside of the Moon in its orbit, in such a way as to also be a Moon centered retrograde orbit. This avoids potentially risky aerobraking in the Earth’s atmosphere (Strange et al, 2013) (Pires et al, 2020).

This figure shows a possible trajectory - a mission concept from 2024 worked out for a return of a Mars sample to DRO in 2023 (Lock et al, 2014)

:

[Figure needs permission]

Figure 54: Example Lunar Distant Retrograde Orbit capture for 2022 Mars-Earth sample return, figure 6 from (Lock et al, 2014:8)

The DRO orbits are known to be stable for at least 100 years (Lock et al, 2014:8), it is then easy to transfer to LL2 (Ming, 2009), the gravitational point of balance beyond the far side of the Moon as seen from Earth. From there it can then transfer to above GEO, using lunar flybys in fuel efficient ballistic transfer trajectories again, to reduce the total delta v requirements.

The DRO capture's Earth close approach at an altitude of 28,500 km does not seem to be a significant risk to Earth, especially for a low continuous thrust spacecraft such as ESA's Earth Return Orbiter, so long as it is trajectory biased away from Earth at all times during the approach. In practice close flybys are normally flawlessly executed, with many examples now for numerous missions.

Another alternative is to return to LL2 via an LL2 halo orbit which is an unstable orbit around Earth which slowly circles LL2. If a spacecraft is left in a LL2 halo orbit slightly further away from Earth than LL2, it will slowly spiral outwards into deep space into more and more distant and wider halo orbits, through the three body interactions with the sun, Moon and Earth, all the time gaining in delta v through the three body gravity assist, until it escapes into interplanetary space. This reduces the delta v for deep space missions. A spaceship returning from deep space can use this process in the opposite direction, entering a large circular distant halo orbit at high delta v and then use the three body interactions with the Moon, Earth and sun to spiral back down to LL2, to reduce the delta v for a return from deep space (Nakamiya et al, 2010).

There are two ways to get to the LL2 halo orbit from Mars that optimize it even further. The first is an Earth flyby, followed by LL2 halo orbit capture. Kakoi et al study this in reverse for missions from Earth to Mars optimizing on fuel (Kakoi et al, 2014). It would need to be re-analysed for the reverse direction but the principle is the same.

[Figure needs permission]

Figure 55: Example Earth Moon L2 halo followed by Earth flyby for transfer from Earth Moon L2 to Mars, the same could be used in reverse for the sample return (figure 13 from Kakoi et al, 2014)

The other approach is to use the Sun - Earth L2 halo and transfer from that to the Earth Moon L2 halo orbit.

[Figure needs permission]

Figure 56: Example Earth Moon L2 halo followed by Sun Earth halo for transfer from Earth Moon L2 to Mars, the same method could be used in reverse for capture (figure 10 from Kakoi et al, 2014)

The researchers discovered that there are more opportunities for the Earth flyby method than the method joining the two halo orbits together.

This double halo capture orbit avoids the need for the Earth close approach of the DRO capture method or the Earth flyby then halo capture. However, even the DRO capture's Earth close approach at an altitude of 28,500 km does not seem to be a significant risk to Earth, especially for a low continuous thrust spacecraft such as ESA's Earth Return Orbiter, so long as it is trajectory biased away from Earth at all times during the approach. In practice close flybys are normally flawlessly executed, with many examples now for numerous missions.

Either approach could be selected, or any other option for fuel efficient return to above GEO that avoids aerobraking and permits trajectories from Mars to Earth that are always biased away from Earth.

A robotic spaceship from Earth can rendezvous for preliminary study of the returned sample above GEO

A spaceship from Earth can then rendezvous with the orbiter above GEO. Its main job at this stage is to open the capsule, curate the samples, make preliminary observations and remove a small amount of material from each sample.

Later once the preliminary observations are done, a small spaceship sent from Earth can dock to retrieve any materials selected for examination on Earth, sterilize its own interior and the material with an X-ray or gamma ray source, and return the samples to Earth.

If preliminary investigations suggest the samples are unlikely to contain life, they can be sterilized as for the sterilized sample return, and returned to Earth. This would not impact on their geological or geochemical interest, and if there is undetected life in the samples, it would still be recognizable as such. If some contain life and others don't, the abiotic samples can be sterilized and returned in their entirety.

If life is found, preliminary studies can continue telerobotically in orbit above GEO using instruments designed for in situ life detection on Mars

If preliminary examination finds life in the samples, then, depending on its characteristics and whether it is thought to have potential to impact on the environment of Earth, it can be studied in situ as part of a growing telerobotic facility.

The instruments we send to orbit can include the instruments already mentioned, designed for in situ missions to Mars, such as the Life Marker Chip (Davila et al, 2010), Astrobionibbler (Schirber, 2013) (Noell et al, 2016), chiral labelled release (Parro et al, 2011), electron microscope (Gaskin et al, 2012) , gene sequencer (Mojarro et al, 2016) etc.. This should be at least as good as in situ preliminary characterization on Mars of the returned sample, and probably a lot better because of the reduced latency.

See: Modern miniaturized instruments designed to detect life in situ on Mars - could also be used to examine returned samples in an orbital telerobotic laboratory

All instruments would be sterilized of course, as for a space mission, and sent to the spaceship one way, to study unsterilized samples telerobotically and remove interesting rock sections for sterilization and return to Earth.

Although we wouldn't have the flexibility of a surface laboratory, the easy access means that we could send new instruments to the facility designed to do follow up observations depending on what the findings are. These telerobotic investigations can use all the instruments that may be sent to Mars for future in situ work, and others that are too heavy to send all the way to Mars or that can only be operated with low latency.

There is one major advantage of telerobotic study in orbit. The experience of previous sample return missions show that some level of contamination is inevitable even in the cleanest of terrestrial facilities (Chan, 2020). That may be easier to control in a telerobotically operated orbital facility.

Advantages of telerobotic study above geo over terrestrial study

This

- Streamlines the legal process based on capabilities of a known sample since samples are only returned to Earth once sterilized – and if eventually Martian life is returned to Earth for study it is well understood first simplifying the legal situation.
- Eliminates the approximately half billion dollar upfront cost for a receiving facility. We only build what is needed at every step
- Eliminates upfront costs for initial telerobotic study - these are postponed to future budgets in the early 2030s. At that point they will also be low risk as the sample mission by then is a proven success.
- Eliminates upfront costs for in situ telerobotic searches for life signatures in the samples as these are not sent there until there is a clear indication that they are needed – indeed – much of the cost might be born by universities constructing the instruments to send to the facility
- Totally eliminates any possibility to contaminate Earth's biosphere
- Also permits study of the samples in simulated Martian gravity in a centrifuge (which is not possible in a terrestrial laboratory) – which may be important e.g. for simulating chemical or biological processes
- Reduces possibilities for contaminating Martian samples with terrestrial life.

On that last point, spacecraft sent to orbit to study the samples could sterilize themselves with X-rays followed by ethylene dioxide to remove any internal organics before receiving the samples. This would make the risk of forward contamination of the sample very low.

The ESA Earth Return Orbiter could also sterilize itself, or it could be retrieved by another satellite that would sterilize the Earth Return Orbiter in this way while collecting the sample.

Possibility of early discovery of extraterrestrial microbes of no risk to Earth such as pre-Darwinian life as suggested by Weiss – if microbial challenge experiments show they are quickly destroyed by pervasive terrestrial microbes

In some cases extraterrestrial life could be assessed to be of no risk quickly. Mars could have newly evolved life which has evolved from prebiotic chemicals recently with just a few million years of evolution, in habitats isolated from the subsurface. In Cockell's trajectory 2, potential habitats form temporarily but there is no life to inhabit them. However he also remarks (Cockell, 2014)

> *There are other trajectories of greater complexity that can be envisaged. Examples include an inhabited Mars on which life becomes extinct and then reoriginates (or is transferred from Earth) at some later time.*

For his trajectory 2, Cockell envisions an uninhabited habitat created by a meteorite impact:

[Figure needs permission, redraw or new source]

Figure 57: concept diagram of an uninhabited habitat created by a meteorite impact (Cockell, 2014)

There may be many short lived habitats of this sort. For instance, lakes formed 210 million years ago on one of the flanks of Arsia Mons as a result of volcanism, two of around 40 cubic kilometers of water each, and a third one of 20 cubic kilometers of water. They probably stayed liquid for hundreds, or even thousands of years, covered in ice and kept warm by a subsurface hydrothermal system (Scanlon et al, 2014). We don’t know how quickly life can evolve - is that enough time? Could Mars have newly evolved life from 210 million years ago?

210 million years ago is relatively recent in geological time and there are other signs that Mars is still geologically active, so there may be subsurface hot spots today perhaps creating conditions for subsurface caves filled with water or sulfuric acid (Boston, 2010). These may well not be detectable from orbit. These may provide temporary conditions similar to the hydrothermal vents proposed as one of the candidates for the origin of life.

Depending how fast life evolves, perhaps life could evolve from scratch in one of these temporary habitats, then find its way to the surface, perhaps in the RSLs, and then be transported in the wind. Perhaps it may adapt to surface conditions and spread for a while in the brine layers found by Curiosity. This is just one scenario, to motivate the possibility of newly evolved life on Mars. There may be many ways this can happen.

Another way that Mars could still have early life today is that evolution slowed down on Mars and proceeded far slower on Mars than on Earth, after Mars became less habitable. Even after billions of years of slow evolution, it may still be at an early stage. According to one idea, the earliest life, all the way through to the last universal common ancestor (LUCA), might have been simple “modifiable cells” capable of taking up “naked” genetic material which evolved through lateral transfer, by Lamarckian rather than Darwinian evolution (Woese, 2002) (Woese, 1998) (Brown, 2003) (Jheeta, 2013).

If Mars has pre-Darwinian life, then, although cells themselves would not yet be in competition with each other, the genomes within them would be. These might evolve some degree of *genomic protection*, in small vesicles. As described by Koonin:

> *"Conceivably, such primitive units of evolution could have been represented by small, virus-like replicons that populated abiogenic lipid vesicles or inorganic compartments and were subject to selection for replication efficiency" (Koonin, 2014)*

This first stage may have continued for several hundred million years of evolution, through to as late as 3.5 - 3.8 billion years ago (Doolittle, 2000).

On this hypothesis for evolution of early life on Earth, the transition to Darwinian evolution of whole cells on Earth occurred at roughly the same time as the end of the Noachian period on Mars, right at the end of its period of most abundant water and seas.

This suggests a hypothesis that the Noachian period on Mars might not have been long enough time for life to evolve beyond pre-LUCA replicons in vesicles in undifferentiated cells, sharing genetic material with each other readily.

Though primitive, such life could still be adapted to the conditions it inhabits, with many specialist enzymes and other adaptations to help it to function in its extreme environment. Evolving through massively parallel Lamarckian evolution, the priority of all the cells would be easy and fast uptake of capabilities from its neighbours. Such life might have little by way of defences against modern Earth microbes.

Simpler life must have existed on Earth in the past and no trace of it now remains, and it may still exist on Mars. This section is exploring pre-Darwninian life as one example but simpler life might take many different forms.

If we find recently evolved pre-Darwinian life on Mars, or other forms of primitive early life in undifferentiated cells, we'd likely conclude early on that there is no risk to Earth's biosphere. In this case we could try terrestrial microbe challenge experiments in orbit to confirm that the Martian life is completely destroyed by Earth life. If this happens we might conclude early on that it is of no risk to Earth.

The aim wouldn't be to prove that the early life is harmless to terrestrial life, but to show that it is completely destroyed by microbes found anywhere on Earth that it might encounter. It may be hard to prove that life can spread through Earth's ecosystems and yet be harmless to terrestrial life because of the wide range of potential interactions, as with the example of mirror life nanobacteria, see:

- If Mars has nanobacteria 0.01 microns in diameter – and these are returned to Earth – could they spread through Earth in a shadow biosphere? Answer seems yes, possibly

But it may be possible to show that early life is safe if it can't compete with terrestrial life anywhere and will soon be destroyed, as may well be the case for pre-Darwinian transformable cells.

This is a similar idea to the microbe challenge experiments for the lunar samples from Apollo in the 1960s (BUCM, 1967). This could be far more thorough and detailed with modern technology. We could for instance test for any Martian genetic material left after the encounters between Martian life and terrestrial microbes.

If we have a known to be non hazardous sample of early life, in principle it could be handled as an unrestricted Earth return, through COSPAR. There might be general agreement that there are no risks to Earth's biosphere to be assessed.

In this situation concerns would be in the opposite direction, see:

- Vulnerability of early life on Mars in forwards direction - legal protection is weak, but strengthened by the laws for backwards protection of Earth

For the argument in the opposite direction, that life on Mars could be more complex than terrestrial life, see Scenario: evolution on Mars evolves faster than on Earth because of an oxygen rich atmosphere and frequent freeze / thaws of oceans, leading to life of the same genomic complexity as Earth or even greater, and with multicellularity evolving early

Permitted levels of contamination could make it impossible to prove absence of Martian life in Perseverance's sample tubes – leading to an unnecessary requirement to sterilize returned samples indefinitely

Sadly the Curiosity sample tubes are not 100% sterile. Their measurements suggest they achieved a maximum of

- 8.1 nanograms of organics per tube
- 0.7 nanograms for each of the biosignatures they tested (e.g. DNA)
- 0.00048% chance of a single viable microbe per tube – this means a 0.02% chance that at least one tube has a viable terrestrial microbe in it.

For details see

- Limitations on cleanliness of the Mars sample tubes with estimated 0.7 nanograms contamination per tube each for DNA and other biosignatures and a roughly 0.02% possibility of a viable microbe in at least one of the tubes

As stated in the NASA guide Planetary protection provisions for robotic extraterrestrial missions (NASA, 2005ppp):

> ***A "false positive" could prevent distribution of the sample from containment and could lead to unnecessary increased rigor in the requirements for all later Mars missions.***

There seems a significant possibility of a false positive which could delay certifying the samples as safe for Earth, or make it necessary to sterilize all samples returned indefinitely.

The level of contamination in the samples, though low, may still be high enough to make it hard to prove that there is no Martian life in the samples.

It will be easier to make a positive detection of life in the sample if it is there.

Early discovery of a familiar microbe from Mars such as chroococcidiopsis is not enough to prove the sample is safe – as familiar life can have new capabilities

Suppose, for example, we find a variant of chroococcidiopsis (Billi et al, 2019a) in the sample with the same biology as terrestrial life. This will mean it has a common origin, however we still need to understand its capabilities.

If we find a variant of chroococcidiopsis, it could have been transferred to Mars on a meteorite from Earth any time back to before the Great Oxygenation Event.

With possibly as much as three billion years of independent evolution in the challenging Martian environment since it left Earth, it is likely to have increased tolerance relative to terrestrial strains of:

- Ionizing radiation
- UV
- Perchlorates
- Extreme cold
- Arid conditions
- Low atmospheric pressure

It is likely to have evolved new abilities too, such as perhaps:

- enhanced nitrogen fixation to cope with low levels in the Martian atmosphere.
- Able to use red light for photosynthesis since the dust in the atmosphere absorbs much of the blue light.
- Able to photosynthesize in the ultra low light levels of an approaching or minor dust storm, perhaps using variable size chlorophyll antenna (Negi et al, 2020:15)

We would need to evaluate its capabilities and decide whether any of those capabilities are of sufficient potential impact for any Earth ecosystems or biomes to be careful about introduction to Earth.

Discovery of a familiar microbe like chroococcidiopsis does not prove all life in the sample is familiar – if terrestrial life originated on Mars, it could have extra domains of life that never got to Earth

In addition the presence of a strain of a familiar lifeform like Chroococidiopsis would not mean that all life in the sample has to be familiar.

One hypothesis is that terrestrial life originated on Mars (McKay, 2010) (Mojarro, 2020) (Kirschvink, 2006) (Kirschvink, 2013).

Benner and others have argued that Mars is a likely origin for life because (Schilling, 2015) (Geiling, 2013) (Benner et al., 2015) (Ranjan, 2017:193)

- it had a larger area of dry land for the wet / dry cycles that may have been important for concentrating organics for early life
- dry conditions are needed for RNA which can't form in water (water is corrosive for RNA).
- it had boron, and phosphates important for likely early life processes,
- boron combines with the oxygen in carbohydrates to prevent formation of tars
- It had molybdates which catalyses reactions that can convert the boron stabilized carbohydrates into ribose, needed for the formation of RNA and early life precursors.

If terrestrial life did originate on Mars then life from Mars, possibly including chroococcidiopsis, transferred the other way, from Mars to Earth. See

- Report by the National Research Council couldn't discount the possibility of past mass extinctions caused by Martian life - could the Great Oxygenation Event be an example?

In this case, life on Earth would be pre-filtered to whatever microbial lineages from Mars survived the transition to Earth via meteorite impacts.

There might be more domains of life on Mars than the three domains of life we have on Earth, the archaea, bacteria and eukaryotes.

These could be present in the same Mars sample as familiar life.

The evolution of early life on Mars might also have explored a wider diversity of nucleotides or amino acids than the domains of life that got to Earth. For more on this see

- Potential diversity of extraterrestrial life based on alternatives to DNA such as RNA, PNA, TNA, additional bases and an additional or different set of amino acids

Potential to discover multiple biochemistries such as mirror and non mirror life in the same sample – perhaps evolved in disconnected early Martian habitats – or unfamiliar life mixed with familiar life transferred from Earth to Mars in the past

We saw that it would be possible for mirror nanobacteria from Mars to co-exist with Earth life in the section:

- If Mars has nanobacteria 0.01 microns in diameter – and these are returned to Earth – could they spread through Earth in a shadow biosphere? Answer seems yes, possibly

The same could happen on Mars, nanobacteria might coexist in a shadow biosphere, perhaps with larger microbes of a different biology.

Preliminary studies would be likely to find familiar life first if there is a diversity of biochemistries in the sample. A gene sequencer, for instance, would find the genes of normal life, not mirror life. We need to bear in mind that our ability to sequence genes only works with life that closely resembles terrestrial DNA.

So, discovery of a familiar cousin doesn't mean that all Martian life in the sample is terrestrial. There could be independently originated microbes, or ones that are more distantly related, in the same sample.
So, if we find familiar life in the sample, we will need to keep searching until we understand the sample thoroughly enough to be sure this is the only form of life there.

As an example, Chroococcidiopsis, a normal cyanobacteria, could live in the same Martian microhabitat as a mirror cyanobacteria. This might be independently evolved, or both might have evolved from the same ancestor, perhaps "ambidextrous" life able to replicate both mirror and normal RNA, using something similar to Joyce's enzyme (Joyce, 2007) (Sczepanski, 2014) (Singer, 2014)

The early Mars was less connected than early Earth. The bigger impacts would create a crater lake which might form a short term disconnected habitat for 2 million years, perhaps enough time for life to evolve (Westall et al, 2013). Other habitats would last for perhaps 100 million years but still perhaps not connected to each other, for instance hydrothermal vents in bodies of water not connected to each other (Westall et al, 2013)
.
At times when Mars had its northern sea, the sea itself might be connected but other habitats in Mars might not be, apart from some mixing from subsurface movement of water (Westall et al, 2013). Also habitability would change, as lakes dried out, impact lakes formed, as the axial tilt changed, and so on. At any time some habitats would be inhabited, some uninhabite, and other locations that used to be habitable could now have dormant life (Westall et al, 2013). Amongst all those habitats some might have evolution starting again perhaps re-using organics left by previous experiments or previous life.

It's also possible that familiar life got to Mars after an ancient terrestrial impact on Earth and spread through the planet's microbiomes billions of years ago, mixing with the native life. See:

- Could life get transferred from Earth to Mars? With Earth's high gravity and thick atmosphere the challenges are far greater but may be more possible in the early solar system with impacts large enough to blow out part of Earth's atmosphere

Possibility of discovery of high risk extraterrestrial microbes needing extreme caution

In other cases it might be clear that extreme caution is needed. In this case the legal process for potentially hazardous life is required, however it would involve a known danger with known containment requirements.

If the decision is made that it is safe to return the sample provided the right precautions are taken, the legal process would be simplified, as would the design of any ground facilities. Also there would be less risk of a lapse of containment procedures through inattention, when the scientists and technicians have certain knowledge of serious possible consequences.

In some cases, such as mirror life, the decision might be made that the risk of severe effects in case of a lapse of sample handling is too high to return it to Earth early on. In this case unsterilized samples from Mars should continue to be studied in orbit, telerobotically.

Examples would include mirror life, such as a mirror analogue of the cyanobacteria chroococcidiopsis. This is a nitrogen-fixing photosynthetic polyextremophile ideally suited to conditions on Mars and also at home in many terrestrial ecosystems from the Chinese sea (Xu et al, 2016:111) to hot or cold dry deserts (Bahl et al, 2011).

In this way we never take any chances, however small, with the safety of Earth's biosphere. We also reduce upfront costs and legal complexity and build only what is required at each stage, rather than attempt a multi-purpose facility to return samples with any conceivable astrobiology.

Both the legal process and the technology to contain the sample are greatly simplified.

This is an agile adaptive approach where at each stage the process is directly evidence based, rather based on attempts at extrapolating the capabilities of hypothetical Martian biochemistry from our understanding of Earth life.

Potential for early discoveries of Martian life from samples of Martian meteorites preserved in ice at the lunar poles - likely pre-sterilised by natural processes sufficiently to protect Earth

China has already sent the first robotic missions to the lunar surface since the 1970s, and many more will be sent there in the early 2020s including sample return missions. After that, NASA / ESA have ambitious plans for astronauts to return to the Moon in the mid 2020s.

The first sample return from Mars can't get back to Earth before 2030 so this would seem to give a window of opportunity to return samples of Martian meteorites from Mars before the first samples returned from Mars.

If we are lucky the lunar poles might have sheets or layers of ice undisturbed for the last several billions of years, at liquid nitrogen temperatures. The Meteoritical Bulletin Database shows 31 Martian meteorites out of 45714 meteorites (only 16 of those doubtful) found in Antarctica as of 26 Dec 2021 (Meteoritical Bulletin Database,2021). It's much easier to spot Martian meteorites in Antarctica than in most places, so those are a good first estimate of what we can expect from the lunar poles, about 1 in 1500 of recent meteorites we find there will likely be from Mars.

Out of 332 Martian meteorites, two are of special interest for life, ALH84001, and the Tissint meteorite, Others that may be of interest include Yamato 000593, the Nahkla meteorite, the Shergotty meteorite(Gibson et al, 2001) (Gibson et al, 2012) and maybe ALH-77005 (Gyollai et al, 2019).

So that is 3, perhaps 7 meteorites of interest for life out of 332. That's about 1 to 2 in 100 Martian meteorites, which makes it 1 or 2 meteorites in 150,000 of all meteorites that might have evidence of life from Mars. It could be much more if past Martian meteorite impacts sent a lot of material to Earth after impacting into, say, an ancient habitable Martian ocean with life in it. The ice at the lunar poles would also include debris from the larger terrestrial impacts including the Chicxulub meteorite from 66 million years ago. The ice might also retain large amounts of debris from earlier impacts on Earth - which might therefore include organics from early life on Earth - and then there would also be far more by way of Martian meteorites too. None of our Martian meteorites are older than 20 million years. This is not because of a recent influx of meteorites, it is just that we can't recognize earlier meteorites as coming from Mars. Similarly there may be many rocks that are Earth meteorites from the Chicxulub impact - not bolides, rocks that achieved escape velocity and eventually re-impacted Earth - and the Moon should have many of those too.

These are rough estimates based on the current flux of meteorites. It suggests we would need a way to detect large numbers of meteorites quickly to have a chance to return a Martian meteorite early on. They might be distinctive in appearance to a trained geologist.

If we are lucky those sheets of ice may be meters thick and all the rocks in them would either be from meteorites and micrometeorites or the debris from nearby impacts.

There is a lot of interest in using the ice for In Situ Resource Utilisation (ISRU) to split the ice into hydrogen and oxygen for rocket fuel and oxygen for life support, as well as a source of water for life support. There may be enough ice there to supply us for centuries in that way.

If these optimistic scenarios turn out to be true, we may find large numbers of meteorites as an indirect result of ISRU.

We may be able to detect micrometeorites from Mars on the Moon (impossible for Earth as they burn up in the atmosphere). If we return thousands of micrometeorites from ISRU we may have a chance to recover samples of Martian life early on.

Would there be any planetary protection issues of back contamination from Martian meteorites preserved in the lunar ice? Probably the risk of back contamination is minimal to non existent because:

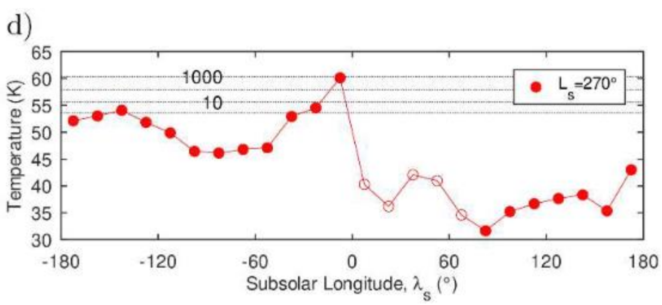
- Recent impacts on Mars are not likely to eject viable life since they can't return the surface dust, ice and salts and there is not likely to be much life below the surface except possibly below the RSLs or geothermal hot spots,
- There have been no recent impacts large enough to return material from the kilometers deep hydrosphere below the cryosphere see: Could Martian life have got to Earth on meteorites? Our Martian meteorites come from at least 3 m below the surface in high altitude regions of Mars
- The shock of ejection from Mars likely kills most life including photosynthetic life (Nicholson, 2009),
- Any life that survives ejection from Mars likely comes from an impact large enough to send meters wide fragments to Earth which gives a way for the life to get to Earth too.
- If the ice comes from near the surface of the Moon, life dormant on the Moon for tens or hundreds of millions of years would also be sterilized by the ionizing cosmic radiation and ionizing radiation from solar storms.
- Life in micrometeorites would be sterilized during the decades long minimum time for transfer from Mars to Earth.

- Life in larger meteorites on the Moon would likely be able to survive entry of Earth's atmosphere

It seems likely a careful analysis will show that there are no planetary protection issues with returning materials from Mars found in the lunar ice deposits at the poles.

Suggestion by Crotts of a subsurface ice layer on the Moon deep enough for liquid water and by Loeb of a subsurface biosphere on the Moon

The surface layers of the Moon are inhospitable for life, as they are too cold. The cold traps at the lunar poles are cold enough even to trap CO_2. The plume of gases thrown up after the LCROSS impact contained H_2O, H_2S, NH_3 (ammonia), $S0_2$, C_2H_2 (ethylene) and CO_2 (in order of abundance). However these sites are extremely cold. At the LCROSS site, surface temperatures vary from 30 to 60 K during the summer solstice and subsurface temperatures would be colder (Schorghofer et al, 2021)



Temperature over one lunation (lunar day) during the summer solstice for the LCROSS impact site in Cabeus Crater which detected water vapour and other volatiles including ethylene, ammonia and CO_2

These organics on the Moon may include organics from comets and organic rich asteroids as well as organics produced in situ by cosmic radiation. It may even contain substantial quantities of CO_2 in ultra cold traps at the lunar poles as a result of a temporary exosphere of CO_2 and water vapour after comet impacts which then condenses at the poles (Schorghofer et al, 2021)
.
At greater depths the temperatures would get warm enough for liquid water as Carl Sagan suggested. See:

- Carl Sagan's hypothesis of a subsurface habitable layer on the Moon at a depth of tens of meters – which could risk backwards contamination of Earth – and originally there was thought to be a low risk of forwards contamination

Lingam et al have suggested there could be a subsurface hydrosphere on the Moon with liquid water capable of hosting biochemistry or life, as for Mars (Lingam et al., 2020)

Arlin Crotts of Columbia University raised the possibility that the Moon might have some subsurface indigenous ice, which could form at depths deep enough for liquid water. He studied transient lunar phenomena which he postulates may be due to releases of volatiles. The Apollo missions measured outgassing of argon and radon gas which must have come from below the surface. He postulated that the transient lunar phenomena may be due to releases of pockets of gas from below the surface. If these are responsible for outgassing of volatiles, there could be local sources of high levels of volatiles enough to maintain layers of ice below the surface at a similar depth to Sagan's postulated habitable area (Crotts et al., 2009:section 1).

The Moon could have some indigenous water from hydrated minerals during formation which could survive 1000 K of heating, or if either the Earth or Theia had water rich satellites before the collision that formed the Moon and got incorporated into the forming moon after the collision (Crotts et al., 2009:section 3.3). Water is stable below 13 meters even in equatorial regions where it reaches the triple point of water. Assuming total outgassing of 7 grams per second with 0.1 grams per second of water, a plausible rate from the Apollo experiments, he finds that this could maintain an ice layer one meter thick and 125 meters in diameter in equatorial regions – and would be enough to maintain an ice layer hundreds of kilometers in diameter at the base of the regolith in polar regions (Crotts et al., 2009: section 3.1). In two other papers, he looks at existing data including evidence for outgassing and potential association with the TLPs (Crotts, 2007) and ways that the ice could be detected (Crotts, 2008).

If such a biosphere does exist it might be possible to access the liquid water by drilling, and that then would need planetary protection measures if there is a possibility of life in the water.

Could Martian life have got to Earth on meteorites? Our Martian meteorites come from at least 3 m below the surface in high altitude regions of Mars

If Earth frequently encounters Martian life, then we have no need to protect Earth with special precautions, by Greenberg's "Natural Contamination Standard" (Greenberg et al, 2001).

However, our Martian meteorites all come from at least 3 meters below the surface (Head et al, 2002), and left Mars over a period spanning 20 million years. They were probably thrown up into space after glancing collisions into the Elysium or Tharsis regions, high altitude southern uplands (Tornabene et al, 2006). The atmosphere for these high altitude regions on Mars is thin, making ejection to Earth easier. With such a thin atmosphere, present day life at those altitudes is unlikely (except perhaps for deep subsurface geothermal hot spots).

So it seems unlikely that any life has got to Earth in the last few million years. The Martian meteorites we have are from one of the least likely to be habitable regions on Mars, the sub-surface of the high altitude Martian uplands.

Larger impacts could send material to Earth - but unlikely to transfer fragile surface dirt, ice and salts

Larger impacts in the recent geological past could send material to Earth from other potentially more habitable parts of Mars. However:

- **Many proposed habitats are in surface layers of dirt, ice and salts**. These would likely never get into space
- **Other proposed habitats are millimeters below the surface of rocks**. These layers would ablate away during entry into the Earth's atmosphere
- **Life on Mars could be extremely localized to only a few square kilometers over the entire planet,** for instance, only to the RSL's, or only above geological hot spots, making it less likely that the habitats are hit by an asteroid able to send material all the way to Earth in the large chunks needed for protection from cosmic radiation during the transfer.
.

It was easier for Mars to exchange life with Earth in the early solar system. However even the ejecta from an impact into a Martian ocean need not necessarily transmit life to Earth.
To get to Earth, Martian life has to withstand the shock of ejection. Chroococcidiopsis is killed at 10 GPa and the shock of ejection from Mars is 5 - 55 GPa based on analysis of the Mars meteorites. More shock resistant microbes can survive better. Of the order of 1 in 10,000 of microbes of b. subtilis and the photobiont and microbiont partners in the lichen X Elegans could survive 40 to 50 GPa (Nicholson, 2009). In one paper, samples of a marine photosynthetic algae nannochloropsis oculata frozen in ice were able to survive 6.93 km / sec impacts into water with approximate shock pressure of 40 GPa (Pasini, 2014).

For larger impacts such as happened in the early solar system, some deep subsurface layers are sent to orbit with much less shock. These low levels of shock arises from interaction between the shock wave moving away from the forming crater and a reflected shock wave moving backwards. The shock moving back is 180 degrees out of phase so the two shock waves cancel, creating a lightly shocked "spall" zone where the two interact. The spall zone depth is proportional to the radius of the impactor, so a large impactor would have a thicker spall zone. Some of the ejecta would survive shock of less than 1 GPa (Mileikowsky, 2000: 393)

The Martian life then has to survive the fireball of exit from the Martian atmosphere. The lower gravity reduces the Martian escape velocity from 11.19 to 5.03 km / sec (NASA, n.d.mfs), but the Martian atmosphere has to have nearly three times the mass of the Earth’s atmosphere for the same surface pressure, and the Martian atmosphere was likely several bars for early Mars (Mileikowsky et al, 2000: 423).

It then has to survive the cold and vacuum conditions of space and cosmic radiation. Cosmic radiation sterilizes the surface of a meteorite to a depth of 2 cm within 100,000 years by breaking up the nucleic acids . That's below the maximum depth you'd expect to find photosynthetic life in normal circumstances, even in fine cracks.

It is theoretically possible for some rocks to get to Earth as soon as ten years after ejection from Mars. But most take between a hundred thousand and ten million years to get there. Assuming a maximum ejection velocity of 6 km / sec, in a simulation with 2100 particles, incorporating the gravitational effects of all the planets from Venus through to Neptune, most took over 100,000 years in transit. The fastest transfer in the simulation was 16,000 years (Gladman et al, 1996).

It also has to survive the fireball of re-entry to Earth, Cockell inculcated an artificial gneiss rock with Chrooccoccidiopsis at a depth where it occurs naturally, and affixed it to the re-entry shield of a Soyuz rocket. None survived re-entry, nor did any organics. He concluded that it might not be impossible for photosynthetic life to get to Earth from Mars, but it would need an extraordinary combination of events (Cockell, 2008)

Some terrestrial extremophiles might survive these processes but the fireball of re-entry would sterilize most of them.

The interior of a rock can be better protected. The interior of ALH84001 never got hotter than 40°C during entry into our atmosphere (Weiss et al, 2000). But how does the photosynthetic life get deep into a Martian rock? It can flourish in cracks, if light filters in through them - but that also would give cracks that channel hot gases into the interior of the rock during re-entry. Cracks like that would also be places where the rocks are quite likely to break apart during ejection from Mars or re-entry to Earth.

Charles Cockell's concludes that it might not be impossible for photosynthetic life to get to Earth from Mars, but it would need a rather extraordinary combination of events (Cockell, 2008):

> "Thus, the planetary exchange of photosynthesis might not be impossible, but quite specific physical situations and/or evolutionary innovations are required to create conditions where a photosynthetic organism happens to be buried deep within a rock during ejection to survive atmospheric transit."

His final conclusion is that photosynthetic life has the potential to make dramatic changes to a planet, but that this transfer of photosynthetic life is less likely than for heterotrophs (which use organic carbon) or chemotrophs (which use chemical reactions as a source of energy and synthesize all their organics from carbon dioxide, living in places such as hydrothermal vents).

In addition, panspermia experiments are based on capabilities of terrestrial life. Capabilities of any native Martian life are unknown. Many Earth microbes could not survive this journey.

It’s not impossible that Martian life made the transition. However, even if there has been some transfer of llife from Mars to Earth, there are likely to be many species of Martian life that don’t have the capability to get to Earth in this way, as for their Earth counterparts, either because they live in fragile habitats like dust and salts that can’t be transferred via meteorites, or because they don’t have the extremophile adaptations needed to be able to survive the transfer.

So we can’t apply the Greenberg “Natural Contamination Standard” (Greenberg et. al, 2001) for microbial life from Mars. It’s possible that a sample return could return microbes that wouldn’t be able to get to Earth on meteorite impacts.

Could life get transferred from Earth to Mars? With Earth’s high gravity and thick atmosphere the challenges are far greater but may be more possible in the early solar system with impacts large enough to blow out part of Earth’s atmosphere

In the opposite direction from Earth to Mars, the challenges are far greater. When meteorites hit Earth, the deceleration during reentry slows down the initial impact velocity of kilometers per second to meters per second. In the opposite direction, an ejected rock has to be travelling fast enough to pass through the atmosphere fast enough to leave it at 11.2 km / second, the escape velocity of Earth. This means it has to leave Earth’s surface at far higher than 11.2 km / second. Although ejection at that speed is possible, and the Chicxulub impact sent meteorite fragments as far as Mars, the shock of ejection and the fireball of exit from Earth’s atmosphere would have likely sterilized any life on the meteorites before they left Earth’s atmosphere.

The best opportunity for transfer from Earth to Mars is after very large impact events, large enough to blow out at least part of Earth’s atmosphere, so leaving a low pressure region for the ejection fragments to exit through (Stöffler et al, 2007)..

> *“'Lithopanspermia' also includes a potential transfer of microorganisms in the opposite direction, i.e., from Earth to Mars. A direct transfer scenario is severely limited because very high ejection velocities in the solid state are required to escape the Earth's gravity field and to pass its dense atmosphere. Favorable transfer conditions may be only achieved by very large impact events, which blow out at least part of the atmosphere. Such impact events happened frequently during the 'early heavy bombardment phase',”*

So it is possible that terrestrial and martian life is related through transfer from Earth to Mars, especially if early life had the capabilities to survive transfer from impacts large enough to blow out part of Earth's atmosphere, but if so the two biospheres have likely evolved separately for billions of years.

There could also be unfamiliar life on Mars that evolved there independently co-existing with life that was transferred from Earth to Mars.

Report by the National Research Council couldn't discount the possibility of past mass extinctions caused by Martian life - could the Great Oxygenation Event be an example?

If it turns out that some terrestrial microbes did originate on Mars and transferred from Mars to Earth in the past, this does not mean it is safe to return material from Mars today. Martian life might have already harmed Earth's biosphere in the past. We wouldn't know.

The National Research Council looked into this question in their "Assessment of Planetary Protection Requirements for a Mars Sample Return". They were unable to rule out the possibility that life from Mars could have caused past mass extinctions on Earth (Board et al, 2009: 48).

They gave no examples, but the Great Oxygenation Event could be relevant. Chroococcidiopsis may be partially responsible for the oxygenation of our atmosphere. One minority view explains the unusual ionizing radiation resistance of Chroococcidiopsis as a natural adaptation of Martian organisms (Pavlov et al, 2006). This is weak evidence since the ionizing radiation resistance of chroococcidiopsis could be a byproduct of the repair mechanisms that chroococcidiopsis uses for UV resistance and desiccation resistance. Cyanobacteria originated in the Precambrian era. It could have developed these mechanisms back then, when, with no oxygen in the atmosphere, there was no ozone layer to shield out UV radiation (Casero et al, 2020) (Rahman et al, 2014)

However, the early Martian atmosphere was rich in oxygen (Lanza et al, 2016) before Earth and though much of that may well be due to ionizing radiation from solar storms splitting the water it's not impossible that it had photosynthetic life.as well.

Some astrobiologists have hypothesized that terrestrial life originated on Mars..

Whether or not chroococcidiopsis caused the Great Oxygenation Event – it gives a practical example of a way life from another Mars-like planet could in principle cause large scale changes to an Earth-like planet

If something like chroococcidiopsis evolved on Mars, it is not impossible it got to Earth via meteorites - though as we saw in the last section, ejection from Mars would be a major challenge (Cockell, 2008).

Whether this happened for Mars and Earth, it does give a practical example of a way that life from another planet such as Mars could in principle cause large scale changes to an Earth-like planet.

So was this an extinction event? The Great Oxygenation Event might have forced rapid evolution rather than extinction. Early anaerobes may have retreated to anaerobic habitats as obligate anaerobes, which we still have today (Lane, 2015).

However, there is some evidence suggesting extinctions. There is evidence of exceptionally large sulfur reducing bacteria from this time, 20 to 265 µm in size, which also occasionally occur in short chains of cells. This may be part of a diverse ecosystem that predated the GOE (Czaja et al, 2016). If such an ecosystem existed, most traces of it are gone now. However it seems not impossible that the GOE had major impacts on a prior diverse ecosystem.

There are many other confirmed mass extinctions in the fossil record. In many cases the cause is not fully known or debated leaving it possible that microbial transfer from Mars could be part of the explanation.

Scenario: evolution on Mars evolves faster than on Earth because of an oxygen rich atmosphere and frequent freeze / thaws of oceans, leading to life of the same genomic complexity as Earth or even greater, and with multicellularity evolving early

We saw that there is a strong case for life on Mars to be at an early stage, see

- Possibility of early discovery of extraterrestrial microbes of no risk to Earth such as pre-Darwinian life as suggested by Weiss – if microbial challenge experiments show they are quickly destroyed by pervasive terrestrial microbes (above)

However we can also argue a strong case in the opposite direction too. in this scenario it goes in the other direction, as advanced as Earth life, perhaps even more complex, with more evolved genomes than for Earth life.

Perhaps rapid evolution would be favoured by the many changes in habitability of early Mars or by the high levels of oxygen in early Mars. For the changes in habitability see:

- Evidence that habitability of Mars frequently changes in brief episodes of warmer conditions (above)

If this is true, it is not impossible Mars developed its first multicellular life billions of years before Earth did.

Genetic complexity needn't mean intelligent life. This could mean microbial life, sponges, lichens, molds and so forth with genes more complex than any equivalent Earth life has yet developed. E.g. the Martian chroococcidiopsis might have had the equivalent of another several billion years of terrestrial evolution and have a wider variety of capabilities than any of the terrestrial strains. Or perhaps multicellular life evolved on Mars many times, and has been able to explore types of multicellular life novel to us and not classifiable as multicellular algae, fungi, animals or plants.

The frequent freezings of the Martian oceans in early Mars, possibly every Martian year when its eccentricity is high, and the ionizing radiation, might have led to populations repeatedly reduced to a fraction of the previous numbers, then rapidly growing again. Boyle et al argued a similar process led to the development of multicellular life on Earth during its "snowball Earth" glaciations (Boyle et al, 2007).

Their suggestion is that during snowball Earth phases, colonies would often be founded by a single cell from the previous generation, the founder effect, leading to habitats colonized by large numbers of almost identical cells. These cells would be confined to small habitats, and so encounter each other more often, increasing the benefits of mutual altruism. The rate of reproduction would also be slow, reducing the benefit to "cheats" that do not contribute to the benefit of the colony as a whole.

In this scenario, they suggest, there would be more importance in mutually beneficial modification of a microhabitat through production of chemicals that are costly for individual cells to produce. They suggest that differentiation of cells, the first steps towards multicellularity, would be especially useful in harsh conditions.

Although they do not apply their theory to Mars, these are conditions that applied to early Mars frequently.

This process would still continue today, with the frequent short term changes in habitability of Mars. See:

- Evidence that habitability of Mars frequently changes in brief episodes of warmer conditions (above)

Another possibility is that oxygen triggered the explosion of multicellular life. The last common ancestor of the eukaryotes may have lived between 1.855 and 1.677 billion years ago. That's at a time when the oceans were only moderately oxygenated. Most of the varieties (clades) of eukaryotes diverged before 1 billion years ago, probably before 1.2 billion years ago. But the huge diversity we have today within those clades only started 800 million years ago when the oceans started to change to their modern chemical (Parfrey et al, 2011). Curiosity's Chemcam instrument found manganese oxides which suggest that at the time of Gale crater lake, three billion years ago (NASA, 2017). the water was oxygen rich (Lanza et al, 2014).

So, perhaps the case can be argued both ways, that the harsh conditions could have slowed down evolution, or that the ionizing radiation and the frequent "snowball Mars" phases, combined with the oxygen rich early atmosphere and frequent localized temporary habitats and the oxygen rich brines of present day Mars, could have triggered a more rapid evolution on Mars, and possibly even complex multicellular life billions of years before it became common on Earth.

If Mars had multicellular life early on, perhaps that multicellular life is still there, as a relic biosphere. Stamenković et al, 2018 research suggests the possibility of enough oxygen for simple animal life such as sponges exploiting the oxygen in extremely cold oxygenated salty brines when the axial tilt of Mars is less than 45 degrees.

As we saw, present day Mars may also have conditions for oxygen rich brines anywhere on the surface, by taking up oxygen from the atmosphere, a process that happens most easily in cold conditions. Extremely cold brines in polar regions could reach oxygen saturation levels similar to those needed for primitive sponges (Stamenković et al, 2018) (Walker, 2019). The south pole subglacial lake (Orosei et al, 2018), (Witze 2018), if they exist, may provide habitats for multicellular life. These habitats may also be oxygen rich, through radiolysis of the ice, favouring animal life (Walker, 2019: section on subglacial lakes), (Stamenković et al, 2018). See:

- Some Martian brines could be oxygen rich permitting aerobes or even primitive sponges or other forms of multicellularity - Stamenković's oxygen-rich briny seeps model (above)

Mars also has times of volcanic activity leading to hydrothermal systems where ice meets with lava. These lead to lakes that last for thousands of years, as happened 210 million years ago on one of the flanks of Arsia Mons, two lakes with around 40 cubic kilometers of water each, and a third one of 20 cubic kilometers of water, liquid for hundreds, or even thousands of years (Scanlon et al, 2014). Our infrared mappers can only directly measure the top few millimeters of the surface, and there could be present day hydrothermal systems at depths of up to tens or hundreds of meters below the surface, where biological activity may still survive (Nisbet et al, 2007, page 108ff).

If it is true that the rapid changes in habitability and or the oxygen speed up evolution of multicellular life, this could also lead to a scenario of frequent extinction and then renewed evolution of multicellularity.

This is similar to Cockell's suggestion (Cockell, 2014):

> *There are other trajectories of greater complexity that can be envisaged. Examples include an inhabited Mars on which life becomes extinct and then reoriginates (or is transferred from Earth) at some later time.*

The step from unicellular to multicellular life could be something that happens on Mars frequently as it fluctuates in habitability. Primitive multicellular creatures such as sponges and lichens might evolve anew during the longer periods of habitability and then go extinct again over and over in Martian history.

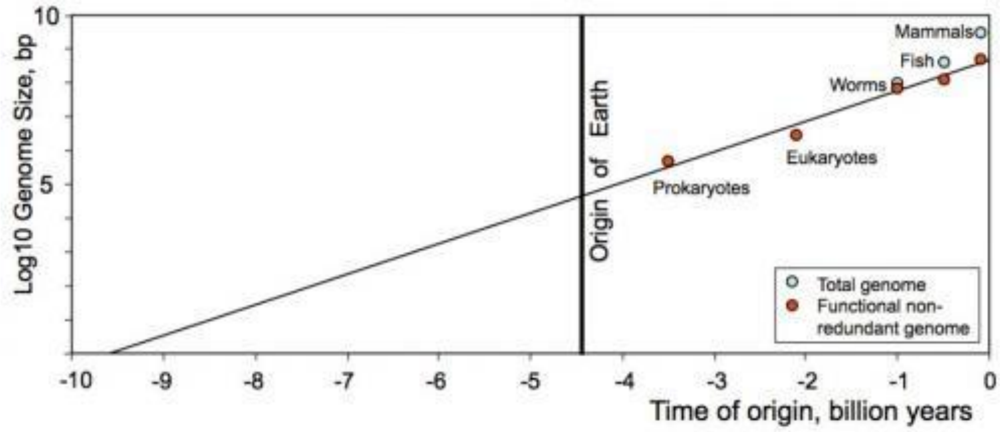
One possibility is life on Mars and Earth has a common origin, seeded from each other. Both could also be seeded from other stars in the birth nebula of our solar system which could exchange life readily when the stars were closer together (Valtonen et al, 2008) (Belbruno et al, 2012). Life in our sun's birth cluster could also originate in a star older than our sun, spread from cluster to cluster by life bearing stars (Adams et al, 2005)

Sharov et al. graphed genetic complexity of non redundant nucleotides against the time of origin of an organism, and found that the complexity of the most complex organism increased at almost the same uniform rate of exponential increase of a 7.8 fold increase in complexity every billion years (log of complexity increased by 0.89 every billion years) (Sharov, 2006).
To get this graph, Sharov et al. first had to deal with the issue of "junk DNA" as it is popularly called, DNA that doesn't encode for proteins. For the prokaryotes, cells without a nucleus including the archaea and bacteria, there is a single genetic sequence in a closed loop and nearly all is functional. However for eukaryotes, cells with a separate nucleus enclosed in a membrane inside the cell, often most of the DNA is non coding, doesn't make proteins.

Some eukaryote microbes have more DNA than a human being - much of that consisting of Transposable Elements (TEs) which sequences that are either copied to RNA and pasted back into the DNA (retrotransposons) or cut and pasted directly from one part to another of the DNA (transposable DNA) (Pray, 2008) (Elliot et al., 2015). They can sometimes take some of the gene sequence along with them when they jump (Pray, 2008). Some of these eventually get incorporated into genes that code for proteins(Elliot et al., 2015), for instance, 2% of the genes encoding proteins in rice are chimeric proteins that have TEs as part of the gene sequence (Sakai et al., 2007).

However, a lot of the gene sequences in eukaryotes seem to serve no function – if they were removed the organism would likely behave much the same way, except that they can slow down replication as there is more gene sequence to copy each time a cell replicates, and they are useful for future evolution of chimeric protiens. This is the so called C Value Enigma (Nicolau et al., 2021). Measuring the DNA by functional non redundant nucleotides deals with that issue.

Projecting back, if evolution of genetic complexity continued at the same rate since the origins of life, Sharov finds that Earth life originated around ten billion years ago. If so, life on Earth could be billions of years older than our solar system (Sharov, 2006).



This diagram shows an estimate for the complexity of each type of organism when it first appears in the record. It uses the complexity of the DNA as measured using the number of functional non redundant nucleotides (Sharov, 2013). This is a better measure of the genetic complexity than the total length of its DNA.

The graph is from (Sharov, 2013:Figure 1) with the section from the origins of Earth onwards also in (Sharov, 2006), which also explains in detail how it was derived.

Notice that the prokaryotes; the simplest primitive cell structures we know; are well over half way in complexity between the potential earliest forms of life and ourselves.

Mammals have around 3.2 billion base pairs or 3.2×10^9, but only 5% is conserved between species. Sharov et al. add another 10% as their estimate for other regions that are likely functional but vary between species for a total of 480 million base pairs in (Sharov, 2006).

The found that the smallest prokaryote base pair has 500,000 base pairs (for Nanoarchaeum equitans and Mycoplasma genitalium) or 5×10^5. These microbes are host dependent and don't make all their proteins, but they used them as an estimate for the size for the first most primitive prokaryotes in (Sharov, 2006),.

Sharov et al. found that plants gain in complexity more slowly than mammals. The first flowering plants had a third of the genetic complexity of mammals yet appear in the record at around the same time. The most complex archaea increased only 1.9 fold every billion years and the most complex Eubacteria increased only 2.5 fold every billion years (Sharov, 2006).

However, if the constant is planet dependent, Martian life could exist at a different stage of complexity. Perhaps abundance of life leads to faster evolution of non redundant genomes. If so Earth might have faster exponential growth in complexity than Mars. Or perhaps the frequent alterations between more and less habitable and disconnected habitats where life can evolve separately could lead to more diversity and faster genomic evolution, then Mars could have faster exponential growth in complexity than Earth.

Martian life could be at a less advanced level or a more advanced level than terrestrial life, with genome sequences either less or more complex than we have on Earth at present. Indeed, it would be a significant coincidence if the genomic complexity of present day independently evolved life on Mars is identical to the genetic complexity of terrestrial life.
For instance, if Mars has chroococcidiopsis with a common ancestor with Earth, is it possible that it could have greater genomic complexity than any of its terrestrial strains?

Also though Mars couldn't have evolved mammals, could it have evolved other forms of life at a similar level of genomic complexity, Or would the maximum complexity be similar to the fastest evolving terrestrial microbes or plants, or be much less than either of those?

Potential diversity of extraterrestrial life based on alternatives to DNA such as RNA, PNA, TNA, additional bases and an additional or different set of amino acids

Martian biochemistry may not resemble Earth life. The simplest form of transformation is mirror life, and we use this as the main example because it is universally recognized as a possibility. Another possibility is RNA world life which doesn't have DNA. Other possibilities include PNA or TNA which have a different backbone from DNA or RNA (NASA, 2001),

However there are many ways now known to construct the backbone of an informational biopolymer. Some of these might also potentially be available to extraterrestrial life.

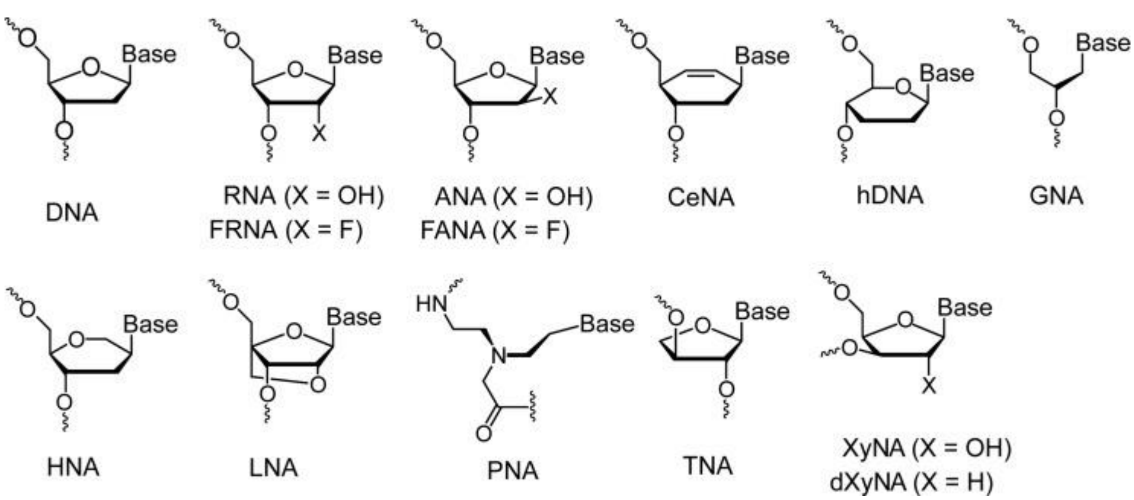


Figure 58: some of the proposed “backbones” for alternatives to DNA for a bioinformational polymer [Figure 2 of (Anosova et al, 2015)]

These can then be combined with each other in a couple of dozen different paring systems.

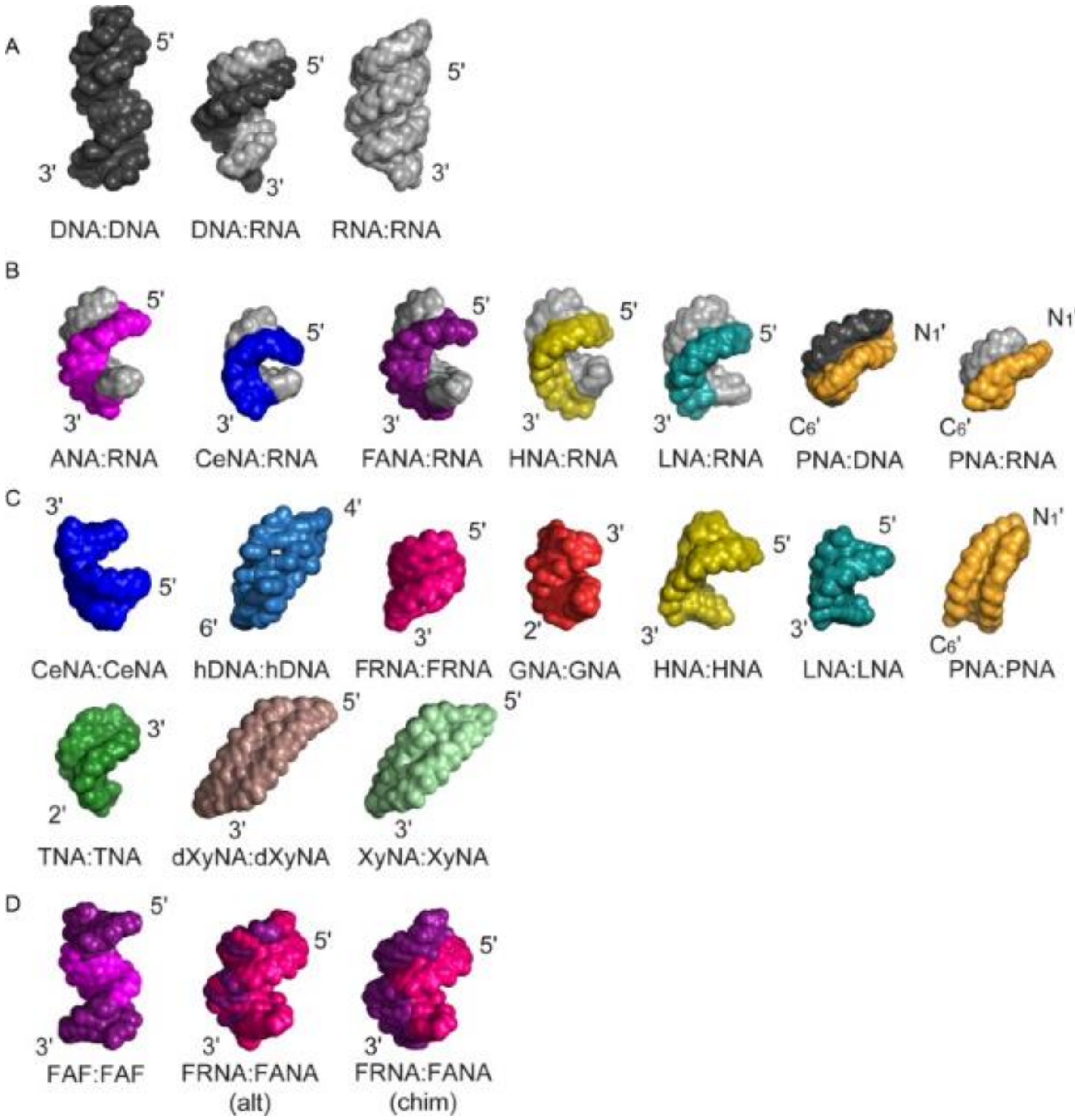


Figure 59: Some of the base pairing systems that could be used for synthetic biopolymers and may be available for extraterrestrial life.

[Figure 3 of (Anosova et al, 2015)]

Then as well as a diversity of backbones, Martian life that uses the same two biopolymers as terrestrial life might use additional bases. Two extra bases “X” and “Y” have been added to DNA, and the resulting microbe could make a fluorescent green protein that included unnatural amino acids (Zhang et al, 2017).

This was later expanded to an eight base system called Hachimoji

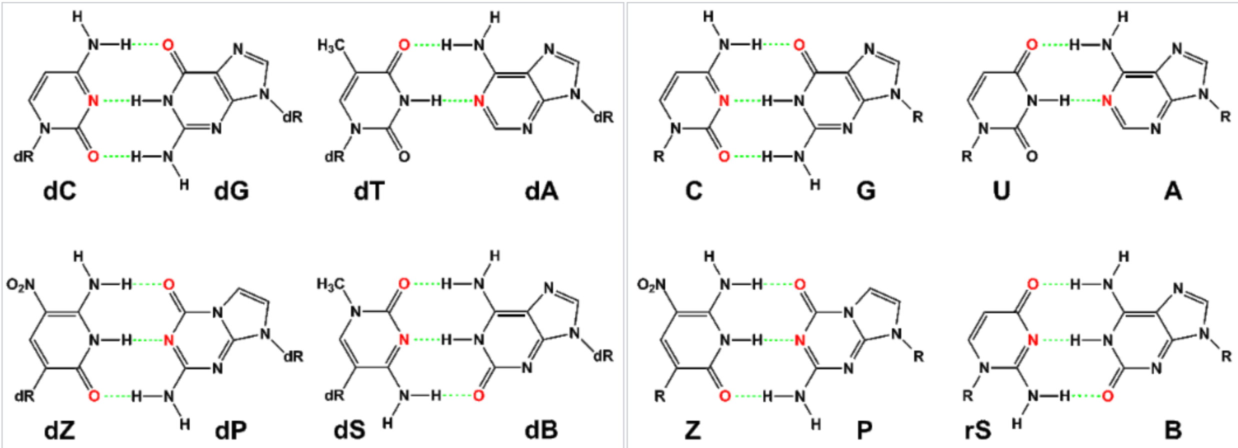


Figure 60: The eight base Hachimoji system which extends the four bases of conventional DNA and RNA.

[Figure 1 of (Hoshika et al, 2019) as redrawn by (WolfmanSF, 2019)]

Then life with the same backbone and the same bases can still have differences in the proteins. Proteins are built from amino acids, and of the 20 amino acids (or 22 including the two non standard amino acids) coded for by RNA, several have changed assignment, which shows that the language is flexible. The ones in blue in this diagram have had changes of assignment in some organisms.

[Figure needs permission, redraw or new source]

Figure 61: Codons shown in red have changed reassignment. The two amino acids coloured in red in the outer circle are non standard amino acids (selenocysteine and pyrrolysine). The black squares denote stop codons

[Figure 1 of (Ambrogelly et al., 2007)]

It's clear that biology could use many more amino acids than the 20 encoded. There are 140 that occur naturally in terrestrial biology, but not in proteins (Ambrogelly et al., 2007). 52 amino acids have been identified in the Murchison meteorite (Cronin, 1983).

A computer search turned up nearly 4,000 biologically reasonable amino acids (Meringer, 2013) (Doyle, 2014). Many of those won't occur in nature, but terrestrial biology also includes non natural amino acids. Meanwhile also many of the natural amino acids don't occur in terrestrial biology and might potentially be used in extraterrestrial biology.

Could present day Martian life harm terrestrial organisms?

First, Martian life could survive on Earth. Oxygen in Earth's atmosphere is not likely to inhibit Martian life given that the Mars surface has highly oxidising peroxides, and hydrogen peroxide. The Martian atmosphere also has 0.13 - 0.19% oxygen (Trainer et al) in the atmosphere and oxygen is also possibly present at higher concentrations in the cold brines (Stamenković et al, 2018). See

- Some Martian brines could be oxygen rich permitting aerobes or even primitive sponges or other forms of multicellularity - Stamenković's oxygen-rich briny seeps model (below)

Martian life is likely to have antioxidants similar to the terrestrial antioxidant enzymes such as superoxide dismutase to convert superoxide radicals into hydrogen peroxide, and catalase to convert hydrogen peroxide into water and oxygen gas (Goodsel, 2004).

Martian life doesn't need to be adapted to terrestrial biology to harm terrestrial organisms. One example here, micro-algae produce secondary metabolites including accidental liver toxins. These often damage the livers of cattle and dogs that eat the algal mats that often form in the Great Lakes (Hoff et al, 2007). Algae also produce accidental neurotoxins and dermatoxin. These secondary metabolites may be used by the microbes to deter microbial competition (Leflaive et al, 2007).

Microbes also produce accidental toxins that harm humans. Warmflash gives examples such as Tetanus, Botulism and Ergot disease all of which are caused by microbes that infect us, or that we ingest, that produce accidental toxins.(Warmflash, 2007). As these examples show, there is no need for Martian life to be adapted to us for it to produce coincidentally toxic substances like this.

Martian microbes could cause infectious diseases too. We mentioned legionnaires' disease as an infection of biofilms that can also infect human lungs (Warmflash, 2007). Let's try to fill this out in a bit more detail.

The adaptations that legionella pneumonia have are specific to terrestrial protists (single cell eukaryotes, with nucleus and organelles, but not part of a fungus, animal or plant). L. pneumonia is able to enter the white blood cells, the phagocytes that normally would eliminate pathogens. It can survive in vacuoles that don't fuse with the lysosomes that would normally destroy the pathogen. It's able to inhibit that fusion (Todar, 2006).

Martian life could be closely related to terrestrial life through panspermia, transferred to Earth on meteorites in the early solar system, or perhaps in the other direction from Earth to Mars. If so,the Martian biosphere could include protists, or closely related organisms. Most protists are aerobes, but there are terrestrial anaerobic protists and even a small animal, loricifera that never uses oxygen at any stage of its life-cycle (Fang, 2010). Also if there are indeed significant amounts of oxygen in the Martian brines (Stamenković et al, 2018), this would expand the possibilities for Martian protists.

So the first possibility is a pathogen of Martian biofilms sufficiently closely related to terrestrial life that it is already adapted to infect protists and the white blood cells. It would use the same mechanisms that let it avoid digestion by protist analogues on Mars to infect similar cells on Earth.

Legionella doesn't form spores, and surely couldn't survive on Mars and wouldn't be in the sample return. However, a Martian analogue would be likely to evolve spores or some other way to survive dust storms and spread to a new habitat. Such a capability would be highly favoured in the Martian environment. Using the same methods it could then survive the journey back to Earth in the sample container.

On the other hand, if Martian life is widely separated from terrestrial life, evolutionarily, with an independent origin or it split off before most of the capabilities of modern life evolved, there is a possibility that the Martian pathogen would not be recognized as life by terrestrial biology and terrestrial life wouldn't mount an immune response *(Lederberg, 1999b)*. In this case the pathogen might be ignored by the white blood cell phagocytes, and it might live in the intercellular spaces in our lungs.

One of the microbes best able to grow in Mars simulation conditions of low atmospheric pressure, CO_2 atmosphere and low temperature is Serratia liquefaciens (Schuerger et al, 2013) (Fajardo-Cavazos et al., 2018).

S. liquefaciens is a widespread bacteria in the environment, found in soil, water, and also associated with plants and animals (Grimont et al, 1978). It is also motile, capable of swimming, biofilm formation and also synchronized biofilm swarming at a rate of up to 1 cm per hour (Eberl et al, 1999).

S. liquefaciens is an opportunistic human pathogen (Fajardo-Cavazos et al., 2018). It can also colonize the human respiratory tract, and urinary tract. S. Liquefaciens is a frequent cause of nosocomial outbreaks (outbreaks in hospitals) usually due to lapses in hygiene and is sometimes fatal (Mahlen, 2011) with examples of deaths of children in Ghana (Ikumapayi et al, 2016) and US recipients of blood contaminated by it (Roth et al, 2000). It has also caused eye infections, urinary tract infections, bloodstream infections, abscesses, septic arthritis, and fatal meningoencephalitis (Mahlen, 2011:769).

Martian microbes could also infect directly as biofilms. Over 80 percent of human microbial infections are associated with invasive biofilms, and moreover, many of these biofilms are particularly antibiotic resistant (NIH, n.d.) (Lebeaux et al, 2013).

Our antibiotics might not work with Martian life. They target specific enzymes and processes within living cells based on Earth's biochemistry (Kapoor et al, 2017). Let's take penicillin as an example. It targets transpeptidase which is essential for cross linking in the final stage of cell wall synthesis to make rigid cell walls (Yocum et al, 1980). It does that by forming a highly stable penicilloyl-enzyme intermediate. One way that microbes develop resistance to this antibiotic is by using different enzymes that perform the same function in the cell (Gordon et al, 2000). For related life, a gene that modifies those processes can give a microbe antibiotic resistance even if it isn't actually originally evolved to develop resistance, indeed, even if it never encountered the antibiotic. Meanwhile an alien biochemistry might have different enzymes already, through independent evolution.

When human pathogens develop antibiotic resistance, this often comes from other microbes by horizontal gene transfer, as they arise too quickly for the microbes to evolve it themselves. These resistance genes are found for every type of antimicrobial (Martínez, 2012).

Many of the naturally occurring antibiotic resistance genes probably originate in microbes that make those antibiotics themselves and need the resistance gene to protect themselves from their own antibiotics. But the gene that gives antibiotic resistance to quinolones, a new non naturally occurring synthetic antibiotic, seems to have originated in a Shewanella algae which doesn't produce antibiotics itself. So it seems likely to have a different role in it (Martínez, 2012).

In the same way, even related Martian microbes could have antibiotic resistance through genes evolved for other purposes on Mars that lead to their internal processes changing in ways that make the antibiotics no longer effective

We mentioned earlier in this article that the endolithic yeast Exophiala jeanselmei can survive simulated Martian conditions, without any source of water except atmospheric humidity (Zakharova et al, 2014). See Experiments with black yeasts, fungi and lichens in Mars simulation conditions suggest life could use the night time humidity directly without liquid water (above)

Exophiala jeanselmei is closely related to opportunistic human pathogens. It can be an opportunistic human pathogen itself, causing superficial and localized infections in humans, in skin, nails, cornea and superficial wounds and is occasionally serious for immunocompromised individuals and is naturally resistant to most antifungals on the market (Urbaniakt al, 2019).

Most healthy people have fungi in their sinuses, but these are harmless to them. Sometimes in patients with normal immune systems, these may form “fungal balls” that occupy the empty spaces in our sinuses.

When the immune system is not functioning properly, fungi can penetrate mucosal barriers and the epithelial layer and invade the host tissues and when this happens the results can be serious (Soler et al, 2012). A diverse range of fungal species can cause a lethal infection in immunocompromised hosts and these are often resistant to antibiotics (Pfaller et al, 2004) Opportunistic fungi kill an estimated 1.5 million people worldwide every year (Brown et al, 2012).

We have only a few effective antifungal medicines, making antifungal resistant microbes a problem (Cowen et al, 2015). Alien life might be naturally antifungal resistant, if they don't have the biochemistry targeted by antifungal medicines.

It may be a similar situation for immune systems. An alien microbe, perhaps a disease of biofilms invading our lungs might not be recognized as a threat by our bodies.

This is how John Rummel put it in the foreword to "When Biospheres Collide":

> *"Likewise, we don't know what would happen if alien organisms were introduced into Earth's biosphere. Would a close relationship (and a benign one) be obvious to all, or will Martian life be so alien as to be unnoticed by both Earth organisms and human defenses? We really have no data to address these questions, and considerate scientists fear conducting these experiments without proper safeguards. After all, this is the only biosphere we currently know - and we do love it!"*

Joshua Lederberg, who got his Nobel prize for his work on microbial genetics was a key figure in the early work on planetary protection (Scharf, 2016). He first began to give it his attention in 1957 (Lederberg, 1959). He put it like this:

> *"Whether a microorganism from Mars exists and could attack us is more conjectural. If so, it might be a zoonosis to beat all others. On the one hand, how could microbes from Mars be pathogenic for hosts on Earth when so many subtle adaptations are needed for any new organisms to come into a host and cause disease? On the other hand, microorganisms make little besides proteins and carbohydrates, and the human or other mammalian immune systems typically respond to peptides or carbohydrates produced by invading pathogens. Thus, although the hypothetical parasite from Mars is not adapted to live in a host from Earth, our immune systems are not equipped to cope with totally alien parasites: a conceptual impasse."* (Lederberg, 1999b)

Our immune system and defenses are keyed to various chemicals produced by Earth life. such as peptides and carbohydrates. Mars life might use different chemicals. In the best case (for us), the Martian microbes are unable to make anything of terrestrial biochemistry and give up. However, in the worst case, it's the other way around. This time, it's our defense systems that are mystified. The microbes don't resemble Earth life and so our defenses don't recognize the attackers as life or attempt to do anything about them.

Carl Sagan put it like this (Sagan, 1973:162):

> *"Precisely because Mars is an environment of great potential biological interest, it is possible that on Mars there are pathogens, organisms which, if transported to the terrestrial environment, might do enormous biological damage - a Martian plague, the twist in the plot of H. G. Wells' War of the Worlds, but in reverse. This is an*

extremely grave point. On the one hand, we can argue that Martian organisms cannot cause any serious problems to terrestrial organisms, because there has been no biological contact for 4.5 billion years between Martian and terrestrial organisms. On the other hand, we can argue equally well that terrestrial organisms have evolved no defenses against potential Martian pathogens, precisely because there has been no such contact for 4.5 billion years. The chance of such an infection may be very small, but the hazards, if it occurs, are certainly very high.

By way of example, it is possible that the skin gives little protection against Martian microbes. Its first line of defence consists of sixteen broad spectrum antimicrobial peptides and the second line of defence consists of T cell responses with inflammatory cascades in the subepithelial tissue (Abdo et al, 2020). The antimicrobials might have no effect on an alien biology and the immune response might not be triggered by it. If this were to happen, Martian life might penetrate these barriers without being noticed by our skin's defences and enter the underlying flesh and bloodstream.

Immunocompromised people are especially at risk from opportunistic pathogens such as fungi, S. Liequefaciens, etc. However for alien life we may all be effectively immunocompromised if the broad spectrum antibiotics in our skin and epithelium have no effect on the alien life, and our innate or adaptive immune systems don't recognize it as pathogenic.

Could a Martian originated pathogen be airborne or otherwise spread human to human?

There are various ways that a Martian originated pathogen could spread from human to human, for instance it could form a skin infection similar to fungal infections, and spread via contact.

In this section we will mainly look at respiratory diseases, using Legionnaires' disease as an example, to explore potential capabilities of a Martian Legionnaires' disease analogue. It might be adapted to protists similar to the terrestrial disease, or it might be a totally alien form of life that evolves on Earth to take advantage of phagocytosis to replicate. See

- Example of technician in quarantine with acute respiratory distress and symptoms similar to Legionnaires' disease – a disease of biofilms and amoebae that adventitiously infects humans – and sometimes mentioned in planetary protection discussions

There are many airborne microbial infections, spread to other humans through the finer droplets of the breath. These include whooping cough, meningitis, tuberculosis and pneumonia (Deacon, 2016).

So could a Martian respiratory disease be airborne? Legionnaires' disease may not seem a good example here. Although it is spread in droplets small enough to breathe in, these don't normally originate in the human breath. Legionnaires' disease is usually spread from droplets in sources such as shower heads or fountains fed by water from a contaminated tank.

However there are rare cases with good empirical evidence of person to person spread of Legionnaires disease. One case is of a mother who got it from her son after eight hours of close-up care when he was coughing (Correia et al, 2016). So, it is possible for a Legionnaires' disease analogue to be airborne.

Legionnaires' disease is symptomless in many individuals, or at least, subclinical (Boshuizen et al, 2001).

By analogy with Legionnaires' disease, an airborne respiratory disease from Mars with symptomless spreaders (Boshuizen et al, 2001) seems not impossible.

An airborne disease with symptomless spreaders would be especially hard to control using quarantine as there would be no indication that the technician is infected. Also, for an unknown pathogen, there would be no way to decide how long a quarantine period should be.

A novel unknown pathogen could be highly infectious. You would not be able to tell how infectious a novel pathogen is, for as long as the technician remains isolated from anyone else

Flu may be a suitable model for a worst case here. Flu is hard to control because much of the transmission is through asymptomatic spreaders (Hayward et al, 2014) (Leung et al, 2015). Also, flu is airborne (Yan et al, 2018) and vaccines are of limited effectiveness.

For a worst case Martian analogue, Hib is a microbial disease that especially affects children under age 5 and is airborne like flu. Many of those who get it are symptomless but it can cause severe pneumonia, and other issues, such as meningitis and death. It is controlled through childhood vaccination (WHO, 2014) (CDC, n.d.)

A Martian originated microbial airborne respiratory disease resistant to antibiotics could be as hard to control as Hib or flu once it leaves quarantine. Indeed, it could be harder since we would have no vaccines initially, and no previous experience of such a disease.

This does not need to be a probable scenario. It is enough if it is a credible worst case scenario for a Martian respiratory pathogen. If so, this would need to be considered in legal discussions of worst case situations for quarantine procedures.

There are many other possibilities for human to human transfer, for instance, one possibility is that Martian fungi could contribute to the opportunistic fungal infections that kill over 1.5 million people a year (Brown et al, 2012). They often invade the sinuses and in immunocompromised people can also cross barriers and infect tissues (Soler et al, 2012). For a martian fungus with an unfamiliar biochemistry we may all resemble immunocompromised people.

A fungus could be transferred human to human for instance via contact or through surfaces, or from humans to the environment and then back to humans.

Microplastics and nanoplastics as an analogue for cells of alien life entering our bodies unrecognized by the immune system

Our immune system could be as mystified by alien life as it is by microplastics and nanoplastics. Microplastics are of course not alive and not adapted to terrestrial life or trying to evade the immune systems in any way. Nor are they able to take advantage of the biochemistry of our bodies. This may be a good analogy for the situation where both forms of life are mystified by each other as described by Rummel, Lederberg and Sagan *(Lederberg, 1999b)* (Sagan, 1973:162) (Meltzer, 2012). For instance even if some Martian analogue of the fungus Exophiala jeanselmei (Zakharova et al, 2014) can invade our sinuses, if the biochemistry is sufficiently different, perhaps it is so mystified by our biochemistry that it can't grow there? We can't know this in advance but it is a possibility.

If an alien biology has similar capabilities to terrestrial biology, and neither form of biology has a significant advantage over the other, it can spread through the terrestrial environment. After a period of time to adapt to terrestrial conditions, evolve, and diversify, the equilibrium state might well have roughly equal numbers of cells of the alien biology in the soil, water, atmosphere and our environment generally.

Even if there were orders of magnitude fewer of the alien cells than terrestrial cells, they would still vastly outnumber nanoplastics. Initially of course there would be more of the terrestrial microbes in every microbiome, but there seems no particular reason why the end state would have more of terrestrial life, indeed there are possible scenarios where the alien biology has capabilities terrestrial life doesn't have, such as

- more efficient photosynthesis
- not requiring some of the limiting elements that terrestrial life requires, such as being able to use phosphorus in the absence of sulfur (Davies et al, 2009),
- a biology that can adapt to a wider range of temperature conditions and grow faster in cold conditions,
- if the alien biology has smaller cells on average with a more efficient, simpler biology, the alien cells might be more numerous than terrestrial cells.

We go into this in more detail in the section Martian microbes better adapted to terrestrial conditions than terrestrial life, example of more efficient photosynthesis (below)

So we need to consider a situation where alien cells are more pervasive in the environment than the microplastics and nanoplastics. Microplastics of 10 µm (10,000 nm) or less can enter the skin and cross the linings of the lungs and should be able to *"access all organs, cross cell membranes, cross the blood–brain barrier, and enter the placenta, assuming that a distribution of particles in secondary tissues, such as the liver, muscles, and the brain is possible." (Campanale et al, 2020).*

This could also happen for any extraterrestrial microbes that are ignored by the immune system. They would enter our bodies just because of their minute size, since the body is not impervious to nanoscale or microscale particles.

Though microplastics and the smaller nanoplastics are not hugely harmful to humans, they can damage our cells. Small polystyrene nanoparticles were able to stop cells from replicating, and lower cell viability (Campanale et al, 2020) Polystyrene nanoplastics can also form Polystyrene-protein coronas enclosing them, through interaction with blood. This gives them a new biological entity that hides them from the immune system and lets them translocate to all organs. (Gopinath et al, 2019)

These encapsulated nanoplastics can then enter into cells through processes such as phagocytosis where a white blood cell engulfs them to try to destroy them, unsuccessfully. They can also enter by macropinocytosis, where they are mistaken for desirable materials such as fat droplets in the blood, and they can also enter via clathrin coated vesicles, (Gopinath et al, 2019).

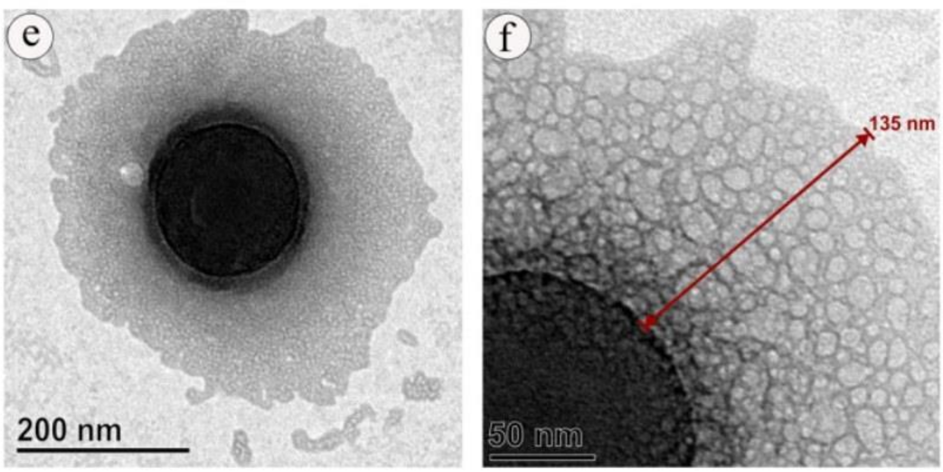


Figure 62: Coronated polystyrene nanoplastics. An alien biology ignored by the immune system might perhaps interact with the blood plasma in the same way on entering the blood and form alien chemicals / protein coronas that would hide it from the immune system and make it more likely for our cells to ingest them through phagocytosis or macro pinocytosis or clathrin coated vesicles.
[Detail from figure 2 of (Gopinath et al, 2019)]

So to summarize what we have so far, if we assume the alien life is mutually mystified and can't make anything of terrestrial life, then first, like microplastics and nanoplastics it would penetrate the epithelium from the skin, sinuses, stomach etc.

Assuming the targeted antibiotics in the epithelium have no effect, alien life would circulate in the body and blood like nanoplastics and microplastics. However, the numbers would be far higher, potentially billions of them entering the body a day, once they reach the point where they are widespread in the terrestrial environment.

This wouldn't happen immediately of course, it would likely take decades to perhaps centuries to build up to these levels, especially if only a few species were introduced to Earth, and the importation of life was stopped immediately, no more sample returns and the life already introduced had to evolve and adapt with new capabilities before it could spread to most ecosystems.

However, this process couldn't be stopped and reversed once started. Also, if a microbe was able to replicate in a terrestrial environment right away, it might overwhelm it quickly. Sagan once calculated that a terrestrial microbe with a generation time of two months could, in the absence of other ecological limitations reproduce to the point where there is as much of it on Mars as in all the terrestrial soils, within a decade (Sagan et al, 1968).

The same could happen on Earth. If a Martian microbe, perhaps one that can be spread in dust storms on Mars, was adapted to terrestrial soils already, a polyextremophile aerobe, as is not impossible, and if it is easily spread in the wind (perhaps because of adaptations to the UV on Mars), then it could be pervasive in all the soils on Earth within its habitat niche well within a decade.

So then- if this microbe is mutually ignored by terrestrial life, we might have exposure to billions of them a day as soon as a decade after the sample return breach in the worst case. It might take decades or centuries if significant adaptation is needed first or it spreads more slowly.

The worst case is that Martian microbes, as they evolve and adapt to terrestrial conditions, eventually pervade all terrestrial ecosystems and also permeate all macroscopic life which are essentially porous to them. This may take years or decades but it is a possible end state.

Given that the body, and all our organs, are likely permeable to such microbes, what happens next?

First, if the alien life can do nothing with terrestrial organics, then it might just circulate harmlessly in our blood and be present harmlessly in our organs. If it doesn't form coronas either then it might have minimal impact.

However, microplastics also give examples of side effects from chemicals released from the microplastics. Although these are for the most part minor in humans, these may be analogues to the chemicals that alien life might release for signaling, protection etc mentioned in the previous section which could potentially be more hazardous. For instance BPA, found in some plastics, is an endocrine disruptor, interfering with the systems that produce hormones. It has a relatively simple structure. $(CH_3)_2C(C_6H_4OH)_2$ (PubChem, n.d.) Amongst other things, it increases the risk of heart attacks in women to levels similar to men (Bruno et al, 2019).

We cover examples like this for potential alien life in the section Exotoxins, protoxins, allergens and opportunistic infection

Then the martian microbes could form a corona around each cell, either naturally, or adapt to do this, by producing chemicals that interact with blood plasma and basically make it sticky as for nanoplastics. These could merge to make larger accumulations of cells since they would stick to each other, again like nanoplastics, and this could cause blockages in the circulation of the blood, similarly to plaque formation in the bloodstream.

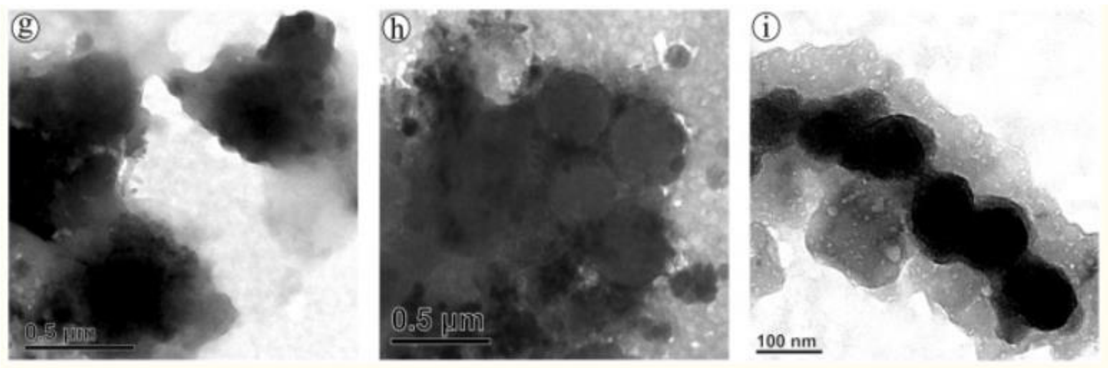


Figure 63: Coalescence of protein coronas of nanoplastics and microplastics in human blood plasma. The levels of nanoplastics in our bodies are low enough for this to not be a serious issue. However if coronated alien cells do this then it could lead to circulation issues and heart attacks.
[Detail from figure 2 of (Gopinath et al, 2019)]

At present coronated micro and nanoplastics are few in number, and don't seem to cause us noticeable problems. However when the environment is filled with trillions of alien cells, with our body essentially permeable to them, if these cells then form similar coronas in our blood stream they may be more of an issue. If those coronas stick together then conglomerates of coronated alien cells in our arteries may well cause problems such as heart attacks.

Nanomaterial exposure can also cause sterile inflammation (Leso et al, 2018) as a result of the secretion of alarmins similarly to asbestosis. Nanomaterials can also cause gout, when monosodium urate crystals trigger responses from the innate immune system in response to damage (Busso et al, 2010).

Non pathogenic alien cells circulating in our bodies and reaching to every organ could perhaps cause minor damage leading to similar responses to gout. Yong et al describe a possible process here for nanoplastics which perhaps is also a possibility for alien life that passes into our permeable bodies:

However, the components of the innate immune system, such as the Toll-like receptors (TLRs), could also respond to a set of endogenous or secreted molecules collectively known as alarmins, or damage-associated molecular patterns (DAMP), and the outcome is what is termed sterile-inflammation, i.e. inflammatory responses without pathogenic infection. In the body, pro-inflammatory cytokines released from such localized inflammations would attract circulating immune cells, and this could worsen the local inflammation, and cause cell and tissue death.
(Yong et al, 2020)

Then the next risk is that the coronated alien microbes could be taken up by cells of the body by macropineosis, or by the white blood cells.

The clathrate coated vesicles surrounding nanoplastics form as a result of accidental triggering of receptor mediated endocytosis by the nanoplastic in carboxyl-functionalized polystyrene (Jiang et al, 2011).

[Figure needs permission, or new source]

Figure 64: Upper figure shows macropinocytosis of 100 nm polystyrene particles, and lower figure shows receptor mediated endocytosis by carboxyl-functionalized polystyrene, in clathrate covered vesicles. The endocytosis is faster. Both types of particle are coated in anionic detergent for stabilization.
[Figure from abstract of (Jiang et al, 2011).]

The phagocytosis is not unlike the way that Legionnaires disease uses phagocytosis mentioned before *(Alberts et al 2002)*, but the corona enveloped microplastics are not adapted to avoid destruction by protists. Instead, microplastics are hydrophobic and in this way resist breakdown by the enzymes that catalyse hydrolysis in the acid conditions of the lysosomes. Once inside cells, they can rupture blood cells (Hemolysis), kill cells (cytotoxicity), and damage genes (genotoxicity) (Gopinath et al, 2019). To resist digestion in this way Martian life would need to form hydrophobic cell walls.

Microbes that evade phagocytosis actively resist digestion. The microbe Legionella pneumonia, the causative agent for Legionnaires disease, survives inside the macrophage because it remains in vacuoles and disables fusion with lysosomes, the vesicles containing the digestive enzymes of the cell, Other microbes use other methods to bypass these defences (Todar, 2006).

The enzymes inside the lysosomes can break up proteins, nucleic acids, carbohydrates and lipids. Perhaps the alien life cell wall might not be made of any of these and can resist the enzymes, but it seems likely it would be destroyed. This would be true for all the processes described, the lysosomes that destroy the vesicles that form during macropinocytosis and endocytosis as well as phagocytosis.

These would most likely be digested by the enzymes inside lysosomes unless they accidentally triggered some chemical effect that inhibited fusion.

The lysosomes would be able to digest DNA, RNA, proteins, and lipids in alien life. However, if the cell wall of the alien life is made of something that the terrestrial enzymes can't digest, like the hydrophobic nanoplastics, then it would resist this. This perhaps is not impossible but seems unlikely.

So then the next risk is that the chemicals that are the byproduct of this microbial digestion harm the cells, damage the DNA or change cell processes. Martian life might for instance have perchlorates and hydroxides inside in addition to the chlorides of terrestrial microbes or it might have unfamiliar or mirrored amino acids that could be misincorporated in terrestrial biology.

If this happens it could lead to the cell dying or the immune system recognizing that it has been damaged and attacking it. At this point then it's a question of what happens to the macrophages after they digest the alien biochemistry. Perhaps they also are damaged and if so this could lead to wider issues.

If any of this happens, with billions of alien cells entering the body, there could be an inflammatory response and possibly autoimmune disease responses similar to those for AIDS.

However, this is not the end of the story. If mutually mystified at first, later the alien life, unlike microplastics, can evolve to attack terrestrial hosts and other microbes. Meanwhile the hosts are not likely to develop new capabilities for their immune systems fast enough to stop an alien pathogen.

So - even if initially terrestrial and martian life forms are mutually mystified, the microbial martian life has an advantage over multicellular animals and plants because of its faster evolution rate. It may at any time, perhaps through multiple evolutionary steps, develop the ability to metabolize terrestrial life, and use its biochemicals, and perhaps even hijack cell processes such as phagocytosis or macro pinocytosis in various ways to grow and to replicate.

Meanwhile, once there is enough of the Martian life in the environment to provide selection pressure, terrestrial microbes would surely evolve in turn to metabolize the Martian life too, and use it, or develop symbiosis with it, or defend against it in various ways.

Even with beneficial symbiosis with alien life, the resulting microbiomes would be different in microbial composition and may function differently from the ones we have now, Neabwguke macroscopic life might find it harder to adapt to the novel situation with their slower replication rate. Macroscopic life might also be attacked directly by the extraterrestrial microbes as just described, or it might trigger autoimmune responses and other problems.

It is also possible that the alien life is already pre-adapted to be able to use the unfamiliar biochemistry of terrestrial life, as with the example of mirror life that has already evolved isomerases in order to digest organics from meteorite infall.

It does remain possible that alien life is completely harmless to terrestrial life, that it spreads through our bodies but does nothing, and is just like inert matter, like water, not even having as much effect as the nanoplastics.

Alien life could also be beneficial to us. The archaea provide an example of an entire realm of terrestrial biology that is not known to cause disease in either humans or any animals or plants, not even as opportunistic pathogens (Kumondorova et al, 2019) (Chong, 2017). Extraterrestrial life might perhaps be similar.

However the reasoning given here suggests that the situation where the two forms of biology are mutually mystified and essentially ignore each other has more potential issues than one might at first think.These need careful consideration in discussions of worst case outcomes of the unintended release of an alien biology into the terrestrial environment.

Exotoxins, protoxins, allergens and opportunistic infection

Other issues may arise from secondary metabolites, for instance, *Wallemia, an* airborne extremophile fungus, is found in food, especially highly salted or sweetened food such as salted fish, jams and cake. It is adapted to low water activity, and produces the secondary toxic metabolites wallimidione, walleminol and walleminon. W. sebi is a common cause for spoiled food through its production of secondary metabolites. The most toxic of these is wallimidione (Desroches et al, 2014).

Mars conditions are likely to favour life adapted to low water activity levels, and so could be a nuisance particularly for highly salted or sugary foods, where they also might produce secondary metabolites, similarly to w. sebi.

Martian life could cause allergic reactions. W. sebi has been found to cause allergic sensitization (Desroches et al, 2014). Another example is the fungus Aspergillus which can trigger asthma, and as an opportunistic infection can also cause the more serious illness of aspergillosis, and death (Latgé, 1999).

The common allergic reaction to poison ivy is due to Urushiol, a Catichol $C_6H_4(OH)_2$ with one or more alkyl chains substituted in the 3 position. It forms antigens by binding to surface proteins of the dermis or epidermis so forming an antigen, which leads to an allergic response on the second exposure (Bryson, 1996, page 680). This again is a simple enough chemical so that it may occur in an alien biology, or something else similar.

For another example, sesquiterpines is a toxic signaling chemical (semiochemical) produced by potatoes under stress (Matthews et al, 2006). Could semiochemicals produced by an alien biochemistry be accidentally toxic to Earth life.

Alien biochemistries could also produce, or contain protoxins, which when metabolized break down into toxic products. For instance hypoglycin A, which is not itself toxic, is broken down into the highly toxic MCPA-CoA on digestion and can lead to the fatal Jamaican vomiting sickness after eating the unripe fruit of the Ackee tree, a national foodstuff in Jamaica (Holson, 2015). A more commonplace example is methanol which is converted into toxins when digested (Mégarbane, 2005).

Again, toxicity may be more common if the secondary metabolites or protoxins are based on a different biochemistry.

The chemistry of alien cells may itself be toxic to Earth life. One suggestion is that Martian life might use hydrogen peroxide and perchlorates in its intracellular fluids in place of the chlorides used by Earth life, similarly to the composition of the brines it inhabits (Schulze-Makuch et al, 2010a). This could adversely affect Earth microbes that interact with Martian cells or scavenge dead Martian life.

Waste products and metabolic intermediaries could also be accidentally toxic or allergenic.

As before all, if humans are unaffected, these effects could still harm other creatures in Earth's biosphere, and harm us indirectly, if other creatures we depend on are affected.

Accidental similarity of amino acids forming neurotoxins such as BMAA

Certain algae blooms, including Chroococcidiopsis produce β-N-methylamino-L-alanine or BMAA (table 2 of Cox et al, 2005) which is a neurotoxin which can contaminate drinking water and in worst cases cause death (Cox et al, 2005).

In laboratory experiments BMAA can get misincorporated into proteins in human cells, and is a putative cause for the motor neurone disease ALS, or Lou Gherig's disease (Dunlop et al, 2013). This time BMAA is not produced as an exotoxin. The poisoning is accidental, it gets misincorporated because of its accidental partial resemblance to l-serine.

If two biospheres collide that are based on a different vocabulary of amino acids, there may be many such accidental similarities. In the case of BMAA, it's been suggested that proteobacteria in our gut provide some protection by removing it (Baugh et al, 2017). However there might be no helpful microbes to protect us by removing similarly close analogs of our amino acids from an alien biochemistry.

Martian microbes better adapted to terrestrial conditions than terrestrial life, example of more efficient photosynthesis

Alien life doesn't have to invade our bodies, or even create accidental toxins to harm us. It can also harm by competition with our microbes. To take an example, photosynthetic life on Earth operates at well below its theoretical peak efficiency for photosynthesis. Martian photosynthesis could be more efficient than terrestrial photosynthesis.

Martian life would then be like an invasive weed. If the result isn't a "drop in replacement" for the photosynthetic life already in our ecosystems this might change how the ecosystem functions, which could be beneficial or harmful, but it would be different.

Photosynthesis proceeds through the reaction: (Mellis, 2009)

$CO_2 + H_2O + 8\ hv \longrightarrow (1/6)\ C_6H_{12}O_6 + O_2$

The coefficients here are moles, so this means that with 8 moles of photons are needed for conversion of one mole (44 grams) of CO_2 and one mole (18 grams) of H_2O into a sixth of a mole (30 grams) of $C_6H_{12}O_6$ biomass and one mole (32 grams) of O_2.

One way to measure the efficiency is to measure the evolved oxygen for given levels of sunlight. At low light levels, the green alga chlorella vulgaris is able to achieve 84% efficiency at using photons to generate oxygen, absorbing 9.5 moles of photons for each mole of evolved oxygen.

However the efficiency rapidly falls as the light intensity increases, eventually saturating at less than half full sunlight intensity and producing no more oxygen as the sunlight levels increase further (Mellis, 2009:273). This graph from the paper shows how saturation is reached in measurements of a wild-type microalgae

[Figure needs permission]

Figure 65: Empirical data from wild-type microalgae. The vertical axis is the amount of oxygen produced per second by each mole of chlorophyll. It starts below zero because in the dark, respiration consumes about 3 mmol of oxygen / mol Chl / sec

The oxygen production increases linearly up to about 400 μmol $m^{-2}\ s^{-1}$ of light, and then it saturates. After about 1000 μmol $m^{-2}\ s^{-1}$ no more oxygen can be produced by increasing the light intensity. Full sunlight is 2200 to 2500 μmol $m^{-2}\ s^{-1}$ (or 2.2 to 2.5 mmol $m^{-2}\ s^{-1}$)

This is due to limitations in the speed of photosynthesis.
[Figure 1 of (Mellis, 2009:273)]

There are other losses in cellular processes and inefficiencies in photosynthesis, with the result that only part of the energy from photosynthesis is converted into usable energy by the algae. The theoretical maximum is that 8-10% is converted into biomass in conditions of full sunlight (Mellis, 2009:274).

The best case scenarios in labs and small scale microalgal cultivation achieve 3% efficiency under normal illumination (Mellis, 2009:274).

This low efficiency is due to the large numbers of chlorophyll antenna molecules attached to each reaction center, to absorb the light, which means terrestrial life absorbs much more light than it can process at high light levels and then has to re-radiate it as heat or photoluminescence. A smaller antenna size with fewer molecules per reaction center means light can penetrate deeper into a culture at the same cell density and more of the light is used. The cultures for smaller antenna sizes use less chlorophyll, so are lighter green at the same cell density (Schenk et al, 2008:37).

According to Mellis, it would be possible to increase the typical 3% efficiency of green algae another three fold, close to the theoretical maximum of 8 to 10% by truncating the light-harvesting chlorophyll antenna size (Mellis, 2009). Experiments back this up, though with smaller improvements (instead of tripling, they achieve modest increases of 55% to 60%) (Kirst, 2014)

So, why do terrestrial microbes capture more light than they need, shading other cells even of their own species, that would be able to use the excess light? It might be to inhibit competition from other species, that at high light levels a phototroph captures light that would otherwise be used by competing phototrophs. Also a larger antenna size allows it to capture more light at lower light levels with lower cell densities (Ort et al, 2015:8530) (Negi et al, 2020:15).

Reducing light antenna size has a trade off. A small antenna with fewer chlorophyll molecules increases efficiency at high light levels but if the cell density is low it reduces the efficiency at low light levels.

However modified cells have been designed that adjust the antenna size depending on the light intensity so that they achieve high efficiency both at low and high light levels compared to wild-type strains, doubling and even tripling the yields of the wild-type strains (Negi et al, 2020:15).

A Martian photosynthetic organism would experience large changes in light levels with a need to capture light during dust storms if possible, and also to capture as much as possible during conditions of bright sunlight, so it might already have an adjustable antenna size.

There are many other points in this complex process of oxygenic photosynthesis where efficiencies can be increased in principle.

Photosynthesis using an alternative form of carbon fixation could have a faster kinetic rate. CO_2 assimilation is often limited by the low catalysis rate of Rubisco. One proposed theoretical synthetic form of oxygenic photosynthesis could be two to three times faster than the Calvin–Benson cycle (Bar-Even et al, 2010).

Terrestrial photosynthesis rejects 50% of the incoming sunlight, mainly in the red part of the spectrum, leading to the distinctive “red edge”. The purple bacteria and lichens don’t have this “red edge” and Martian life would be likely to use red light like the purple bacteria, because of the high absorption of blue light by dust (Kiang, 2007).

Oxygenic photosynthesis goes through two photosystems, 1 and 2, and both use the same frequencies of light. The efficiency could be doubled by using red light for one of the two systems (Blankenship et al, 2011:808).

Martian life might also be able to use the full range of the spectrum. Terrestrial seaweeds are dark brown in colour because they use accessory pigments like fucoxanthin to gather the blue-green component of light rejected by chlorophyll. These then transfer the energy to the chlorophyll and so to the photosynthetic reaction centers. They do this so that they can use sunlight at only 1% of surface levels and to use the blue-green light that passes through seawater (Caron et al, 2001).

There is no need for terrestrial plants to do this because they already get more light than they can use for photosynthesis. However, a hypothetical Martian microbe with faster photosynthesis might find it useful to capture the full spectrum, especially in the low light levels on Mars. This would double its theoretical efficiency compared to terrestrial life.

Oxygenic photosynthesis also uses the Calvin cycle. This has evolved only once. All the organisms with the capability for oxygenic photosynthesis belong to a single clade, all evolved from a single hypothetical ancestor. This is the least efficient of the six known pathways for carbon fixation, both in terms of energy, and in terms of the number of electrons needed for each mole of fixed CO_2 (Bains et al, 2016).

So, why is terrestrial oxygenic photosynthesis so inefficient? Perhaps it is just hard to evolve this form of carbon fixation? Bains et al suggest this may be a many pathways event. Perhaps oxygenic photosynthesis could evolve in many ways, but with very low probability of achieving all the necessary steps so terrestrial life only happened to evolve it once.

Bains et all suggest as a perhaps more plausible alternative, that it could be a "pulling up the ladder" event where once the niche was filled, a photosynthesizer not limited by the need for an electron donor such as sulfide, Fe(II) or hydrogen then it was hard for a new photosynthesizer to evolve again (Bains et al, 2016).

Either explanation would let Martian photosynthesizers achieve a more efficient form of photosynthesis than we have today, by randomly arriving at more efficient photosynthesis, or they might have "pulled up the ladder" on a more efficient form of photosynthesis.

In short Martian photoautotrophs

- Would be likely to absorb red light and use it for photosynthesis, and may use the full range of visible light potentially doubling light to biomass conversion at low light levels compared to terrestrial blue-green algae.
- May have adjustable light antenna size in order to cope with fluctuations of sunlight in the Martian solar storms so permitting high efficiency at high light levels
- May have photosynthesis that achieves faster reactions than terrestrial photosynthesis through an accident of evolution or because Martian conditions favour it, permitting it to use more energy with a large antenna size
- May have more efficient carbon fixation for photosynthesis than the Calvin cycle in terms of the electrons needed or the energy needed per mole of fixed CO_2

Each of these separately could increase biomass yields and it might have several of them combined.

A Martian photoautotroph would only need a small improvement in efficiency compared to terrestrial life to be competitive with our photoautotrophs in the oceans, and there seem to be possibilities for major increases in efficiency. This Martian photoautotroph then might replace the natural species in our oceans.

This could be harmless, even beneficial in some situations if it is compatible with terrestrial biology. However differences in biology could make it inedible, accidentally toxic, etc.

Example of a mirror life analogue of chroococcidiopsis, a photosynthetic nitrogen fixing polyextremophile

Many radically different forms of exobiology have been proposed such as XNA based life or life with different bases or amino acids (Schmidt, 2010). However there is one possibility that is not speculative, but a clear widely accepted possibility for a radically different exobiology.

There is clear evidence that mirror life (with L DNA and D amino acids) is physically and biochemically possible, and some of the processes have been created in the laboratory (Weidmann, 2019). Some astrobiologists such as Church think it is possible that we may eventually be able to make synthetic mirror life (Peplow, 2016).

> ***Church's ultimate goal, to make a mirror-image cell, faces enormous challenges. In nature, RNA is translated into proteins by the ribosome, a complex molecular machine. "Reconstructing a mirror-image of the ribosome would be a daunting task," says Zhu. Instead, Church is trying to mutate a normal ribosome so that it can handle mirror-RNA.***

> ***Church says that it is anyone's guess as to which approach might pay off. But he notes that a growing number of researchers are working on looking-glass versions of biochemical processes. "For a while it was a non-field," says Church. "But now it seems very vibrant."***

In 2021, Fan et all were able to synthesize the 775 amino acid chain of Pyrococcus furiosus DNA polymerase, a DNA copying enzyme used for PCR. Using this they were able to assemble a 1,500 chain mirror DNA sequence, a record at the time (Fan et al, 2021).

This suggests the possibility that Mars could have mirror life, or a mix of mirror and non mirror life.

A mirror analogue of chroococcidiopsis from Mars could flourish almost anywhere from Antarctic cliffs to the Atacama desert (Bahl et al, 2011) or from Sri Lankan reservoirs (Magana-Arachchi et al, 2013) to the Chinese sea (Xu et al, 201q26:111), and form the foundation of a mirror ecosystem.

Chroococcidiopsis, which is one of our best analogs for a possible Martian polyextremophile is an ancient polyextremophile with numerous alternative metabolic pathways it can utilize, including nitrogen fixation, methanotrophy, sulfate reduction, nitrate reduction etc (KEGG, n.d.), even able to grow in complete darkness using a hydrogen-based lithoautotrophic metabolism with viable populations found over 600 meters below the surface (Puente-Sánchez et al, 2018) and in another case 750 meters below the Atlantic sea bed (Li et al, 2020).

In the same way a mirror Martian polyextremophile might retain numerous metabolic pathways from its evolutionary history on Mars that it could use to colonize diverse habitats on Earth. The Martian history would include hydrothermal vents, oxygen rich lakes, and almost any climate condition it could encounter on Earth as well as some conditions not present here naturally such as ultra low temperatures and ultra low atmospheric pressures and far higher levels of UV and ionizing radiation than life encounters on Earth.

Mirror starches, proteins and many fats would be largely indigestible to normal life (Dinan et al, 2007) which might give these microbes a competitive advantage.

If, after mirror life were to spread through the terrestrial biosphere, until half the microbes in some habitats consisted of largely inedible mirror life, possibly also accidentally toxic to terrestrial life or producing allergens, it seems unlikely that our ecosystems would continue to function in the same way.

Example of mirror life nanobacteria spreading through terrestrial ecosystems

A mirror nanobacteria would have the same survival advantages in the wild as other nanobacteria due to its small size (Ghuneim et al, 2018) including a selection advantage in microhabitats with low nutrient concentrations because of the large surface to volume ratio, and selection advantage in the presence of large secondary consumers that preferentially prey on larger microbes. They would also not be infected by terrestrial phages - in this case that would be impossible because of the mirror biochemistry. (Davies et al, 2009).

It is enough for a mirror nanobacteria to find some initial niche on Earth where it can survive in low numbers. Of course it wouldn't need to remain a nanobacteria in size after it escapes containment. Indeed the small size could be a response to low nutrient availability in the original habitat.

A Martian mirror nanobacteria could be present at a low level in the terrestrial environment for some time, until it makes the necessary adaptations to terrestrial conditions to start to spread widely through terrestrial biomes. It could adapt to novel terrestrial environments through varying gene expression, expressing latent capabilities it already has. Martian life could also be related to Earth life in the distant past. If so, it could rapidly take up capabilities from terrestrial life via gene transfer agents to help them to adapt to environments they encounter on our planet. This can happen overnight in seawater transferring capabilities between microbes that are far apart genetically (Maxmen, 2010).

Microbes would also develop new capabilities through evolution. This progresses rapidly in microbes with short generation times.

These changes could happen many years after a microbe of mirror life escapes from the facility. As an example of such a process, in the E. coli long-term evolution experiment, it took 20 years and 31,500 generations for e.coli to evolve the ability to use citrate in aerobic conditions (Blount, 2008). One of the defining characteristics for E. Coli is that it tests negative in the citrate utilization test (Sapkota, 2020) (EvoEd, n.d.)

This e.coli mutation to metabolize citrate occurred in only one of twelve initially identical strains, and was multi-step, historically contingent on previous mutations through to generation 20,000. In attempts to replay the mutation, the mutant cells couldn't arise in one step (Lenski, 2017:2185).

A minimal size free living autotrophic cell, smaller than DNA based life, could still bring a novel biochemistry to Earth such as mirror life. Such life, if able to survive alongside terrestrial life in any habitat would then be able to evolve and adapt to terrestrial conditions. The long term effects of introducing a novel biochemistry as a permanent addition to Earth's biosphere would be hard to predict.

This could happen even if the initial mirror nanobe seems to have no apparent cause for concern initially. Once Martian life is spread sufficiently widely, for instance in deserts, freshwater lakes, the sea or soil, or plant or animal microbiomes, this process would be impossible to stop.

So we should introduce microbes with a novel extraterrestrial biology to Earth with great caution, because of the speed of evolution, and the impossibility of controlling microbial evolution once released into the sea soil, air and other habitats that are present globally and interconnected through movement of water, wind, etc.

Possibility of extraterrestrial Martian life setting up a "Diminished Gaia" on Earth

If Lovelock's original Gaia hypothesis was true, then whatever the effects of returning extraterrestrial life to Earth, at least it would modify the planet to be close to optimally habitable for itself (Lovelock, 1975). As long as extraterrestrial life has similar requirements to terrestrial life, then by the strong Gaia hypothesis, it would keep Earth in a close to optimally habitable state for us too.

However, we suggested earlier that Mars could be an example of a planet with a "Swansong biosphere" where life made the planet less habitable than it would be without life (see above). Whether or not Mars is such a planet, the proposal leads to the possibility that introduction of extraterrestrial life could also introduce a novel homeostasis that even physically in terms of atmospheric composition, temperature etc, maintains Earth at a state significantly less habitable for us than it is now which for the purposes of this article we could call "Diminished Gaia"

We will start with a suggestion by Kasting. In a discussion of the need to be careful in experiments in biological engineering to try to make mirror life, he has suggested that mirror photosynthetic microbes with no predators could rapidly sequester CO_2 from the atmosphere depleting it for terrestrial life over a period of centuries (Kasting, cited in Bohannon, 2010). C3 plants would no longer be able to survive once levels drop to below 10 to 60 ppm depending on the CO_2 compensation point of the plant, the point where more CO_2 is lost through photorespiration than gained through photosynthesis. Land life would be severely depleted except for C4 plants like maize and sugarcane which retain the CO_2 from photorespiration and would still be able to grow at close to 0 ppm (Gerhart et al, 2010:679).

If mirror life somehow got the isomerases needed to convert normal organics to mirror organics, and break down normal fats, sugars and proteins, it could slowly convert familiar edible matter into mirrored molecules that normal life can't digest (Bohannon, 2010).

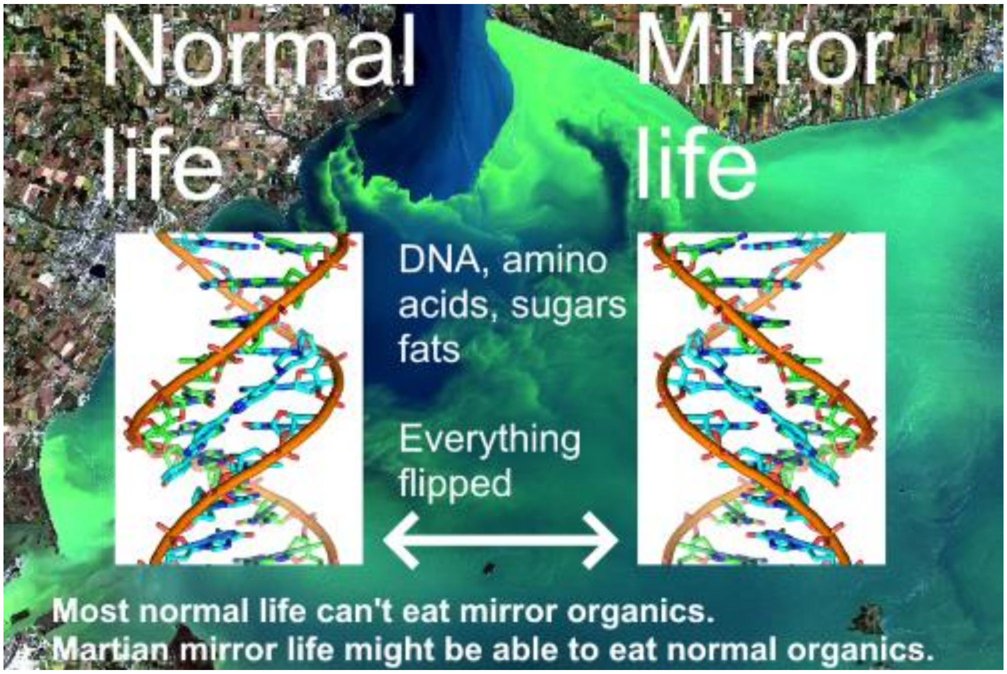


Figure 66: Normal life, Mirror life, DNA, amino acids, sugars, fats, everything flipped. Most normal life can’t eat mirror organics. Martian mirror life might be able to eat normal organics.

Background image from (NOAA, n.d.cwcu), DNA spiral from (Pusey, 2012)

Mars life might have this capability already. Martian life might metabolize the achiral sugars from meteorite infal (Frantseva et al, 2018) (Goetz et al, 2016:247)l, or Mars might have life in both forms, mirror and non mirror life.

Perhaps the C4 plants also would be destroyed in the process, if they have no defences against mirror life. They might be directly consumed, or conversion of organics to mirror organics might make the soil, water, or the environment uninhabitable to them.

Extrapolating further, the climate would cool down to a new global ice age and slowly over tens of thousands of years oxygen levels in the atmosphere would also be reduced. Both in terms of temperature and atmospheric composition this new “Swansong Gaia” might be significantly less habitable to life. It would also be a self maintaining homeostasis. Any increase in CO_2 levels would lead to more of the mirror life cyanobacteria which would then sequester the CO_2 until it is less habitable again.

This would be a stable end point if evolution is ignored. However, it would not be the end of the process as far as life is concerned. Secondary mirror life consumers would be likely to evolve eventually, or it might be that they were accidentally imported from Mars along with the original mirror life primary producers. Some terrestrial microbes would be likely to develop the ability to metabolize mirror organics, with their short generation time. Some small multicellular organisms with short generation times might also develop the ability to metabolize mirror organics.

The outcome might depend on how fast these secondary consumers evolve and what their properties are. If this was later at a point with low oxygen levels, these might be methanogens, Though methane has greater warming potential than CO_2, it is removed from the atmosphere rapidly, unless the atmosphere is already reducing.

This might be an alternative equilibrium state for Earth with methanogens, cyanobacteria and methanotrophs with an atmosphere of a mix of nitrogen, methane and low levels of oxygen.

If such an end state was possible, it would be maintained under homeostasis but would be significantly less habitable.

In this way, in the very worst case of unfortunate non beneficial interactions, an accidental introduction of Martian life to our biosphere might transform Earth's self reinforcing "Gaia", into a self limiting "Diminished Gaia" which over millennia and millions of years reduces the habitability of our planet, not only to terrestrial life but to all forms of life.

Humans would surely intervene in some way in this process, for instance by bioengineering or by paraterraforming. With modern technology we would not go extinct, but this might be the worst case without intervention.

Other similar Swansong Gaia interactions could be imagined that could be set up by accidentally introducing extraterrestrial life.

The result would not be as limited as the Martian swansong biosphere hypothesis on a significantly less habitable planet, but would have reduced surface biomass and greatly reduced ecosystem complexity and far less species diversity compared to the current terrestrial biosphere.

Worst case scenario where terrestrial life has no defences to an alien biology - humans survive by 'paraterraforming' a severely diminished Gaia

The physicist Claudius Gros looks at a clash of interpenetrating biospheres in his paper on a "Genesis project" to develop ecospheres on transiently habitable planets. Gros reasons that the key to functioning of the immune system of multicellular organisms, plants or animals, is recognition of "non-self". He presumes that biological defense mechanisms evolve only when the threat is actually present and they don't evolve to respond to a never encountered theoretical possibility (Gros, 2016).

In such a worst case, where terrestrial life is naive and offers no resistance when eaten by Martian life, after a clash with life from an alien biosphere, almost all multicellular organisms on Earth could be eradicated. All that would be left would be some small rapidly evolving organisms.

This is an argument similar to the worst cases of Lederberg, Rummel and Sagan *(Lederberg, 1999b)* (Sagan, 1973:162) (Meltzer, 2012).

Even in this scenario, it would be possible to preserve higher life in enclosed habitats protected by use of technology. The habitats could have self contained biospheres based on plants grown for food, and oxygen, which in turn take up carbon dioxide and water from humans. This seems feasible as we have already designed almost completely self-sustaining habitats that should work in space, a more challenging situation (Salisbury et al, 1997).

So long as our seed banks were protected from the invasive Martian life, whatever it is, we could gradually re-establish plant life inside these habitats too, and populate our habitats with any animal life rescued from deteriorating ecosystems. The seed banks preserve most plant species (apart from some tropical plants such as mangoes which can't be preserved for long as seeds). Eventually much of the world could be covered in expanding joined together habitats in a process similar to paraterraforming. Perhaps a similar process would work for parts of the sea bed too, and the sea shore

In this way, at least some humans could survive any of these scenarios. However in this worst cases, our biosphere would be severely affected, for a significant period of time.

This scenario and some of the previous ones such as the introduction of mirror life may seem like a scene from a science fiction book or movie. Hopefully that is exactly what they are. Hopefully these are not future possibilities.

However, the idea of returning a sample from Mars itself would seem a science fiction scenario as recently as the early 1960s. We are entering a future where what used to be science fiction is becoming a reality, and we have to seriously consider real world outcomes from such scenarios. Unlike a movie script, we can't rewrite this story to a happy ending if we don't like the outcome.

Our intuitions about what is credible or incredible based on past experience can easily lead us astray in novel situations like this, never encountered by any previous human civilization.

Worst case where alien life unrecognized by terrestrial immune systems spreads to pervade all terrestrial ecosystems

Humans wouldn't go extinct in such a scenario, as we would have time to recognize what is happening and build habitats to survive in. Also, we would be able to preserve much of the Earth's biodiversity including all the plants with preservable seeds (which is most of them).

However such a paraterraformed Earth would severely diminish life prospects for several generations.

Eventually life outside the habitats would reach an equilibrium, with small microscopic single cell and multicellular terrestrial lifeforms able to evolve fast enough to take advantage of the new microbial environments. Over millions of years, perhaps faster with assistance from humans, there would be higher life forms again able to survive in an environment with both kinds of biology. Perhaps humans also could artificially adapt our progeny to survive outside the habitats or find ways to supplement their own immune systems so that they are protected from the extraterrestrial microbes that our naive immune systems don't recognize as life. But essentially this process would turn Earth into an alien planet for macroscopic terrestrial biology in its current (original) form.

Although we have technology we could use to survive this scenario today, it would have been much harder with the early technology of the 1960s. The first "bubble boy" David Vetter who lived his life in an isolation room was born in 1971 (Gannon, 2012). Without experience of such technology, it would be that much harder for 1960s humans to survive back contamination of Earth's biosphere with life that our biology is not able to protect itself against naturally.

We can't know, but we may be lucky with our Moon, that there was no extraterrestrial life there. This might be an extinction risk that extraterrestrials have already encountered at a similar technology level to 1960s humans. If an intelligent alien species returned alien life to their planet with inadequate planetary protection, at the level of technological development of the Apollo mission, they could go extinct. They might not manage to develop the technology for self-sustaining habitats in time to keep out the alien microbes. It's not impossible that this has already made some other alien intelligent species extinct on one of the billions of exoplanets in our galaxy or in the billions of other galaxies in the observable universe.

Could Martian microbes be harmless to terrestrial organisms?

It is striking that identified human microbial diseases are all bacteria or eukaryotes (e.g fungi). Earth's third domain of life, the archaea, are not known to cause diseases in humans, animals or plants. The archaea could be implicated as opportunistic pathogens in some diseases like tooth decay, and diverticulosis, but the evidence is circumstantial. The archaea are present but it's not clear they are a cause (Kumondorova et al, 2019) (Chong, 2017).

Whether or not there are genuine archaeal diseases, the experience of almost complete harmlessness of the archaea suggests it is possible that Martian microbes could also be harmless to terrestrial life, or almost completely harmless. An entire domain of life from Mars could perhaps be harmless, even beneficial, to terrestrial life. After all, a microbe normally has no incentive to harm its host. Although this is not true for all diseases (polio, and smallpox are examples or diseases that have never evolved to be less deadly), for most microbes, keeping its host alive is its priority and harming its host is maladaptive (Chong, 2017).

Interestingly, archaea are more closely related to animals and plants than bacteria, though less closely related to them than fungi. It seems that an evolutionary distance for Martian microbes would be no protection, nor would evolutionary closeness be protection either.

That leads to an interesting question, if we find a new domain of life on Mars that we believe may be harmless to terrestrial life, how could we prove it? How could we prove the archaea to be harmless, in a hypothetical scenario where we introduce them to Earth from Mars for the first time this century? It wouldn't be possible to test interactions exhaustively, though some of the most important interactions could perhaps be tested in experiments, also how would one predict how it could evolve?

This is a question we may have to address at some point in the future, for instance if we find related life on Mars but in a new domain not yet present on Earth.

Enhanced Gaia - could Martian life be beneficial to Earth's biosphere?

So far we've focused on situations where biosphere collisions are harmful, since the topic is planetary protection, so we need to focus on scenarios where there is indeed a need to protect Earth. However we should also recognize that the introduction of extraterrestrial life to our biosphere could also be beneficial, as Rummel mentioned in his foreword to "When Biospheres Collide" (Meltzer, 2012)

We have examples from multicellular life to show that invasive species aren't always harmful. Schlaepfer et al did a survey of invasive species and in their table 1 they find many non native species that are actually beneficial. Some were deliberately introduced for their value for conservation, but many of the best examples were introduced unintentionally (Schlaepfer et al, 2011).

Schlaepfer doesn't list any microbial examples. What could benign interactions with terrestrial life look like for Martian microbes? Here are a few suggestions:

- More efficient photosynthetic life from Mars could increase the rate of sequestration of CO_2 in the sea and on land, improve soil organic content, and perhaps help with reduction of CO_2 levels in the atmosphere
- More efficient photosynthesis could increase the productivity of oceans
- Most of the surface layers of our oceans are deserts, except near to the coasts, because of the limitation of nitrogen, phosphorus, iron and silica (needed for diatom shells) (Bristow et al, 2017). If extraterrestrial life has different nutrient requirements, it may be able to inhabit these deserts and form the basis of an expanded food web.

- Martian microbes could be better at nitrogen fixation, phosphorus and iron mobilization, and so improve our soils, and help with crop yields as endophytes. Just as Martian microbes could enter the human microbiome, they could also enter plant microbiomes as endophytes and those interactions need not be harmful, many could be beneficial. (Afzal et al, 2019)
- New forms of yeast could be of interest in the food industry (Sarmiento et al, 2015).
- Martian life could increase species richness by gene transfer to Earth microbes, leading to more biodiverse microbial populations.
- Martian extremophiles could colonize microhabitats in deserts and eroded landscapes barely habitable to terrestrial life, helping with reversal of desertification
- More efficient Martian microbes might be useful to generate biofuels from sunlight and water (Schenk et al, 2008)
- Martian life might be accidentally toxic and control harmful microbes or insects
- Martian life might aid digestion or enter into other beneficial forms of symbiosis.
- Martian life could produce beneficial bioactive molecules as part of the human microbiome. These could include molecules that are antiviral, antibacterial, antifungal, insecticides, molecules that kill cancer cells, immunosuppressants, and antioxidants (Borges et al, 2009).
- It could add a new domain of life with almost entirely beneficial interactions similarly to the Archaea
- It could add new forms of multicellular life based on a different biochemistry, or multicellular life in a different domain of life from the eukaryotes, with a more ancient common ancestor.

However even if introducing terrestrial life is largely beneficial we still need caution. There would be not just one encounter in one ecosystem. Martian conditions may well favour polyextremophiles able to survive in a wide range of conditions.

Chroococcidiopsis is perhaps our best analogue for a Martian cyanobacteria and it is a polyextremophile and found in many habitats throughout the world. Also the microbes would evolve eventually, and perhaps quickly, or change gene expression, and eventually find new habitats that they can colonize.

Maybe some of these encounters would be beneficial in some ecosystems, while other ecosystems are degraded, possibly even by the same interactions with the same microbe. Similarly for organisms, some organisms may be benefited and others harmed.

The same Martian microbe may also have both harmful and beneficial effects on the same organism, or in the same ecosystem. Generally there might well be a mix of some beneficial and some harmful interactions.

On the other hand the interactions could all be beneficial. To take an example, our planet is not necessarily optimal for global biomass (Kleidon, 2002). Perhaps extraterrestrial life with additional capabilities could do the opposite of triggering a Swansong Gaia.

Return of Martian life might create a new enhanced Gaia system that has significantly more surface biomass and biodiversity than the one we have today. It might even add new beneficial domains of life like the archaea or a new form of multicellularity which only enhances the diversity of our biosphere.

We have nothing by way of previous experience to guide us here.

Amongst a million extraterrestrial civilizations that return a sample from a nearby biosphere with limited technological capabilities to contain it, we don't know how many would find they have harmed the biosphere of their home world. It might be that

- it is never seriously harmful, it usually leads to an enhanced Gaia, and is almost always a beneficial process.
- Or even that most extraterrestrial biospheres are seriously degraded after their first unsterilized sample return from a nearby independently evolved biosphere

There is no way to know.

A simple titanium sphere could contain an unsterilized sample for safe return to Earth's surface even with the technology of 1969 - but how do you open this "Pandora's box"?

We were lucky that our nearest destination for space exploration, the Moon, was not inhabited by an alien biochemistry. Suppose we had applied the Apollo guidelines correctly, and submitted them to a proper peer review. Back in the 1960s we didn't have the scientific understanding necessary for a safe sample return.

In an alternate timeline where the Apollo guidelines went through legal review, a likely decision in 1969 was that human quarantine can't protect Earth for the reasons explained in: : Complexities of quarantine for technicians accidentally exposed to sample materials.

In this alternate timeline the US would likely have done a robotic sample return first before sending humans to the Moon. In our timeline this was achieved a little over a year later with the Soviet mission Luna 16, the first robotic sample return from outside of Earth (NASA, 2018luna).

We would have thought our robotic sample returnprocedures were safe in 1969, but they wouldn't have been. Back then we didn't have the knowledge of extremophiles and the limits of size for life needed to contain alien life. Even in 2009 we didn't have modern understanding of the limits of size as we saw in: First restricted (potentially life bearing) sample return since Apollo, however, science reviews in 2009 and 2012 have lead to increasing requirements on such a mission – especially as the result of discovery of the very small starvation mode nanobacteriaia

However, even with the technology of the 1960s, we ***could*** have returned an unsterilized sample to Earth's surface with a zero risk of any harm to our biosphere. One way would be to seal it within a spherical shell of titanium, thick enough to be unbreachable during re-entry. If we never opened it once it reached the surface, Earth's biosphere would be protected, for as long as it remained intact.

Spherical fuel tanks from rockets typically survive re-entry into our atmosphere undamaged. This is because of the high area to mass ratio, the high melting point of titanium of 1,668 °C, and the resistance to ablation of a spherical structure.

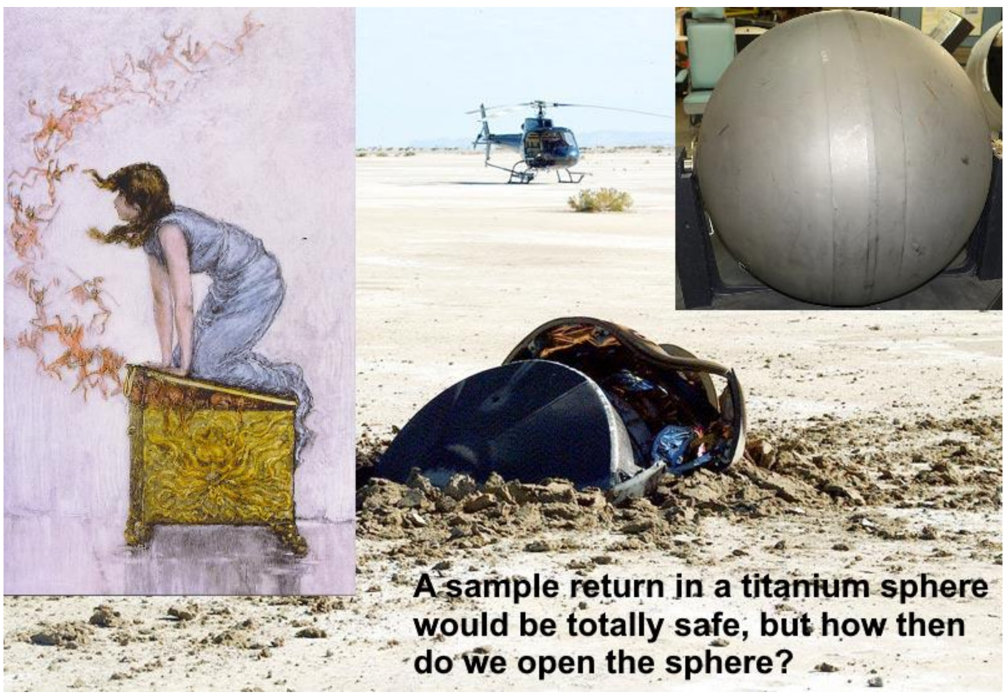


Figure 67, A sample return in a titanium sphere would be totally safe, but how then do we open the sphere? Top right image shows a titanium sphere that survived re-entry. Top left image shows Pandora trying to close the box that she opened in the Greek legend.

Main image - Genesis return capsule on the ground after it crashed (NASA, 2008grcg).
Top left, Opening Pandora's box (Church, n.d.)
Top right - space ball after re-entry - probably from the equipment module of Gemini 3, 4 or 5. (Daderot, 2017)

We can do the same today. Enclose the samples in a sealed titanium sphere, and it can then be delivered safely to the Earth's surface, so long as the outer surface is sterilized, or had no chain of contact with the Martian surface. However, if we wish to open the sample, and study it within our own biosphere, containment is far harder.

How do we open the sphere to study its contents? There doesn't seem to be any way to do this that guarantees this same high level of certainty that we can protect the biosphere of Earth (Ammann et al, 2012:25).

> *It is not possible to demonstrate that the return of a Mars sample presents no appreciable risk of harm*

There is nothing in the basic physics to prevent study of an unsterilized sample of an unknown alien biology on the Earth's surface with no appreciable risk of harm. If we had 100% perfect nanoscale filters we could do it - so long as we can also replace them when needed with no appreciable risk of escape of a nanoscale particle, and so long as we can eliminate any appreciable risk of human error, accidents and malicious damage.

However, though we can take many precautions, it seems that our technology needs to be developed further before we can study a sample within our biosphere with the same level of biosafety that we would achieve for a sample return in a sealed titanium sphere.

At least we can't achieve such levels of containment in a normal biosafety laboratory design even with improvements to the filters. I have a proposal below for a radically different form of laboratory that may be way to achieve titanium sphere levels of containment for a biosafe laboratory that might be worth considering, see:

- Proposal: a sketch for a biosafe laboratory on Earth designed for 100% containment of even nanoscale mirror life using telerobotics, a sump heated to 300°C with heat and vacuum stable light oil, and built in heat sterilization at end of life of the facility - could this be a safe way to open "Pandora's box"?

But following Amman et al, let's assume for now that we use normal biosafety laboratory designs and that we can't achieve the perfect safety of a titanium sphere. The question then is whether the level of safety we can achieve is sufficient.

Which variation on the precautionary principle is appropriate for a Mars sample return?

The precautionary principle was developed to help deal with some of the new unprecedented challenges faced by humans. The aim is to help guide decision making in situations (like a Mars sample return) where we have to make decisions although we don't yet know the potential effects of our actions and where some possible outcomes could be severe. This is one variation on it:

> *When an activity raises threats of harm to human health or the environment, precautionary measures should be taken even if some cause and effect relationships are not fully established scientifically.*
>
> *"In this context the proponent of an activity, rather than the public, should bear the burden of proof.*
>
> *"The process of applying the Precautionary Principle must be open, informed and democratic and must include potentially affected parties. It must also involve an examination of the full range of alternatives,* ***including no action****."*
>
> *(Raffensperger, 1998)*

There are many other variations on this principle. The European Space Foundation study considered four variations on the precautionary principle (Ammann et al, 2012:25) following an analysis of the principle by Stewart (Stewart, 2002)

- **Non-preclusion Precautionary Principle**: Scientific uncertainty should not automatically preclude regulation of activities that pose a potential risk of significant harm.•
- **Margin of Safety Precautionary Principle**: Regulatory controls should incorporate a margin of safety; activities should be limited below the level at which no adverse effect has been observed or predicted.
- **Best Available Technology Precautionary Principle**: Activities that present an uncertain potential for significant harm should be subject to best technology available requirements to minimise the risk of harm unless the proponent of the activity shows that they present no appreciable risk of harm.
- **Prohibitory Precautionary Principle**: Activities that present an uncertain potential for significant harm should be prohibited unless the proponent of the activity shows that they present no appreciable risk of harm

The ESF ruled out the non-preclusion variation since the potential negative impact on the biosphere can't be discarded, and neither the public or policy makers would accept a program without controls. They ruled out the margin of safety variation because the consequences can't be estimated and there are no previous observations that we can use to predict adverse effects.

The ESF then ruled out the Prohibitory Precautionary Principle. The reasoning here may be less compelling than the reasoning for excluding the other versions of the principle. They explain that it is impossible to demonstrate that the sample return produces no appreciable risk of harm. If we used the Prohibitory variation this would lead to cancellation of the MSR mission, so they argued that we can't use it (Ammann et al, 2012:25).

It is not possible to demonstrate that the return of a Mars sample presents no appreciable risk of harm. Therefore, if applied, the Prohibitory Precautionary Principle approach would simply lead to the cancellation of the MSR mission.

Based on Stewart's structure, the only model relevant to apply the Precautionary Principle would be the Best Available Technology Precautionary Principle.

However Stewart, elsewhere in that same paper, suggests that there may be situations where prohibition may be needed. This is possible since society places very high value on the environment and its protection (Stewart, 2002:15).

In critiquing strong versions of PP [Precautionary Principle], this essay does not argue that stringent preventive environmental regulation should never be adopted. … As society places a very high value on the environment and its protection, stringent preventive regulation of uncertain environmental risks is often justified and appropriate.

In his conclusion he discusses whether there may be criteria we can use to decide which of the precautionary principles apply in any given situation, and if they exist, suggest they need to be identified and justified (Stewart, 2002:48):

...If there are indeed criteria, consistent with PP premises, to guide selective application of the PP regulatory prescriptions and a balancing approach so as to avoid unduly rigid and costly regulation, those criteria need to be identified and justified.

Stewart doesn't attempt to outline criteria to use to decide between the variations on the principle. Instead presents this as a challenge for proponents of the Precautionary Principles to resolve.

So, should we use the Best Available Technology principle or the Prohibitory principle and can we develop criteria to help decide which version to use? This is an ethical decision, and not a decision for scientists or engineers to make for others without a voice in the decision. As Randolph put it (Randolph, 2009:292).

While NASA and other space agencies have certainly maintained due diligence in protecting against back contamination, there remains a significant moral issue that I have not seen addressed in any of the literature.

The risk of back contamination is not zero. There is always some risk. In this case, the problem of risk - even extremely low risk - is exacerbated because the consequences of back contamination could be quite severe. ***Without being overly dramatic, the consequences might well include the extinction of species and the destruction of whole ecosystems****. Humans could also be threatened with death or a significant decrease in life prospects*

In this situation, what is an ethically acceptable level of risk, even if it is quite low? This is not a technical question for scientists and engineers. Rather it is a moral question concerning accepting risk.

Currently, the vast majority of the people exposed to this risk do not have a voice or vote in the decision to accept it. Most of the literature on back contamination is framed as a discourse amongst experts in planetary protection. Yet, as I've already argued, space exploration is inescapably a social endeavor done on behalf of the human race. Astronauts and all the supporting engineers and scientists work as representatives of all human persons...

The ESF study's mandate had an underlying assumption that the mission will happen as they were tasked with recommending a level of assurance to enable it in their mandate: (Ammann et al, 2012:1).

"Recommend the level of assurance for the exclusion of an unintended release of a potential Mars life form into the Earth's biosphere for a Mars Sample Return mission"

This is why the only version of the principle available to them was the Best Available Technology

However, there is no such mandate for the legal process. The legal process is therefore likely to involve discussion of Stewart's question, to attempt to outline criteria for when the prohibitory version of the principle applies. We will look at one possible criterion for applying the Prohibitory version in the next section.

Formulating Sagan's statement that "we cannot take even a small risk with a billion lives" as a criterion for the prohibitory version of the precautionary principle

One possible criterion for applying the prohibitory principle is that it always applies when worst cases include severe degradation of the biosphere of Earth, or impact severely on large numbers of human beings. There can hardly be a clearer example of this than a worst case that can impact on the lives or livelihoods of a billion people. As Carl Sagan once put it (Sagan, 1973:130)

The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives.

It is likely that some members of the general public and some of the experts involved in the discussions have similar views to Sagan on this matter. The criterion might be:

> ***Sagan's criterion:*** *If it is impossible to show that there is no appreciable risk of significant harm to the lives or livelihoods of a billion people the Prohibitory version of the Precautionary Principle must always be used*

Here the threshold of a billion is arbitrary - but the exact figure may not matter. Any mission that has even a very minute risk of large-scale modification of the terrestrial biosphere will potentially endanger lives or livelihoods of at least a billion people.

There may well be other situations where we also need to apply the prohibitory principle. It would be a sufficient but not a necessary condition, as logicians put it.

If, for instance, it is impossible to show that there is less than one chance in a million that half a billion people die in some future proposed scientific experiment we surely still use the Prohibitory version, but there is no need to try to define an exact lower threshold here.

The suggestion is, we always use the Prohibitory version of the principle if Sagan's criterion can't be met applies.

During the legal process for a Mars sample return, we can expect proposals along lines like this, and public discussion of whether Sagan's criterion should be used (or something similar). It seems not impossible that a criterion similar to Sagan's criterion is adopted as a result of the legal review. If that is the outcome, no unsterilized return will be permitted until we know enough to guarantee that this particular experiment is risk free, at least to the level where there is no appreciable risk that a billion people can be seriously adversely affected by the mission.

This idea doesn't seem to have been considered in the planetary protection literature - that after legal review we might adopt something like Sagan's criterion, or that criteria adopted during legal review could lead to the decision to adopt the Prohibitory version of the Precautionary principle. However, given that Carl Sagan had this view, others of influence in the debates may too. Synthetic biologists have already expressed similar views for their discipline, as we'll see in the next section.

A requirement for similar levels of safety to those used for experiments with synthetic life would lead to the Prohibitory version of the Precautionary Principle and make unsterilized sample return impossible with current technology and current understanding of Mars

Synthetic biology already permits the creation of inheritable synthetic life such as life with hachimoji DNA (Hoshika et al, 2019). They make sure that this is safe by designing nucleotides that depend on chemicals only available in the laboratory.

Synthetic biologists have suggested that a safety mechanism to contain synthetic life should be many orders of magnitude safer than any contemporary biosafety device.

Schmidt puts it like this (Schmidt, 2010)

> *The ultimate goal would be a safety device with a probability to fail below 10^{-40}, which equals approximately the number of cells that ever lived on earth (and never produced a non-DNA non-RNA life form). Of course, 10^{-40} sounds utterly dystopic (and we could never test it in a life time), maybe 10^{-20} is more than enough. The probability also needs to reflect the potential impact, in our case the establishment of an XNA ecosystem in the environment, and how threatening we believe this is.*
>
> *The most important aspect, however, is that the new safety mechanism should be several orders of magnitude safer than any contemporary biosafety mechanism.*

Though Schmidt's paper doesn't make a connection with the legal principle, in effect this is an application of the Prohibitory version of the Precautionary Principle as mentioned above (Ammann et al, 2012:25) (Stewart, 2002).

- **Prohibitory Precautionary Principle**: Activities that present an uncertain potential for significant harm should be prohibited unless the proponent of the activity shows that they present no appreciable risk of harm

(see Which variation on the precautionary principle is appropriate for a Mars sample return? above)

It seems not impossible that such a view might prevail in legal discussions of a Mars sample return too. It has similar risks of returning unfamiliar life with the potential for *"the establishment of an XNA ecosystem in the environment"*.

If this is the legal outcome, it is for us, as proponents of the activity, to find a way to do it safely. The question then becomes, can we preserve the science value of the mission while running no appreciable risk of harm to Earth, similar to the risks from escape of synthetic life from a lab, at this level of a billion people's lives or livelihoods seriously harmed?

When the ESF study stated that it would be impossible to demonstrate no appreciable risk of harm for a Mars sample return facility, this was based on an analysis of the risks. The main categories of risk listed in the study are (Ammann et al, 2012:33) :

- *A break-up of the container during atmospheric entry (due to a design fault or sabotage),*

- *An unsuccessful full sterilisation of the Earth Entry Capsule, potentially having Mars particles attached to its outside surfaces,*
- *Damage to the vehicle due to heavy impact with the Earth,*
- *Escape of material during transport or from the laboratory*

There are many examples of escapes of pathogens from conventional biosafety laboratories that were thought to be safe for those pathogens (Furmanski, 2014). It's often because of human error. Also it can be equipment failure with unexpected failure modes, like the gloves for the Apollo samples (Meltzer, 2012:485) (Meltzer, 2012:241) , or a problem with the design or construction of the facility.

The risk of laboratory escape could also include such external events as a small plane or helicopter crashing into the facility, a terrorist attack, theft, or arson, that someone burns the facility down.

If we have to use the Prohibitory version of the Precautionary Principle, it is probably impossible to eliminate those risks to a sufficient level of assurance with current technology.

But see:

- Proposal: a sketch for a biosafe laboratory on Earth designed for 100% containment of even nanoscale mirror life using telerobotics, a sump heated to 300°C with heat and vacuum stable light oil, and built in heat sterilization at end of life of the facility - could this be a safe way to open "Pandora's box"?

Origins of the one in a million "gold standard" – as originally proposed it was 1 in 100 million and EPA uses numbers between 1 in 10,000 and 1 in 10 million – with administrative discretion - no magic number can substitute for informed and thoughtful consideration, working with the public

Kelly et al (Kelly, 1991) traced the one in a million criterion back to a 1961 article by Mantel et al which proposed a 1 in 100 million chance of developing cancer as a criterion for the purpose of discussion (Mantel et al, 1961). When asked by Kelly why he chose this figure he replied ***"We just pulled it out of a hat"*** (Kelly, 1991). The FDA adopted this 1 in 100 million criterion in 1973 but by the time the final rule was issued it became 1 in a million. This became the "maximum lifetime risk that is essentially zero," or the level below which no further regulatory consideration would be given regarding the safety of residues of a carcinogenic animal drug.

Graham (Graham, 1993) says that in practice, EPA recommends a range of risk levels from 1 in 100,000 to 1 in 10 million, and sometimes approves at a level of 1 in 10,000. EPA's air office tries to reduce the risk to as many people as possible to 1 in a million and the maximally exposed individual to 1 in 10,000.

Graham says that there are many factors involved in a decision such as the risk of the exposed population, the resource cost and the scientific quality of risk assessments and concludes (Graham, 1993):

> Administrative discretion is necessary to weight these factors on a case-by-case basis. No magic risk number can substitute for informed and thoughtful consideration by accountable officials who work with the public to make balanced decisions.

Kelly says that acceptability of a risk is properly made by those exposed to the hazard, or their health officials. It's not scientifically derived or a decision that can be made by outsiders to the process. The acceptability is based on many factors.

> The general consensus in the literature is that "acceptability" of a risk is a judgment decision properly made by those exposed to the hazard or their designated health officials. It is not a scientifically derived value or a decision made by outsiders to the process. Acceptability is based on many factors, such as the number of people exposed, the consequences of the risk, the degree of control over exposure, and 40 or so other factors.

This is similar to Randolph's point mentioned above (Randolph, 2009:292)

> ***In this situation, what is an ethically acceptable level of risk, even if it is quite low? This is not a technical question for scientists and engineers. Rather it is a moral question concerning accepting risk.***

The EPA science advisory board lists some of the factors in 1990, including (EPA, 1990:10)

Number of people and other organisms exposed to he risk

- likelihood of the environmental problem actually occurring
- severity of effects including economic losses and other damages if it does occur
- length of time over which the problem is caused, recognized and mitigated
- extent of the geographical area affected by it.

The EPA science advisory board likens the extra expense needed for precautions against long-term and widespread environmental problems to an insurance premium(EPA, 1990:10)

> "some long-term and widespread environmental problems should be considered relatively high-risk even if the data on which the risk assessment is based are somewhat incomplete and uncertain. Some risks are potentially so serious, and the time for recovery so long, that risk reduction actions should be viewed as a kind of insurance premium and initiated in the face of incomplete and uncertain data. The risks entailed in

> postponing action can be greater than the risks entailed in taking inefficient or unnecessary action."

What counts as "no appreciable risk"? Needs to be decided by ethics not science, but science can help clarify discussion - idea of expected number of people severely affected

In the prohibitory version of the precautionary principle, "appreciable risk" is left undefined. These things can't be decided on purely scientific grounds, it depends on ethical systems. These can vary by country and by religious or for philosophical reasons. Randolph gave this as one reason why it's important to have ethicists from a wide range of backgrounds involved in the decision process early on.

So, we can't decide this scientifically but we can break down the problem to provide a clearer framework for decision making. The Drake equation gives an approach that may be helpful.

We need to work out, what is an acceptable probability for "appreciable level of risk"? Is it 1 in a billion, 1 in a trillion, or even higher? Some synthetic biologists have come up with numbers far greater than a trillion there [cite]

One proposal by Nick Bostron for a scenario like this is to use the expected number of deaths or people severely affected in a typical worst case. Bostrom has suggested for large scale permanent effects we also need to look at future generations. So considered that way, a single release of mirror life could impact on the lives of not only us but all future generations on Earth.

Take one example, if human civilization expands to a trillion people and lasts for a million future generations, introducing mirror life impacts on the home planet of a quintillion people (10^18) who no longer can enjoy a planet free of mirror life.

So then, we need to replace the one in a billion level to one in a sextillion (10^24) to reduce the expected level of harm to equivalent to a biosafety laboratory which is handling pathogens that couldn't cause harm for future generations in the same way.

This is just one proposed way of thinking about such ideas. Again it would be for dialog, if we need to take account of future generations in this way. But it can help clarify thought.

Many accidental releases from biosafety laboratories don't lead to any deaths or severe impacts. So it's not about multiplying the worst case scenario deaths by the one in a million per facility with the aim to achieve an expected number of deaths.

We can also use this in a comparative way, to aim to achieve comparable assurances to current biosafety facilities. If a one in a million chance of escape is permitted for a case where escape could in a typical worst case severely impact on the lives of, say, 1000 people, it would need to be a 1 in a trillion chance if the worst case is the same level of risk of impact on a billion people to reduce the expected number of deaths to the same level as for an experiment that risks impacting on a 1000 people.

Some jobs and some forms of recreation have far higher levels of risk, such as test pilots, construction industry, base jumping. However the difference there is that it is a choice for the people who take those risks. Most people wouldn’t choose such a high risk job or recreation

Currently there are 59 BSL-4 laboratories operating or planned (Lentzos et al, 2021), with more than 50 of those operational (Goad, 2021).

Even with a comparatively small number of facilities, there are releases even with that 1 in a million chance of release. However these are usually through human error rather than issues in the lab design or protocols. Example, in 2003 in Taiwan, SARS was released from a BSL4 facility through human error. The technician found a spill in a cabinet and instead of filling it with hydrogen peroxide and waiting for some hours as was the normal procedure, he wiped it with ethanol, and put his head into the cabinet to do this. He did this because the standard procedure would make him late for a conference (Demaneuf, 2020)

Similar incidents might happen in a BSL-4 laboratory for a Mars sample return, especially since the technicians might well think it is unlikely to contain life and relax or skip procedures as happened often with the lunar sample returns. The recommendation of two years training before the sample return would hopefully eliminate this, but if we need the higher level of assurance of no appreciable risk this is harder to achieve.

Adaptive approach - return an unsterilized sample to Earth’s biosphere only when you know what is in it

The process outlined in this article in the section **Recommendation** to return a sample for teleoperated ‘in situ’ study above Geosynchronous Equatorial Orbit (GEO) in the Laplace plane, where particles in a ring system would orbit may be a solution. In summary:

1. **If preliminary studies suggest the chance of viable life in the sample is small, we sterilize it** with the equivalent of several millions or tens of millions of years of Mars surface ionizing radiation to be sure, return it to Earth, and study it in terrestrial labs as an unrestricted sample return.
2. **If preliminary studies suggest a significant possibility of viable life in the sample, we return it to above GEO**, sterilize some sections of the sample for return to Earth and study the rest telerobotically in orbit.

Our discoveries about the sample then determine what we do next.

3. **If viable life is found, then precautions are taken appropriate for whatever is discovered.** This can range from returning the sample unsterilized with no action needed to protect Earth, for instance in the case of an early pre-Darwinian form of life that has been shown to be no match for terrestrial life, through to perhaps total prohibition of returning it to Earth with current levels of technology, if what we find is a mirror-life nanobe.

This process not only enables us to examine an unsterilized sample far sooner than would be legally possible through attempts to return it directly to the Earth's surface, it also does so in a way that never at any stage runs any appreciable risk of harm.

There is no appreciable risk involved to the biosphere of Earth. There is also no appreciable risk for ourselves, or any of the other organisms that inhabit our planet.

This then can be a template for future sample returns from Europa, Enceladus, or any other location in our solar system that might have non terrestrial life.

Proposal: a sketch for a biosafe laboratory on Earth designed for 100% containment of even nanoscale mirror life using telerobotics, a sump heated to 300°C with heat and vacuum stable light oil, and built in heat sterilization at end of life of the facility - could this be a safe way to open "Pandora's box"?

We saw that (Ammann et al, 2012:25) said it's impossible to demonstrate that a Mars sample return presents no appreciable risk of harm.

> *It is not possible to demonstrate that the return of a Mars sample presents no appreciable risk of harm. Therefore, if applied, the Prohibitory Precautionary Principle approach would simply lead to the cancellation of the MSR mission.*

However, with studies of xenobiology in the labs they do hope to achieve high levels of containment as we saw (Schmidt, 2010)

> *The ultimate goal would be a safety device with a probability to fail below 10^{-40}, which equals approximately the number of cells that ever lived on earth (and never produced a non-DNA non-RNA life form). Of course, 10^{-40} sounds utterly dystopic (and we could never test it in a life time), maybe 10^{-20} is more than enough. The probability also needs*

to reflect the potential impact, in our case the establishment of an XNA ecosystem in the environment, and how threatening we believe this is.

The most important aspect, however, is that the new safety mechanism should be several orders of magnitude safer than any contemporary biosafety mechanism.

With a Mars sample return we can't use the same methods xenobiologists plan to use, to design the life itself so it can't survive outside the laboratory.

But perhaps we need to look at this more closely. Could there be a way that we might be able to achieve 100% containment on Earth to the same degree of safety as an orbital facility above GEO?

The current paper proposes a way to do this, which also uses present day technology, so that the facility build can start right away. The aim would be to make a design that can be approved in advance by all interested agencies and international organizations. This could be a sufficient guarantee to start on the build even before the legal process is underway.

Then the sample can be returned in the early 2030s as NASA could start the legal process with a high level of confidence that their design can withstand all legal challenges.

My aim here is to show that such a design may be feasible, but not to try to minimize costs. So this design will be over engineered and can surely be done with less cost.

The first step is to return the samples to a safe orbit in the Laplace plane above GEO as in the section:

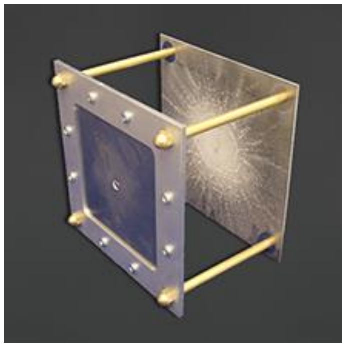
- Recommendation to return a sample for teleoperated 'in situ' study to above Geosynchronous Equatorial Orbit (GEO) in the Laplace plane, where particles in a ring system would orbit

This ensures that no contamination can get from the receiving satellite to Earth.

Then the sample is enclosed in a presterilized titanium sphere that can be inspected from the outside, as described above in A simple titanium sphere could contain an unsterilized sample for safe return to Earth's surface even with the technology of 1969 - but how do you open this "Pandora's box"?

For extra precautions the titanium sphere is covered in a multi-layer Whipple shield to protect from space debris and micrometeorite impacts, or an aluminium honeycomb shield or foam shield.

This is the basic idea of the Whipple shield:



NASA describes the Whipple shield like this (NASA, n.d.sd)

> ***"The Whipple bumper shocks the projectile and creates a debris cloud containing smaller, less lethal, bumper and projectile fragments. The full force of the debris cloud is diluted over a larger area on the spacecraft rearwall"***

More advanced designs have replaced the Whipple shield in many modern spacecraft. These other possibilities they mention include a honeycomb sandwich, metallic foam panel and a compressible foam used for the proposed Mars Module shield for future Mars missions.

For the Mars sample return, a honeycomb sandwich or metallic foam might be easier to use, as it would give a simple way to enclose a spherical container.

Only a large meteoroid could penetrate it, larger than any meteoroid that has impacted on the ISS. For additional assurance, acoustic sensors could be used to detect impacts, and the sample returned on an orbit that is biased away from Earth's atmosphere and then a thruster used to bias it to the impact trajectory if there are no detected impacts in the few hours of the return journey from GEO to Earth's atmosphere.

A large sphere will quickly slow down during re-entry and never reach temperatures that could melt titanium. For additional protection to prevent heating of the sample, and reduce terminal velocity, it could be protected with a spherical ballute, as suggested for crew emergency re-entry vehicles.

This is not needed to protect Earth's biosphere, but will help to prevent heating of the sample, also will reduce the terminal velocity as it falls through the atmosphere, and the shock of impact as it hits the ground, and can help keep the interior at the cold temperatures of the Martian surface. This is a design that was engineered to allow a human being to return safely from space without a parachute so should enable a reasonably soft landing for the sample (Jones et al. 2004).

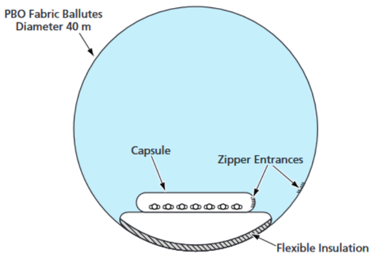


Figure ??: Design for an inflatable spherical ballute for an emergency crewed atmospheric-entry vehicle.

ESA demonstrated an inflatable ballute for re-entry in 2000 (Marraffa et al, 2000). This was a partial success, because of impact with the ballute by one of the components that didn't separate cleanly, there was some localized heating to 200 ° C but the ballute remained inflated, and the interior temperature wasn't changed (Marraffa et al, 200).

For the next stage, to transfer the sample to the research laboratory, we use the mature technology for black box flight recorders. These use insulating materials to make sure that they can survive an aircraft crash.

These can withstand (ATSB, n.d.):

- *an impact producing a 3,400-g deceleration for 6.5 milliseconds (equivalent to an impact velocity of 270 knots and a deceleration or crushing distance of 45 cm)*
- *a penetration force produced by a 227 kilograms (500 pounds) weight which is dropped from a height of 3 metres (10 feet)*
- *a static crush force of 22.25 kN (5,000 pounds) applied continuously for five minutes*
- *a fire of 1,100 degrees Celsius for 60 minutes.*

So, before transport the returned capsule is enclosed in insulating materials and a final outer shell similarly to a black box. It's possible that the Whipple shield could double as heat insulation for this stage.

The facility itself is built inside a decommissioned nuclear bunker so that there is no possibility of harm even by a direct hit by a plane or explosives, nuclear weapons or a meteorite impact up to 10s of meters in diameter.

The aim is to design the facility to be safe even for hazards like mirror life which can never be released during our lifetime or even during the lifetimes of any future generations on Earth. So, there needs to be a way to sterilize the sample and the entire facility when the facility is decommissioned.

So the facility is built inside an oven capable of dry heat to 300°C. This oven is designed so that it can be maintained externally, with the lab inside the oven. This means that the oven can be turned on at any time if there is some breach of containment that can't be resolved in any other way.

300°C is enough to destroy most of the nucleobases and amino acids in minutes. For details see

- Design specifications for 100% sterile rovers for fast safe astrobiological surveys throughout the solar system (below)

So, when the facility is decommissioned, after all the science is done, the entire facility is heated to 300°C for as long as needed - it could be heat sterilized for six months if that was thought necessary.

To get the sample container into the facility - the only concern at this stage is terrestrial contamination. There should be no terrestrial contamination inside the titanium sphere, and it would be possible to unpack everything down to the titanium sphere outside the facility.

The titanium sphere would be cleaned first with ethanol, then placed inside the airlock. Once in the airlock, the outside of it can be treated with carbon dioxide snow and hydrogen peroxide or in whatever way is thought necessary to remove any traces of contamination with Earth life.

It would then be opened inside the facility using telerobotically controlled equipment to cut it open.

The simplest way to design the facility is as a hermetically sealed facility which doesn't need any airlocks. Instead it is built with everything that will ever be needed to study the sample inside the facility, including everything that will ever be needed to repair the equipment, similarly to the design of a space mission to another planet.

Once the sample is placed inside the facility, it is hermetically sealed from the outside. All the work after that is done telerobotically, and nothing leaves the facility until the end of life when the entire facility is sterilized.

The advantage of building even a hermetically sealed facility on Earth rather than placing it in orbit or sending it to Mars would be to include heavy equipment such as particle accelerators.

However – one of the main reasons to bring samples back to Earth is to distribute the samples to multiple laboratories for independent research. It's also important to have the capability to bring new equipment into the facility, either new technology, or equipment designed specifically to follow up observations made by earlier experiments on the samples.

In the forwards direction, we need to be able to bring in new equipment, replacement parts, reagents, growth media etc. In this direction, the aim is to make sure they are free of any terrestrial life.

In the backwards direction we need to be able to remove fragments of the original samples, and perhaps other materials e.g. growth media with mirror life in it. This time the aim is to make sure that it is sterile of any life that could harm Earth's biosphere.

We also need to remove any equipment that is no longer functional, packaging and so on.

To move materials in and out with no risk of release to Earth's biosphere, the design uses two airlocks kept at positive pressure and a sump.

Mercury boils at 356.7°C. Big equipment could be pushed through a mercury sump and out of the building. However the density of mercury would make it hard to push objects through it and the vapors are toxic.

So, instead, this proposed design uses a vacuum stable high temperature oil such as Pentane X2000 which is used in space applications.

Pentane X2000 has a fire point of 335 C and flash point of 315 C (flash point is when the vapour from the oil has a risk of igniting). Density 0.85, vapor pressure 10-12 torr (13- 16 millibars) so it can be used in vacuum conditions with almost no loss of oil through evaporation (Venier et al., 2003)

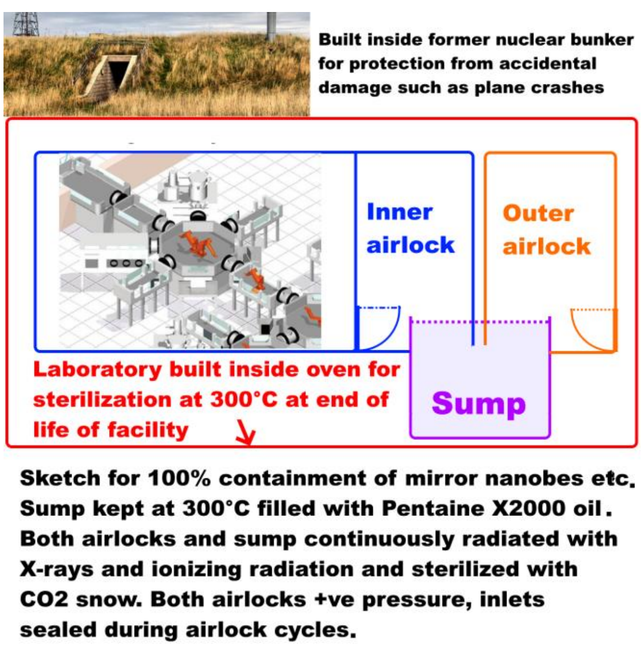


Figure ??: the LAS fully robotic floor plan for a Mars sample receiving facility placed inside an oven for end of laboratory lifetime sterilization of the facility and accessed via two airlocks and a sump for 100% containment of even mirror life nanobes.
Sketch of telerobitic facility Credit NASA / LAS (Hsu, 2009)
Photo of Cultybraggan nuclear bunker (Clark, 2009)

The oil sump is kept at a constant 300°C – this ensures that no viable spores can survive in it. It’s also irradiated constantly with ionizing radiation from cobalt gamma ray emitters placed in the oil, or X-rays or both.

The airlocks are also irradiated constantly with ionizing radiation or X-rays or both, and cycled with carbon dioxide snow to make sure that no biofilms can form in them. The oil in the sump is replenished from outside while keeping it at 300°C throughout.

Any materials to be removed are placed into the inner airlock. This is then pressurized to a positive pressure to keep out any mirror life and the pump’s outlet into the airlock is then sealed – the pump doesn’t operate beyond that point. The pump for pressurizing the inner airlock is operated from inside the facility, with no air connection with the outside.

Similarly the outer airlock is pressurized to a positive pressure and sealed with no air connection from the outer airlock to the inside.

In this way there is no way for air to pass between the inside and the outside of the facility.

For large objects that aren’t heat sensitive, the object is placed in one airlock and the airlocks are heated to 300°C to sterilize both the airlocks and the object of any amino acids or sensitive organic.

The object is then moved through the oil sump to the outer airlock and then both airlocks allowed to cool down – but the oil sump is kept at 300°C to remain as an impenetrable barrier to mirror life and covered with an insulating cover between uses.

The same method is used in the opposite direction to bring any objects into the facility that aren’t heat sensitive.

For heat sensitive objects, first they need a heat insulating container which is sterilized at 300°C along with the airlocks – and then everything is allowed to cool down.

The heat sensitive object is then placed into one of the airlocks, which is then pressurized above atmospheric pressure, and sterilized with ionizing radiation, X-rays, and carbon dioxide snow as appropriate.

The object is then placed in the heat insulating container, all this done telerobotically. The container is then moved through the oil sump which remains at 300°C to the other airlock and then removed.

As before, this works in the same way in both directions.

The whole process would be automated with security fail safes so that no human being can override the process.

If there needs to be a way to override this lockout, perhaps for maintenance, this could be done using security keys that are not made available to the technicians who operate the facility normally so that there is no possibility of anyone trying to take shortcuts.

Instead, staff would need to call in an independent technician who has no involvement in the experiment, who has no motive to rush proceedings. They would then override the airlock opening mechanism, for instance if it gets stuck and can't be opened normally.

In the worst case, if an issue arises in the mechanisms that can’t be fixed in a way that preserves biological containment, experiments continue but nothing can be moved in or out any more and in worst case, if necessary the facility is decommissioned and heat sterilized.

If it becomes clear early on that the design is acceptable to everyone, it might be possible for NASA to start the build on a facility like this well before the end of the legal process, perhaps as early as 2024.

That would leave perhaps the same 9 years estimate for the build and the sample could be returned by 2033. However this assumes everything goes smoothly without any delays.

It would be important to communicate to the public clearly from the start of the legal process, to explain why the plans eliminate any appreciable risk of harm. To do this, it’s important to have the plans independently scrutinized by multiple experts in all the relevant agencies - the CDC, WHO, FAO, etc at an early stage, ideally before starting the legal process.

Pre-vetting like this by multiple experts would help make it clear in communications with the public that it is not just NASA's plan but is a coordinated plan of world experts in all relevant agencies that they all agree it would work. They would all need to agree that this means that there is no appreciable risk of harm to the Earth’s biosphere.

Even in this case 2033 seems to be optimistic, as there would be likely to be delays with such a complex new facility never built before. However, it seems an idea that deserves consideration that could perhaps be completed by the 2030s, would lead to a much simpler accelerated legal process.

It would be understandable if such an elaborate facility is not suitable for Perseverance's samples. However, it could be used later on for other samples from Mars or Europa and other locations.

Perhaps some day we find confirmed exotic life such as mirror life in our solar system. If so, at some point we will surely need a facility like this on Earth to study it, unless by then we have such advanced facilities in space that we never need to return the samples to Earth.

Early life or life precursors on Mars, such as protocells or Woese's pre-Darwinian cells, could be very vulnerable in the forwards direction - legal protection is weak, but strengthened by the laws for backwards protection of Earth

Just as terrestrial life may be vulnerable in the backwards direction, so might Mars life be vulnerable in the forwards direction. An example worst case is an early form of life or a precursor for life on Mars, perhaps resembling the RNA only protocells studied at Szostak labs (Szostak, 2016). Although not yet capable of exact replication, such protocells would be of great interest to astrobiology.

Early pre-Darwinian life might also be especially vulnerable. Woese suggested that in early cells from before LUCA (Last Universal Common Ancestor) lateral transfer might have been the dominant way that genes were transferred between cells. He presents this as a data supported conjecture (Woese, 2002),

The LUCA according to this view can't have been a single organism but rather a

"loosely knit diverse conglomerate of primitive cells that evolved as a unit"

These primitive cells would have swapped genes amongst each other readily. It's also possible that cells might have had a shared metabolism, cross feeding each other metabolically too like a modern bacterial consortium. (Woese, 1998) (Doolittle, 2000

For more on this see section:

- Possibility of early discovery of extraterrestrial microbes of no risk to Earth (above)

For the argument in the opposite direction, that life on Mars could be more complex than terrestrial life, see Scenario: evolution on Mars evolves faster than on Earth because of an oxygen rich atmosphere and frequent freeze / thaws of oceans, leading to life of the same genomic complexity as Earth or even greater, and with multicellularity evolving early

If early pre-Darwinian life still exists on Mars today, it might offer no resistance to colonizing Earth microbial life, and this could lead to gradual and complete extinction of all native life that uses the alien biochemistry as the terrestrial life colonizes its habitats.

Another alternative to complete extinction is that if Martian life is closely related to terrestrial life, it might also be able to take up genetic material from a dead terrestrial cell, which it might be able to replicate in some form, so transforming into some more complex intermediate form of life which is partly terrestrial and partly Martian in origin, Martian life but with some terrestrial genetic material incorporated in it, so that we could no longer study the original less complex early life, or at least not in a living cell.

In these scenarios, after the terrestrial life spreads through Mars, there would likely still be remains of early life left in organic deposits too salty or cold or dry or in other ways uninhabitable to terrestrial life and maybe even the life itself if it could inhabit conditions beyond the reach of terrestrial life. But anywhere on Mars that terrestrial life could inhabit, the life would be gone.

In the forwards direction from Earth to Mars the legal protection is weak, based on one clause in the Outer Space Treaty, article IX requiring States Party to the Treaty, to: (Ireland, 1967)

> ***“***pursue studies of outer space, including the moon and other celestial bodies, and conduct exploration of them so as to **avoid their harmful contamination*****… and, where necessary, shall adopt appropriate measures for this purpose.”***

This clause doesn't specify what "harmful contamination" is. However, it has customarily been taken to include harm to the scientific experiments of other parties to the treaty. Forward contamination with terrestrial life, if it could spread through Mars, would harm experiments by other parties to the treaty to search Mars for present day Martian life..

The treaty continues that if a State

> *“has reason to believe that an activity or experiment planned by it or its nationals in outer space … could cause potentially harmful interference with activities of other States Parties in the peaceful exploration and use of outer space…* ***it shall undertake appropriate international consultations before proceeding with any such activity or experiment.****”*

The space lawyer Laura Montgomery is of the view that in US law, this clause is only enforceable on entities that are acting as part of a national program like NASA. For private companies like SpaceX, she doesn't think it implies an obligation on the State to stop them from harmful contamination though she does agree that states have an obligation to inform other member states of their activities (Montgomery, 2016) (Klang et al, 2017).

To take an example, if Elon Musk had plans to send humans on a SpaceX Starship to Mars, risking a crash on Mars – all the space lawyers agree, including Montgomery, that the US would be obliged to tell all the other States party to the treaty about these plans. They are obliged to do this, because such a crash landing can potentially interfere with their scientific observations on Mars and their search for life, by introducing Earth microbes.

However, according to Mongtomery’s reading, that's as far as their obligation goes under the treaty. Montgomery does not think the US government would be obliged to stop him from doing it, not without more clarification. She says it would need to be clarified in Congress and expresses a hope that Congress would decide against this interpretation that the US government would have an obligation to stop him.

Another space lawyer, Pamela Meredith, disagrees. She is of the view that by the treaty, the United States is also responsible for ensuring compliance with the treaty by private companies such as SpaceX (Klang et al, 2017).

However, whatever the outcome of that legal discussion, or any legislative work, as we have seen, the law is only weak in the forwards direction. In the backwards direction it is strong.

Elon Musk’s rockets are intended to shuttle back and forth between Mars and Earth. He could send missions in the forwards direction one way to Mars without triggering Earth's environmental laws. However a spaceship designed to carry 100 people is far too large and complex to be sterilized for a return mission from Mars to Earth.

If we need to protect Earth from Mars samples then Earth has to be protected from materials returned on these spaceships in the same way as for the Perseverance Mars samples. The methods used by the Apollo missions for the Moon can’t be used for astronauts returning from Mars, since the Apollo guidelines were rescinded and were never given any legal scrutiny. We saw that no period of quarantine would be enough to protect Earth from mirror life nanobes, say (see : Complexities of quarantine for technicians accidentally exposed to sample materials above). This suggests that we need to have a clear understanding of whether there is any life on Mars that we might return to Earth accidentally, and the exobiology of Mars before we can send humans there, at least if they are going to return to Earth at some point.

Also, we need a clear understanding of Martian exobiology for the forward direction too, irrespective of the legal situation. At least, we do, if we value science and if it is indeed possible that we discover something as vulnerable to terrestrial biology as early life, maybe even Woese's transformable cells. We also need a clear understanding of Martian exobiology to decide whether or not it is safe to send humans to Mars in the forwards direction. Once we have a clear enough understanding of the exobiology of Mars (if any) to send astronauts there safely we may also have a clear enough understanding to at least know what we are doing if we make early life on Mars extinct, and make informed decisions about whether to preserve it / conserve it in habitats on Mars, or outside of Mars, or even to preserve early life over the entire planet.

Elon Musk, though he is so in favour of sending humans to Mars as quickly as possible, does care about the science impact of introducing Earth microbes to Mars.

Elon Musk was asked what he thought about Chris McKay's suggestion that we should explore Mars in a biologically reversible way and if we find life there that we consider removing human presence to allow it to thrive (McKay, 2009). Elon Musk answered (Musk, E., 2015):

> *A.* ***"Well it really doesn't seem like there is any life on Mars, on the surface at least, we are not seeing any sign of that. If we do find some sign of it, then for sure we need to understand what it is and try to ensure that we don't extinguish it, that's important.*** *But I think the reality is that there isn't any life on the surface of Mars. There may be microbial life deep underground, where it is shielded from radiation and the cold. So that's a possibility but in that case I think anything we do on the surface is really not going to have a big impact on the subterranean life.".*

So, as one might expect from someone who values science himself, he does think it is important we don't extinguish any native Mars life.

The study "Safe on Mars" in 2002 proposed a mission similar to Perseverance to test whether it is safe to send astronauts to Mars – however with the modern more complex understanding of Mars, Perseverance's sample won't prove that astronauts are safe in Jezero crater

It's understandable that prospective Mars astronauts and colonists want a mission with a single "Yes / no" test to discover if it is safe to send humans to Mars. This may not be as easy as it seems at first. As we've seen it would need to answer "Yes / no" to the question "Are there Martian mirror life nanobacteria on Mars or in Jezero crater". See:

- Example of mirror life nanobacteria spreading through terrestrial ecosystems (above)

At first sight Perseverance's sample return might seem to be the best way to do this. Indeed, the study ***"Safe on Mars"*** by the National Research Council in 2002 proposed that NASA establishes zones of minimal biological risk on Mars.

The suggestion was to send a precursor mission to determine if organic carbon is present. If organics were found, at or above the life-detection threshold, the suggestion was to use a sample return to find out if there is life present (Board et al, 2002a, chapter 5:38). If no life was found in the sample returned from a region, it would be declared a safe place for humans to land.

However, though Curiosity found organics in 2014 (JPL, 2014), the organics discovered so far are believed to come from meteorite impacts, and the search for life is far more complex than just investigating the first organics found on Mars. We now have a far more complex picture of Mars as covered earlier in the current paper, for instance in:

- 2015 review: maps can only represent the current incomplete state of knowledge for a specific time – with knowledge gaps on survival of terrestrial life in dust storms and potential for life to survive in habitats hard to detect from orbit - so can't yet be used to identify which areas of Mars are of planetary protection concern in the forwards direction

- Most samples from Jezero crater expected to be of no astrobiological interest - past biosignatures degraded - past and present day life low concentration, masked by abiotic organics, and patchy - especially challenging if Martian life never developed photosynthesis and

- Present day and past life may be patchy or inhabit millimeter scale features

The reason ***"Safe on Mars"*** recommended a sample return as the best way to determine safety was because in 2002 they lacked instruments that could do in situ biosignature searches in 2002. The committee said that if such instruments became available they would have the advantage that they would not be limited by the small amount of material available to a sample return (Board et al, 2002a, chapter 5:38).:

> ***"As stated above, there are currently no measurement techniques or capabilities available for such in situ testing. If such capabilities were to become available, one advantage is that the experiment would not be limited by the small amount of material that a Mars sample return mission would provide. What is more, with the use of rovers, an in situ experiment could be conducted over a wide range of locations."***

Indeed, at the time most of the instruments we could send to Mars for in situ testing for biosignatures were bulky and limited in capabilities. This was written only two years after the first sequencing of the human genome, which involved huge efforts, using many workstations, and was largely manual. Now we have handheld devices able to do end to end gene sequencing that you can hold in your hand. Other instruments have shrunk similarly.

We now have greatly enhanced capabilities for in situ instruments, which are also low mass and have low power demands, as we saw in the section:

- Modern miniaturized instruments designed to detect life in situ on Mars - could also be used to examine returned samples in an orbital telerobotic laboratory

"Safe on Mars" *was* also written at a time when the possibility of present day life on the Mars surface was considered to be remote. At the time the surface was thought to have no possibilities for liquid water.

This was before

- the unexpected discovery in 2008 of perchlorates on Mars (Hand, 2008), which made brines possible at lower temperatures,
- the discovery in 2011 of the Recurring Slope Lineae or Warm Seasonal Flows (McEwen, 2011),
- the observations in 2014, of droplets on the legs of the Phoenix rover (Gronstall, 2014),
- the temporary ultracold brines in the sand dunes discovered by Curiosity (Martin-Torres et al, 2015),
- and many new suggestions for surface potential microhabitats, some of which we discussed in: Could Perseverance's samples from Jezero crater in the equatorial regions of Mars contain viable or well preserved present day life?
- It was also published just a few months after the discovery of potential circadian rhythms in the reanalysis of the labelled release data from the Viking missions, in May 2002 (the Miller paper was from February 2002) (Miller et al, 2002)

Based on what was known at the time, the authors of ***"Safe on Mars"*** were so certain that nothing of significance would be found, as the most likely outcome, that they suggested planning for a manned mission should go ahead, even before a sample can be returned. They expected the result of the first sample return to be favorable for a manned mission immediately after it (Board et al, 2002a, chapter 5:41).

> ***There has been some concern that if a sample return is required, the planning for the first human mission to Mars may be delayed until a sample can be obtained. The committee believes that, even should a sample be required because organic carbon has been found, a baseline plan for a mission to Mars and even hardware development may still proceed under the assumption that a sample return will not find anything significant enough with regard to Martian biology to invalidate the baseline mission plan.***

"Safe on Mars" is one of the main Mars related cites in the Decadal survey which in turn was the original motivation for the Mars sample return mission (Board et al, 2012:157).

It is the only cite in the Decadal survey summary for the sentence:

> ***The elements of the Mars Sample Return campaign, beginning with the Mars Science Laboratory, will provide crucial data for landing significant mass, executing surface ascent and return to Earth, and identifying potential hazards and resources."***

One of the white papers for the next Decadal survey makes the same point as "Safe on Mars" that returned samples are critical for planetary protection protocols (McSween et al, 2020):

Returned samples are also critical for developing appropriate planetary protection protocols for both Mars and Earth.

"Breaking the chain of contact" when leaving Mars is technically achievable for robotic missions, but it is not possible for a crewed mission and potential biological hazards must be determined before humans go to Mars.

Sample returns will indeed be needed at a later stage, if we discover life, to learn more about its capabilities. However, sadly, Perseverance mission is not going to settle questions about the safety for Earth's biosphere or astronauts of any present day life on Mars, even in Jezero crater.

Perseverance is:

- **targeting a region of interest for past life rather than present day life** – it won't be able to decide if there are other regions nearby such as RSLs that could produce spores in the dust. See Could local RSL's be habitable and a source of wind dispersed microbial spores? Both dry and wet mechanisms leave unanswered questions - may be a combination of both or some wet and some dry

- **is not equipped to search for biosignatures in situ, past or present**. See Several studies by astrobiologists concluded we need capabilities to identify life in situ, for a reasonable chance to resolve central questions of astrobiology – if they are correct, this would also be necessary to show Mars is safe for Earth's biosphere and for astronauts

- **is not searching for extant life in Jezero crater, for instance, won't sample the expected brine layers in the Jezero crater sand dunes (which could be habitats for more capable Martian life) or much of the dirt, or salts**. See Detection by Curiosity rover of liquid water as perchlorate brines in Gale crater sand dunes and similar conditions are predicted in Jezero crater dunes and How Martian life could make perchlorate brines habitable when they only have enough water activity at -70 °C – biofilms retaining water at higher temperatures - chaotropic agents permitting normal life processes at lower temperatures – and novel biochemistry for ultra low temperatures

- **won't return much by way of dust, which might potentially carry viable dust from distant parts of Mars –** it may have perhaps one sample of regolith, that may contain dust and whatever dust adheres to the outside of the sample tube walls, See: Could Martian life be transported in dust storms or dust devils, and if so, could any of it still be viable when it reaches Perseverance?

In short, the current sample return strategy doesn't have a strong focus on extant life, and is not going to return samples from the most likely places to search for present day life even in Jezero crater such as the dirt, salty brine microhabitats or the Martian dust.

Perseverance is also not sufficiently sterilized to approach any region with potential microhabitats for terrestrial life, such as one of the Recurring Slope Lineae, if it finds one in Jezero crater.

In short, the selection of samples returned by Perseverance is not designed to give even a first idea of whether there might be extant life in Jezero crater.

The current paper suggested the ESF could increase the possibility of finding present day life in Jezero crater if they modify their rover to sample the dust and the brine layers. It made several specific recommendations and proposals which could be considered to increase the possibility of returning extant life:

- **Recommendation:** Extra sample of air and airfall dust to search for Martian life, assess forward contamination issues for terrestrial microbes, dust dangers for astronauts, and to return a random sample of wind-eroded rock from distant parts of Mars
- **Proposal:** magnets could be used to enhance dust collection
- **Proposal:** to use the sample return capsule as a dust collector – keep it open to the atmosphere before adding the sample tubes
- **Proposal:** by Jakovsky et al from the 2020 NASA decadal survey to combine a dust sample with a compressed sample of the Martian atmosphere
- **Recommendation:** modify ESA's sample fetch rover to grab a sample of the near surface temporary brine layers from sand dunes - perhaps Perseverance may be able to do this too with its regolith bit

However these recommendations would still be just a first step. Though they could increase the chance of finding extant life, if it is present in Jezero crater, this wouldn't be enough to prove Mars safe for humans, even in Jezero crater.

To take an example, if life is widespread in the dirt in Jezero crater, as the Viking results might suggest, we might have a reasonable chance of returning it in the first sample of dirt from Mars. If there are many spores in the dust, as for dust blown from terrestrial deserts, we might have a reasonable chance of finding it in the first sample of Martian dust returned from Mars.

However if there is no life in a few grams of dirt from Jezero crater, this only proves that this particular sample is lifeless. It doesn't prove that all the dirt in Jezero crater is lifeless, or even that dirt a few cms away from the sample is lifeless.

By analogy with terrestrial hyperarid deserts with microhabitats for life, there could be life in a patch of dirt or in the rocks just centimetres away from the samples selected for return, especially since Perseverance has minimal in situ life detection capabilities, as we saw in:

- Most samples from Jezero crater expected to be of no astrobiological interest - past biosignatures degraded - past and present day life low concentration, masked by abiotic organics, and patchy - especially challenging if Martian life never developed photosynthesis and

- Present day and past life may be patchy or inhabit millimeter scale features

It's the same with the dust, if no life is detected in a returned sample of a few grams of dust, this may let us obtain a first estimate of an upper bound on the number of viable spores or propagules blown around in the Martian dust, on the assumption that it is reasonably well mixed by the Martian dust storms.

However, a single sample of dust won't prove that there are no viable propagules even in the dust in Jezero crater. Perseverance could easily miss wind blown dust streaks with viable spores, even from an RSL or other microhabitat in Jezero crater. Or the number of spores in the dust could be so low that a larger dust sample is needed to have a reasonable chance of returning them. The spores could also form and spread seasonally or even on longer cycles. With Jakovsky et al.'s experiment to collect dust in an air filter, it could be that if the experiment ran for a few more months it might have returned a viable spore.

So, how can we obtain reasonable assurance that Mars is safe for humans?

To check safety of Mars for astronauts requires widespread in situ biosignature and life detection, and in situ tests of dust for spores and other propagules

With the complex understanding of Mars we have now, it is a possibility that even many lifeless samples returned from Mars, if selected according to the geology, may only find more and more patches of dust and drill sites on rocks on Mars that don't contain life.

Mars has a complex geology with a surface area similar to the total land area of Earth. If we do sample returns alone, with no in situ dedicated biosignature detection, it will be hard to do as much as a preliminary survey for life in the complex landscape of Mars since we don't know where to focus our attention.

It's the same with the dirt. It's not practical to return enough material from Mars to do a survey using sample returns. In situ biosignature detection is a key to rapid progress. We can study far more samples on Mars than we could realistically return to Earth in the near future.

To give a vivid metaphor, if extant life on Mars is very rare, it would be like searching Earth for frog spawn. We could suspect that the best place to look for it is in marshes and ponds. But we could send many rovers to those habitats and if they can't see the frog spawn and just return random samples of water they are highly unlikely to return it. If they can see the frog spawn they have a far higher chance of success. If we also know a bit about the habits of frogs and know the best time of year to look for the frog spawn, and where in a pond we are most likely to find it, we have an even better chance of success. Even so it may take a long time to find it, but we will find it faster than if we don't have the capability to see it.

The current paper suggested that a few grams of dust returned from Mars could give a preliminary bound on the amount of life in the dust globally on Mars

- **Recommendation:** Extra sample of air and airfall dust to search for Martian life, assess forward contamination issues for terrestrial microbes, dust dangers for astronauts, and to return a random sample of wind-eroded rock from distant parts of Mars

That might have a reasonable chance of returning life, if Martian life spreads as spores in the dust and is reasonably widespread, or there are some habitats on Mars that produce large numbers of spores, or local habitats near to Jezero crater with the winds blowing towards the rover.

But if Martian life occurs in very low concentrations, or if it spreads only rarely in the dust, perhaps as fragments of biofilms not particularly adapted to dust dispersal, we may not find it for a long time using that method.

We also need in situ biosignature detection to get a first idea of the variety of habitats for life on Mars, for biosafety testing.

Then, if we are lucky, and somewhere in a half kilogram of samples we find a lifeform from Mars, all it does is give us evidence that a particular species occurs on Mars. It doesn't tell us much about any other species that might also occur on the planet and whether or not they also are safe for humans.

To take an example, if we discover normal non mirror life in a sample returned from Mars doesn't prove that there is no mirror life on Mars. Mirror and non mirror life could co-exist on Mars or be adapted to different habitats from each other.

For another example, the presence of a familiar blue green algae would not be enough to rule out harmful fungal pathogens or a disease of biofilms able to invade human lungs.

The presence of familiar life can't tell us much about the potential for unfamiliar life.

With the hypothesis that life on Earth originated from Mars, it is possible that there is both related and unrelated life on Mars.

It's interesting that life on Earth has so little diversity - with almost identical choices of nucleotides and of amino acids for all modern life. One solution is that terrestrial life originated on a planet with far more diversity and only one particular branch of life got to Earth, a branch of life that was either hardier or just lucky at the time of the panspermia.

That's an example scenario where we might find both familiar and unfamiliar life on Mars. See

- Early discovery of a familiar microbe from Mars such as chroococcidiopsis is not enough to prove the sample is safe - examples include familiar life with new capabilities, or mirror and non mirror life in the same sample

Another way Mars could have a mix of familiar and unfamiliar life is if life originated independently on Mars and some terrestrial life got from Earth to Mars in the early solar system when it was easier for life to be transferred during the huge impacts on Earth, and established itself in the Martian biosphere alongside the Martian originated life.

This could be a plausible scenario if terrestrial life evolved capabilities accidentally that let it be more resilient to planetary transfer via meteorite impacts than Martian life.

There may be some scenarios that would lead to an early positive decision of "Safe on Mars". However we need caution here. If we return a vulnerable form of present day life such as an RNA world cell with no defenses against Earth life, we might swiftly decide that Earth life is problematic for Martian life in the forwards direction.

However, even such a discovery, that Martian life is very vulnerable to Earth life, would still not be enough by itself to show that Martian life is safe for Earth. The vulnerability could go both ways. As an example, there could be normal and mirror early life co-existing on Mars. The normal early life would be vulnerable to terrestrial life, but the mirror early life might be able to co-exist with it. Even early mirror life could be a risk for Earth.

To establish that terrestrial astronauts are safe on Mars in the positive direction requires much more evidence. Even the discovery of familiar life, perhaps a closely related blue green algae closely related to chroococcidiopsis would not establish the absence of mirror life, other forms of non terrestrial biology, opportunistic fungal pathogens, pathogens of biofilms, or other hazardous life on Mars.

It's the same also with microhabitats. If we find an uninhabited microhabitat on Mars, this would not be enough to show that all the microhabitats on Mars are uninhabited since colonization of new microhabitats is likely to be extremely slow.

Small amounts of familiar life in the samples could also be contamination as we saw in:

- Limitations on cleanliness of the Mars sample tubes with estimated 0.7 nanograms contamination per tube each for DNA and other biosignatures and a roughly 0.02% possibility of a viable microbe in at least one of the tubes

Mars could also have less evolved and more advanced life co-existing with each other, for instance, adapted to different habitats (early microbes with their smaller minimal size could be better adapted to deal with nutrient poor microhabitats), they could ignore each other as in the shadow biosphere hypothesis, play different mutually beneficial roles in the same microbial community, the early life could be parasitic or symbiotic with the more evolved life and so on.

There is an asymmetry here - even discovery of extraterrestrial life of no risk to Earth in Jezero crater - such as pre-Darwinian life easily destroyed in microbial challenges with terrestrial life wouldn't immediately prove the whole of Mars is safe for humans - while a single sample of a biohazard such as mirror life COULD be enough to prove Mars unsafe

Perhaps a discovery of easily destroyed transformable cells could show that Martian life in a sample returned from Jezero crater is safe for Earth as we saw in: Possibility of early discovery of extraterrestrial microbes of no risk to Earth such as pre-Darwinian life as suggested by Weiss – if microbial challenge experiments show they are quickly destroyed by pervasive terrestrial microbes

However, to show that the whole of Mars is safe, not just a returned sample we'd need to be very sure that it doesn't co-exist with other potentially more hazardous life. Depending on how connected Mars is for transfer of life, there could be life at different stages of evolution co-existing on a complex Mars, for instance, recently evolved life in one habitat while another habitat in another part of Mars has ancient mirror life that still persists but hasn't had time to spread to the habitat that is colonized by recently evolved life based on the complex picture of Mars we now have with potentially disconnected uninhabited habitats (Cockell, 2014):

This could be especially likely if the ancient Martian mirror life never developed photosynthesis or nitrogen fixation, see:

- Most samples from Jezero crater expected to be of no astrobiological interest - past biosignatures degraded - past and present day life low concentration, masked by abiotic organics, and patchy - especially challenging if Martian life never developed photosynthesis or nitrogen fixation

Also if it never developed specialized spores to survive transport in the dust storms.

There is a striking asymmetry here. It would take only one microbe that can harm astronauts or Earth's biosphere, such as a mirror cyanobacteria or a potentially pathogenic fungus, to show that astronauts are not safe on Mars, or at least, can't return safely to Earth.

However it's not so easy to show that astronauts are safe on Mars, which would require some understanding of the diversity of life on Mars. Indeed with modern understanding of the complex geology of Mars and its complex history of habitability, it’s not easy to find a scenario that would lead to an early decision that astronauts are safe on Mars as a whole or even in Jezero crater. This would require future missions dedicated to the task of finding out if Mars is safe for astronauts or Earth as one of the main objectives.

Several studies by astrobiologists concluded we need capabilities to identify life in situ, for a reasonable chance to resolve central questions of astrobiology – if they are correct, this would also be necessary to show Mars is safe for Earth’s biosphere and for astronauts

Several studies by astrobiologists have concluded that we need capabilities to identify life in situ, to have a reasonable chance of resolving central questions of astrobiology (Paige, 2000), (Bada et al, 2009), (Davila et al, 2010). A more recent update of “Safe on Mars” would surely conclude that the same capabilities are needed to resolve the question of whether astronauts are safe on Mars.

Paige et al., writing two years earlier than “Safe on Mars”, in 2000 argued that we don’t know where to go on Mars to get the samples we need to answer questions about past or present day life on Mars (Paige, 2000).

> ***We don’t know where to go on Mars to get the samples we need to answer the life on Mars question ...***

They argued for a search in situ first for both past and present day life

> ***Phase 3. Deployment of Exobiologically-Focused Experiments, to provide detailed characterizations of the population of organic compounds, and to search for biomarkers of formerly living organisms, and extant life.***
>
> ***Phase 4. Robotic Return of Martian Samples to Earth, to improve the characterization of organic compounds, and to verify any evidence for biomarkers and extant life discovered in Phase 3.***

Bada et al, writing in 2009 in a white paper submitted to the 2012 Decadal review raised the same concern as Paige et al, (Bada et al, 2009):

> ***… we do not yet know enough to intelligently select samples for possible return. In the best possible scenario, advanced instrumentation would identify biomarkers and define for us the nature of potential samples to be***

returned. In the worst scenario, we would mortgage the exploration program to return an arbitrary sample that proves to be as ambiguous with respect to the search for life as ALH84001."

Bada et al. recommended that in situ searches should be highly sensitive to biosignatures at low parts per billion or parts per trillion. They give the example of the Atacama desert, where there is a huge variety in biosignatures at both the micro and macro scale and If the instruments are not sensitive enough, it would be easy to miss the signal of life altogether and return rocks with no life in them, even if there were sites only millimetres away with unambiguous signatures (Bada et al, 2009:7):

> ***Field studies carried out in 2005 as part of Urey instrument development efforts have shown that in extremely arid locations like the Atacama Desert, variations in biodensity are incredibly pronounced on both the macroscale and microscale. If similar levels of biological heterogeneity were expected at one time on Mars, then it is probable that biosignatures could remain elusive during in situ investigation if instruments with inadequate sensitivity were utilized. Similarly, selection of a limited sample size could result in a null result for life detection during MSR missions and poses a high risk of ultimate failure.***

By analogy with their test missions in the Atacama desert, for the best chance of success, the instruments have to be sensitive to organics at levels that would not be detected by any of the instruments sent to Mars so far.

Davila et al, writing in 2010 stressed the importance of searching for present day and past life in situ to inform decisions about the samples to return, with the sample return at a later stage, saying (Davila et al, 2010)

> ***Sample return would be most efficient and logical once we have information from a variety of environments, particularly if evidence of extant or extinct life is found at any of these sites"***

With the single exception of “Safe on Mars”, all the papers I found stressed the importance of in situ searches first. As we saw in the previous two sections, “Safe on Mars” recommends sample return first only due to the bulky nature of the instruments we could send to Mars with the technology of the year 2000. With our new more complex understanding of Mars, a sample return like this can’t prove Mars safe for astronauts even in Jezero creater, see:

- The study “Safe on Mars” in 2002 proposed a mission similar to Perseverance to test whether it is safe to send astronauts to Mars – however with the modern more complex understanding of Mars, Perseverance’s sample won’t prove that astronauts are safe in Jezero crater
- To check safety of Mars for astronauts requires widespread in situ biosignature and life detection, and in situ tests of dust for spores and other propagules - though there is an

asymmetry here, a single sample of a biohazard such as mirror life COULD be enough to prove Mars unsafe

-

The Decadal review summing up doesn't cite Bada et al.'s paper, or mention the conclusions presented in the paper, although it is listed in Appendix D in the list of papers submitted. The review relies instead on "Safe on Mars" as one of its most cited sources (Board et al., 2012:16, 63, 157). It says (Board et al., 2012:17).

> ***The Mars community, in their inputs to the decadal survey, was emphatic in their view that a sample return mission is the logical next step in Mars exploration. Mars science has reached a level of sophistication such that fundamental advances in addressing the important questions above will come only from analysis of returned samples.***

The decision to return a sample from Mars as top priority may be the result of this heavy reliance on Safe on Mars, a single out of date source that supports this conclusion. It's hard to see why a 2002 paper would be given so much weight over later papers based on a more modern understanding of Mars. Perhaps it's because of its focus on evaluating the safety of Mars for human astronauts, which is a high priority for engineers and scientists who want to send astronauts to Mars?

Sample return as a valuable technology demo for astrobiology – and proposals to keep the first sample returns simple, a scoop of dirt or skimming the atmosphere to return micron sized dust samples

The only astrobiologist I found to recommend an early sample return in the last two decades is Chris McKay (NASA, n.d.cm), and his proposal is a technology demo. In an interview with SpaceNews, he recommends we grab a sample of the Mars soil to show what we can do and return it to Earth. Spend one day on the surface. Design the simplest lowest cost way to return a sample from Mars, no Mars 2020, no rover. Just grab it and return (David, 2015).

> *"**The first thing is getting a mission that scoops up a bunch of loose dirt, puts it in a box and brings it back to Earth**. If I was an astronaut, what I would be worried about is not the rocks. It's the dirt. The discovery [by NASA's Phoenix lander] of perchlorate in the dirt is cause to worry. It's toxic, and the second cause to worry is the fact that it took us so much by surprise. There was no prediction or premonition that there would be perchlorate in the soil. The fact that it took us completely by surprise makes me wonder if there are other surprises in the soil. In fact, I would be surprised if there are no other surprises. Bringing back dirt is easy because it's everywhere you land. You don't need*

precision landing. You don't need a rover. You land, grab some dirt and launch it back to Earth. The ground time on Mars could be one day."

"...I've said for many years that the sample return should be motivated by a combination of human exploration and science. The science community, I think, does itself a disservice by taking the attitude that there will be just one sample return ever in the history of the universe, so it has to be perfect. And a sample return mission that falls short of perfect shouldn't be considered. I don't understand where the logic is behind that. Let's make a first sample return a quick and easy sample grab, demonstrate the key technologies. It builds enthusiasm for the idea of round-trips to Mars. It would also make getting a second sample return easier, both programmatically and technically. That argument falls on deaf ears when I try and bring it up in the community."

One of his main concerns is that there is no alignment at present between the NASA Mars strategy and astrobiology. He covers this twice in the interview - near the beginning, and towards the end (emphasis mine):

"If we're going to search for life, let's search for life. I've been saying this to the point of exhaustion in the Mars community. The geologists win hands down as they are entrenched in the Mars program. The favorite trick is to form a committee to decide what to do. The people that are put on the committee, of course, are people who are funded to study rocks. So the committee recommends that we study rocks. They'll say these rocks will give us the context of how to search for life on Mars. Then you say, well, that's not right. But NASA Headquarters will say they asked the science community and they told us that this is what we ought to do. It's kind of circular. The reason the committee told you that — it's because you put a committee together of people who study rocks. It's almost a Catch-22. "

*"...Right now, **as far as I'm concerned, there is no alignment between the Mars strategy and astrobiology**. What we have learned from studying Mars is that astrobiology has to go underground. You've got to start drilling. Curiosity has a drill and it had problems and we are now very cautious about using it. We've got to get back on that horse and send a bigger drill."*

Chris McKay doesn't suggest his "grab sample return of dirt from Mars" mission is likely to be of astrobiological interest. Rather he sees it as of interest for understanding the conditions in the current Mars dirt for future missions to the surface, and human missions particularly, as the dirt is thought to include chemicals harmful to humans.

Its interest for astrobiology would be as a technology demo to show we can return a sample from Mars, at a later stage, once we know how to select the samples intelligently.
China plans a similar approach, a little more complex, two rockets one to land, the other to retrieve the sample (Jones, 2021)

There's another even lower cost proposal, the "Sample collection to investigate Mars" or SCIM mission. The proposal is to dip into the Mars atmosphere during its dusty season, and pick up a sample of dusty air, to return to Earth. It would use a "free return" trajectory. As soon as it leaves Earth's vicinity, it's on a trajectory to skim the Mars atmosphere and return to Earth with only minor course corrections after that (Leshing, 2002) (Savage, 2002).

Video: SCIM Mission to Mars narrated

BoldlyGo is a Colorado based privately funded non profit. They have ambitious plans to raise a billion dollars for this and other scientific space projects, partly through wealthy philanthropists, for private exploration missions (Billings, 2015) (Foust, 2014).

This is mainly a geological mission. Laurie Leshing, one of the directors of the Boldly Go institute, interviewed by Space.com, says (Tillman, 2014)

> *"Think of it as a microscopic average rock collection from Mars"*

Only tiny micron scale rocks get that high into the atmosphere. However, the Stardust sample analysis has shown how much science return you can get from tiny samples. Papers continue to be published leading to new results about comets including the discovery in 2011 that some comets get warm enough for liquid water to form (Berger et al, 2011).

Such small dust particles high in the Martian atmosphere would be sterilized by the UV in the Martian atmosphere, and may have no planetary protection issues – or if they do they could be sterilized during the return mission with ionizing radiation which would have little effect on the geology as suggested in Sterilized sample return as aspirational technology demonstration for a future astrobiology mission (above).

The dirt in Chris McKay's proposal would also include some dust and larger particles that got there from distant parts of Mars during the dust storms, so her remark would apply to his idea as well.

In the current paper we argue that though returned samples will be essential eventually, the samples returned from Perseverance will not be sufficient to determine potential biological hazards of life on Mars, either for the astronauts or for Earth because of the variations in biodensity and the impossibility of sampling a diverse enough number of locations ex situ.

The issues are the same as for detecting life on Mars at all as outlined in the papers we cited (Paige, 2000), (Bada et al, 2009), (Davila et al, 2010).

If life is found in situ, returned samples from multiple habitats will be necessary to evaluate the biosafety.

The current paper argues that at this point the samples are sufficient only as a technological demo and to establish some of the parameters for a follow up in situ search and later sample returns.

If the reasoning in this section is accepted as correct, how can we resolve this issue quickly? This needs missions dedicated to the task as a high priority. Humans in the vicinity would help speed up the search considerably with their superior decision making capabilities, but we can't send them to the Martian surface until we know that they will be safe on Mars. The current paper proposes that the solution is to do searches from orbit around Mars via telepresence.

Resolving these issues with a rapid astrobiological survey, with astronauts teleoperating rovers from orbit around Mars

Mars can be explored robotically, and then telerobotically, with humans in orbit and on its two moons. This would involve both humans and robots, each doing what it does best, in a valued partnership. The astronauts would be involved in the search for life, controlling robots directly through telepresence and haptic feedback whenever there is a need for on the spot decisions using human intelligence. The robots are our collective sense organs on other planets. Torrence V. Johnson, Galileo Chief Scientist, put it like this in the foreword to Meltzer's "Mission to Jupiter" (Meltzer, 2007)

> *"There is always a tension in the national debate about how much robotic exploration (such as Galileo) we should do versus so-called human exploration (such as Apollo). This misses the point! What we call robotic exploration is in fact human exploration. The crews sitting in the control room at Jet Propulsion Laboratory as well as everyone out there who can log on to the Internet can take a look at what's going on. So, in effect, we are all standing on the bridge of Starship Enterprise"*

SpaceX's new technologies can greatly accelerate the pace of astrobiological exploration of Mars. We can send many rovers to Mars, our mobile sense organs in the solar system.

Once we have the capability, humans in orbit around Mars can direct the rovers on the surface of Mars via telepresence from orbit, with binocular vision and haptic feedback as for the HERRO study (Oleson et al, 2013) (Valinia, 2012) and the first part of the Lockheed Martin "Stepping Stones" to Mars (Hopkins et al, 2011) (Kwong et al 2011) and Mars Base Camp (Cichan et al,

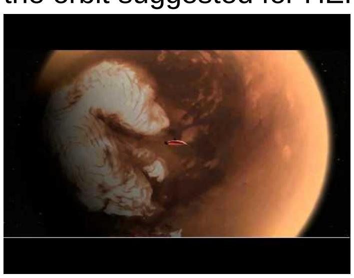
2017) studies as far as the human base camp on the moons of Mars exploring the surface via telepresence,

Figure ??:
NASA, 2012 "*Safely tucked inside orbiting habitat, space explorers use telepresence to operate machinery on Mars, even lobbing a sample of the Red Planet to the outpost for detailed study."*

HERRO image of a tele-operated Centaur as an insert.

The sun-synchronous orbit proposed by HERRO is a spectacular one, comes in over the poles twice every Martian day and flies above opposite sides of Mars HERRO study (Oleson et al, 2013) (Valinia, 2012)

In this video, I use a futuristic spacecraft called the "Delta Flier" in Orbiter as that was the easiest way to do it in the program I used to make the video. Apart from that, it is the same as the orbit suggested for HERRO.



Video: One Orbit Flyby, Time 100x: Mars Molniya Orbit Telerobotic Exploration in HERRO Mission

Figure ??:

Most of it is speeded up 1000 times, the complete orbit would take 12 hours. I slow down to 100 times and 10 times during some of the close passes of Mars as otherwise it's just a blur as you go past as you see with the first time around. Uses the "Delta flier" a futuristic spacecraft from the Orbiter simulator

To set this up in the Orbiter simulator I used
Orbit reference MARS
Frame ref equator
Epoch Current
semimajor axis 12880
Eccentricity 0.726451
Inclination 116
LAN 70
LPe 70
eps 272

There the LAN, LPe and eps are guesses. Chosen so that it approaches on the sunny side but not sure if it approaches closest exactly at the point closest to the sun. The Eccentricity - I adjusted that until the periapsis was the same as given in the paper. Inclination and semimajor axis just as given in the paper (Schmidt et al, 2012).

And this is what it might look like from inside the spacecraft

Composite of photo from the Cupola of the ISS (Coleman, C, 2011) and Hubble photo of Mars (Hubble, 2003)

Our astronauts can also explore Phobos for the samples from throughout the history of Mars that are predicted to lie in its regolith as a result of impacts on early Mars. Our astrobiological understanding of Mars should expand rapidly in a few years once we can do this.

In this way humans and robots can work together to unravel the astrobiology of Mars in a way that avoids all possibility of such worst case consequences either for Earth, or for the astronauts themselves.

Value of telerobotic exploration for a planet with complex chemistry developed over billions of years – need for forward protection of uninhabited habitats

This approach of exploring Mars from orbit first with tele-operated rovers and landers also avoids worst case consequences in the scenario of a lifeless Mars with uninhabited habitats (Cockell, 2014). One might wonder, why is forward protection needed for uninhabited habitats? Does it matter if terrestrial life colonizes an uninhabited habitat on Mars?

The value of preserving uninhabited habitats, at least initially, is that if we can preserve their unique chemistry, at least for a while, it lets us study the effects of over 4 billion years of chemical evolution on another terrestrial planet in the absence of biology.

Prebiotic chemistry has value too. This could tell us much about the early prebiotic stages of evolution, and about the prospects of life around other planets in our galaxy. It can also help us to disentangle the effects of chemistry and of biology on our own planet by comparison with habitats in which only chemical reactions operate.

We can expect some of the astrobiological missions to yield initially ambiguous results as for Viking, since after all, it is our first ever astrobiological search anywhere. We can’t expect to get everything right at the first try. Scientific experiments often lead to ambiguous leads that need to be clarified. But with a vigorous program of exploration this should not be a problem.

The Europa Lander report set the requirement (Hand et al, 2017). ,

- *"Life-detection experiments should provide valuable information regardless of the biology results"*

We need to get away from this approach if we are going to have really serious in situ searches in our solar system. Many of the best astrobiological life detection instruments could not be sent with that requirement, or if they had some abiotic value, planetary geologists would not rank them high, judged according to their benefit for geochemical studies.

We should treat uninhabited habitats as an interesting potential discovery in their own right (Cockell, 2014). On Earth, rocks from volcanoes, soon after they cool down, are inhabitable but uninhabited. Some regions of our harshest deserts don't have life, for instance gypsum pillars in the very driest areas of the Atacama desert, but that's because they are too dry for any Earth life. Just possibly Don Juan lake in Antarctica is uninhabited too - it has microbes but they probably don't grow there, but if so, it's because it is too salty for Earth life [NEEDS CITE].

If we only search for life once we know it is there, how can we expect to find life, unless it is an easily recognizable biofossil or macroscopic lifeform? A null result is of scientific value and can help focus the search (Kite et al, 2018). In addition a potential habitat with no life in it is also of scientific interest in itself

If we do discover a potential habitat in space, one which on Earth would be colonized by microbes, and get a null result from the life detection experiments, this might suggest an uninhabited habitat in space. This should be treated as a major discovery in its own right. It would be the first discovery in our solar system of what may be a common situation in our galaxy.

Places that are outside the normal range for Earth life are of astrobiological interest too, because we don't know of the limits for non Earth life. Is there life on Mars that can live in such habitats though Earth life can't? The answer to that is also interesting both ways, whether we find it, or don't. If Earth life hasn’t adapted to those conditions, maybe Mars life has?

Scenario of a pre-biotic uncontaminated Mars of great scientific value - microhabitats with autopoetic cells, Ostwald crystals breaking the mirror symmetry of organics, or naked genes, adsorbed on mineral particles with impenetrable membrane caps, but not yet quite life

We can’t simulate in our laboratories the effect of millions, or billions of years of prebiotic chemistry on another world. Perhaps such habitats could have RNA and autopoetic cells (Stano et al, 2010) , but no life. Different habitable regions could differ in the type and complexity of the prebiotic chemistry, again in ways we can never simulate or study once the regions are taken over by Earth life.

Some of the habitats such as hydrothermal vents, fumaroles, or the liquid water in Richardson crater associated with the “spider” markings that form in spring on Mars might have complex chemistry of great interest. See:

- Proposed surface microhabitats on Mars that could achieve higher densities of life and be a source for propagules in the dust – including brines that form rapidly when ice overlays salt at high latitudes, caves that vent to the surface, fumaroles, and fresh water melting around heated grains of dust trapped in ice layers through the solid state greenhouse effect

Even the reactions of the dirt in the Viking experiments are hard to understand as chemistry and if they don't have biological explanations may involve chemical reactions of interest to the origins of life.

- Puzzles from the Viking landers – why some think Viking detected life already in the 1970s – evolved gases in the labelled release experiment offset from temperature fluctuations by as much as two hours, more typical of a circadian rhythm than a chemical reaction

To give a few examples, some of these "uninhabited habitats" might have autopoetic cells (Stano et al, 2010) and others Ostwald crystals (Cartwright et al, 2007), formed by crystallization of achiral organic solutions assisted by solution phase racemization (Blackmond, 2010). Or we might find 'naked genes' adsorbed on the surface of mineral particles, and perhaps with impermeable membrane caps 'invented' by the genetic system (Leslie, 2004).

Some of these habitats might have one chirality and others the other chirality, and this could help to elucidate the origins of the homochirality of modern life.

We could study these processes actually in action, study naked genes as they are adsorbed on surfaces, growth of Ostwald crystals, or the activity of protocells in the native environment in which they developed.

If these habitats with prebiotic chemistry are habitable to Earth microbes, how long would they remain in a state suitable for study by astrobiologists and geochemists after infection by even one microbe or dormant spore capable of replicating in them?

Perhaps even an intact microbe or microbial spore is not needed. Infection with fragments of RNA or enzymes from Earth microbes could be enough to give protobionts in these habitats at a late stage in chemical evolution the missing key to become a simple form of replicating life. The resulting life could be interesting in its own right, but this process could erase all traces of the pre-existing protobionts, so that we never get to study them in their original state.

Study of the Martian meteorite NWA 5790 has revealed small vesicles that may provide bioreactors for early life to evolve even in the more recent Amazonian period on Mars (Viennet et al, 2021). Suppose they are, but life has not yet evolved? What a wonderful opportunity to study this process? There may be no other planet within light years to gain such insights from.

Mars could also have very early life, perhaps recently evolved in temporary surface habitats cut off from its deep hydrosphere as we discussed in the section. This earliest life could predate Darwinian evolution of cells based on Weiss's idea of simple modifiable cells with no barriers to uptake of genetic material from other cells evolving through Lanarckian evolution with Darwinian evolution only of the genetic material itself:

- Possibility of early discovery of extraterrestrial microbes of no risk to Earth

In the best case (from the point of view of the study of early life) Mars could have multiple unconnected potential habitats, uninhabited and inhabited, preserving different stages of evolution from complex chemistry to life.

This would give a fine grained understanding of stages in the processes of development of life from non life. We could use present day Mars like a time machine to take us back to the early stages of life and perhaps get some idea of what happened on our own planet right at the beginning before life evolved.

If life is still at an extremely early stage of evolution on Mars with early life with modifiable cells, or prebiotic with life not yet evolved, it might be only a matter of time after the first human boots on Mars before introduced terrestrial life reigns supreme in all the habitats on such a world, both previously uninhabited and habited.

If we prioritize an astrobiological survey from orbit first, we may soon be able to make informed decisions about whether to send humans to the Mars surface. If the decision is then made to land astronauts on Mars, the astrobiological survey can help us to do it in a way that preserves the interest of native Martian complex chemistry, protocells, early life or complex life and is safe for our astronauts, and Earth itself.

Depending on our future plans, if the decision is made to colonize an early life or prebiotic Mars, at least we know in advance what we are doing.

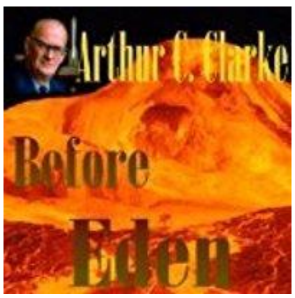
The preliminary astrobiological survey would still give us an opportunity to "rescue" early life and prebiotic chemistry on Mars, to attempt to reproduce it, perhaps in space habitats outside of Mars, or there may be things we can do on the planet to protect small microcosms of uncontaminated Mars, even if just in small-scale habitats a few meters across, before the processes are erased on the rest of the planet itself.
Also, even if there is early or pre-biotic life on Mars, and we extinguish / destroy it, the measurements made during this survey would remain as a record for future humanity, thousands of years into our future of what Mars was like before we initiate the anthropocene geological era on Mars with surface processes altered by terrestrial biology.

We will also have many assets on the surface that we placed there to do the survey, of value to astronauts ,and in case that we decide to land humans there, we will have a better understanding of the Martian surface environment and the best place to build our bases.

The main thing is that we would make our decisions in knowledge of what we do. We’d avoid the situation of landing there, destroying early Mars and then having regret for what we destroyed. We’d be replacing pre-biotic or early life on Mars with terrestrial life in full knowledge of what we are doing and the consequences and preserving what we feel needs to be preserved for ourselves and for future generations.

Arthur C. Clarke's story "Before Eden" exploring the theme of accidental extinction of extraterrestrial life in the forwards direction

Few science fiction authors tackled the theme of forward contamination of other parts of our solar system by Earth microbes, but there's one poignant sad story, by Arthur C. Clarke, "Before Eden", published in Amazing Stories, June 1961. Back then, though they knew Venus was hot, scientists thought it was still possible that Venus could have water on its surface, perhaps at the top of its mountains.



One of the covers for Arthur C. Clarke's "Before Eden" -a poignant sad story about forward contamination of Venus, published in 1961 at a time when surface life there was still a remote scientific possibility. You can hear the complete story read as an audio book here.

These adventurers are exploring a completely dry Venus, or so they think. Up to then (in the story), everyone thought Venus had no water, and was sterile of life. That was a natural thought, because the temperatures they encountered were always above the boiling point of water. But the heroes of the story are stranded near the not quite so hot South pole, and find mountainous cliffs there. On those mountains they find a dried up waterfall - and then - a lake!

> *"Yet for all this, it was a miracle—the first free water that men had ever found on Venus. Hutchins was already on his knees, almost in an attitude of prayer. But he was only collecting drops of the precious liquid to examine through his pocket microscope.... He sealed a test tube and placed it in his collecting bag, as tenderly as any prospector who had just found a nugget laced with gold. It might be – it probably was – nothing more than plain water. But it might also be a universe of unknown, living creatures on the first stage of their billion-year journey to intelligence...."*

> *"...What they were watching was a dark tide, a crawling carpet, sweeping slowly but inexorably toward them over the top of the ridge. The moment of sheer, unreasoning panic lasted, mercifully, no more than a few seconds. Garfield's first terror began to fade as soon as he recognised its cause...."*

“… But whatever this tide might be, it was moving too slowly to be a real danger, unless it cut off their line of retreat. Hutchins was staring at it intently through their only pair of binoculars; he was the biologist, and he was holding his ground. No point in making a fool of myself, thought Jerry, by running like a scalded cat, if it isn’t necessary. ‘For heaven’s sake,’ he said at last, when the moving carpet was only a hundred yards away and Hutchins had not uttered a word or stirred a muscle. ‘What is it?’ Hutchins slowly unfroze, like a statue coming to life. ‘Sorry,’ he said. ‘I’d forgotten all about you. It’s a plant, of course. At least, I suppose we’d better call it that.’ ‘But it’s moving! ’ ‘Why should that surprise you? So do terrestrial plants. Ever seen speeded-up movies of ivy in action?’ ‘That still stays in one place – it doesn’t crawl all over the landscape.’ ”

“‘Then what about the plankton plants of the sea? They can swim when they have to.’ Jerry gave up; in any case, the approaching wonder had robbed him of words... ”

“... ‘Let’s see how it reacts to light,’ said Hutchins. He switched on his chest lamp, and the green auroral glow was instantly banished by the flood of pure white radiance. Until Man had come to this planet, no white light had ever shone upon the surface of Venus, even by day. As in the seas of Earth, there was only a green twilight, deepening slowly to utter darkness. The transformation was so stunning that neither man could check a cry of astonishment. Gone in a flash was the deep, sombre black of the thickpiled velvet carpet at their feet. Instead, as far as their lights carried, lay a blazing pattern of glorious, vivid reds, laced with streaks of gold. No Persian prince could ever have commanded so opulent a tapestry from his weavers, yet this was the accidental product of biological forces. Indeed, until they had switched on their floods, these superb colours had not even existed, and they would vanish once more when the alien light of Earth ceased to conjure them into being...”

“...For the first time, as they relaxed inside their tiny plastic hemisphere, the true wonder and importance of the discovery forced itself upon their minds. This world around them was no longer the same; Venus was no longer dead – it had joined Earth and Mars. For life called to life, across the gulfs of space. Everything that grew or moved upon the face of any planet was a portent, a promise that Man was not alone in this universe of blazing suns and swirling nebulae. If as yet he had found no companions with whom he could speak, that was only to be expected, for the lightyears and the ages still stretched before him, waiting to be explored. Meanwhile, he must guard and cherish the life he found, whether it be upon Earth or Mars or Venus. So Graham Hutchins, the happiest biologist in the solar system, told himself as he helped Garfield collect their refuse and seal it into a plastic disposal bag. When they deflated the tent and started on the homeward journey, there was no sign of the creature they had been examining. That was just as well; they might have been tempted to linger for more experiments, and already it was getting uncomfortably close to their deadline. No matter; in a few months they would be back with a team of assistants, far more adequately equipped and with the eyes of the world upon them. Evolution had laboured for a billion years to make this meeting possible; it could wait a little longer.”

"...For a while nothing moved in the greenly glimmering, fog-bound landscape; it was deserted by man and crimson carpet alike. Then, flowing over the wind-carved hills, the creature reappeared. Or perhaps it was another of the same strange species; no one would ever know. It flowed past the little cairn of stones where Hutchins and Garfield had buried their wastes. And then it stopped. It was not puzzled, for it had no mind. But the chemical urges that drove it relentlessly over the polar plateau were crying: Here, here! Somewhere close at hand was the most precious of all the foods it needed – phosphorous, the element without which the spark of life could never ignite..."

" ... And then it feasted, on food more concentrated than any it had ever known. It absorbed the carbohydrates and the proteins and the phosphates, the nicotine from the cigarette ends, the cellulose from the paper cups and spoons. All these it broke down and assimilated into its strange body, without difficulty and without harm. Likewise it absorbed a whole microcosm of living creatures—the bacteria and viruses which, on an older planet, had evolved into a thousand deadly strains. Though only a very few could survive in this heat and this atmosphere, they were sufficient. As the carpet crawled back to the lake, it carried contagion to all its world. Even as the Morning Star set its course for her distant home, Venus was dying. The films and photographs and specimens that Hutchins was carrying in triumph were more precious even than he knew. They were the only record that would ever exist of life's third attempt to gain a foothold in the solar system. Beneath the clouds of Venus, the story of Creation was ended."

How sad it would be if future explorers on Mars get glimpses of early forms of life on Mars, and they go extinct soon after they are discovered. Or indeed, even before, maybe they are extinct before anyone finds them, and all we find are traces of the signs of past life right up to the present but gone within a decade or two at the start of the Martian Anthropocene (if dust storms can spread terrestrial life throughout the planet).

Design specifications for 100% sterile rovers for fast safe astrobiological surveys throughout the solar system

Even a preliminary astrobiological survey is likely to require a large number of landers and rovers, to explore a wide variety of potential habitats, at least for a planet as complex as Mars. This increases the risk of forward contamination. Some authors have suggested that we relax planetary protection measures for our rovers, in order to study potential habitats on Mars rapidly, knowing that this is likely to introduce Earth life to them irreversibly (Fairén et al, 2017), Others suggest we still have time to send adequately sterilized rovers to the planet to learn something about astrobiology on Mars before humans get there (Rummel et al, 2017)

This article proposes that we respond to the challenges by being more ambitious rather than less so in the field of planetary protection. Our technology has advanced since the Viking landers. The Viking landers were baked for 112 °C for 30 hours, enough for a million-fold reduction of the originally low population (Beauchamp, 2012). As a result of research into a

Venus lander, we now have preliminary specifications for a rover that can safely be baked at 200 - 300 °C for months.

This design of rover is based on commercial components including microprocessors and memory devices, that already function at 200 - 300 °C. They are used in oil wells, aviation and electric cars. Their heat resilience means they don't have to be cooled, and they can be placed closer to heat sources such as engines. This helps with cost, weight and most important, reliability (Watson et al, 2012). Using these components along with high temperature mechanical components, sensors and cameras researchers have sketched out a design for a complete rover able to withstand temperatures of 300 °C (and then to survive at that temperature for long term missions on the surface of Venus with active cooling). This rover specification was developed as part of Venus Rover studies. The researchers propose that the same approach could be useful for planetary protection (Sauder et al, 2017, section 6.2).

The batteries and solar panels are best replaced by RTG's (Radioisotope Thermal Generators) which can withstand high temperatures. Radio communication can be done with high temperature components and high temperature mechanical components are also possible.

Since these temperatures are only used for sterilization, the instruments do not have to operate at 200 - 300 °C. They just have to be able to survive heating at that temperature for several months on the journey to Mars. Once there, they would operate at normal temperatures. Future instruments to be deployed on this rover will need to use chips, solders and other components that work up to high enough temperatures for 100% sterilization.

With this rover specification, the whole spacecraft can be sterilized, as for Viking, but at a far higher temperature using modern more heat resistant electronics.

The simplest way to do this might be to use the spacecraft to heat itself during the journey out to Mars, to save the need to enclose it with a barrier before launch. If it uses an RTG as a source for power, this can also be used as a heat source too. Typically an RTG has an active cooling system or heat radiators. By disconnecting or regulating the active cooling, the RTG could heat the entire spacecraft during the journey out to any desired temperature.

Brian Wilcox has designed an ice melt probe for the Europan ocean which can be sterilized during the cruise phase, in this case at 500 °C, a temperature high enough to pyrolyze and decompose all large organics (Wilcox, 2017:2). The approach would be to have an inert gas circulate inside and outside the probe to maintain a nearly uniform temperature. It would be surrounded by a foil barrier which is also sterilized inside and out, and then the probe penetrates the foil barrier during deployment (Wilcox, 2017:8).

With such a high temperature as 500 °C, Brian Wilcox's probe has no electronics on board, and is a simple remotely deployed probe. However at the significantly lower 300 °C, electronics can be used, and this seems to be a high enough temperature for protecting any native Martian life from terrestrial microbes, and also preventing forwards contamination by genetic sequences and proteins.

- 200 °C is enough to sterilize cells.
- At 250 °C the half life of the RNA bases under hydrolysis is between 1 and 35 minutes, with U the most stable, G and A of intermediate stability and C decomposing most rapidly.
- At 350 °C the half-lives are between 2 and 15 seconds (Levy et al, 1998).
- So long as some water vapour is available for hydrolysis of the bases, there is not likely to be any genetic material remaining by the time it reaches Mars after six months at 300 °C.
- 300 °C should be enough to destroy proteins too. Eight of the 20 amino acids, G, C, D, N, E, Q, R and H, have been proven to not just evaporate or liquify but to decompose at temperatures between 185 for Q (Glutamine) to 280 for H (Histamine) They were not able to completely characterize the gases emitted for the other twelve amino acids (Weiss et al, 2018).

Once the design for such a rover has been developed, modifications of the basic design can be used anywhere in the solar system including Europa, Enceladus and other proposed locations to search for life.

In our decisions about whether and how to protect Martian life from forwards contamination, we need to be able to evaluate not just what we would gain with humans on the surface, but also, what we would lose, if the trillions of microbes that travel with us extinguish native life on Mars.

There may be treasures of immense value, preserved on Mars for billions of years, and yet, vulnerable to destruction in just a few years if we are careless in our procedures to prevent forward contamination. The treasure of extraterrestrial life on Mars, if it exists, is as much a part of our common shared heritage as any of our artistic or architectural treasures preserved from ancient civilizations. This suggestion of 100% sterile rovers is a way to protect our heritage in the solar system.

Mars not habitable for humans in any ordinary sense of the word - less habitable than a plateau higher than Mount Everest, so high our lungs need a pressure suit to function – not significantly more habitable than the Moon

Mars is not habitable in any ordinary sense of the word. The atmospheric pressure of 6 to 7 millibars is well below the Armstrong limit of 6.3% where water and body fluids boil at body temperature (Murray et al, 2013), so we would not be able to survive there even with bottled oxygen. The atmospheric pressure on Mars is too low for our lungs to function. We would go unconscious in seconds, and require a full body pressure suit to breathe.

Suppose we had a plateau on Earth, at a height of 45 kilometers (NASA, n.d.ame), five times higher than Mount Everest at 8,848.86 km (Dwyer, 2020). The pressure would be typical for Mars, but it would still be far more habitable than Mars.

We could survive on such a plateau simply by raising the pressure of the air inside habitats. On Mars colonists would have to generate all their own oxygen and scrub their habitats of the CO_2 (possibly using plants or algae to do this). They would need to add some other gas such as nitrogen to the cabin atmosphere, as the CO_2 in the Martian atmosphere raised to terrestrial pressures is lethal above 10% leading to rapid loss of consciousness and death, irrespective of how much oxygen there is (IVHN, n.d.)

Colonists on Mars would also need extra protection from solar storms - even on such a high plateau on Earth we would have some protection due to Earth's magnetic field as for astronauts in the ISS. We also don't know what difference the lower Martian gravity makes to long term health, and then there's half the sunlight we get on Earth.

It would be far easier to colonize such a high plateau than Mars. Yet if such a plateau occurred on Earth, we would likely have few living there permanently except to extract resources or for scientific study. We don't colonize most deserts, or the shallow continental shelves, which are far more habitable.

Then we have the problem of the dust.

Dust as one of the greatest inhibitors to nominal operation on the Moon - and likely on Mars too

Lunar dust isn't laced with perchlorates like the Martian dust, but it was still a major issue for lunar astronauts. They all reported difficulties with the dust (Stubbs et al, 2007).

Astronaut Eugene Cernan, the last man to walk on the Moon to date (NASA, 2017rgc), described the lunar dust as one of the greatest inhibitors to a nominal operation on the Moon (Levine, 2020):

> ***"I think dust is probably one of the greatest inhibitors to a nominal operation on the Moon. I think we can overcome other physiological or physical or mechanical problems except dust.***

One of the most aggravating, restricting facets of lunar surface exploration is the dust and its adherence to everything no matter what kind of material, whether it be skin, suit material, metal, no matter what it be and its restrictive friction-like action to everything it gets on., metal, no matter what it be and its restrictive friction-like action to everything it gets on,”

Figure 68: Gene Cernan inside the lunar module (NASA, n.d.ecilm). The dust got everywhere. He was particularly dusty because of an incident with a broken fender on the lunar rover (Phillips, 2008).

The lunar dust got embedded deep in the woven fabric of the suits causing significant damage after just three days EVA on the surface. The dirt got into the knees and the seat of the suit, and the upper arms too, perhaps when the astronauts supported themselves when they fell over on the lunar surface. This is something that needs to be considered in future lunar and Mars spacesuit designs (Christoffersen et al, 2009).

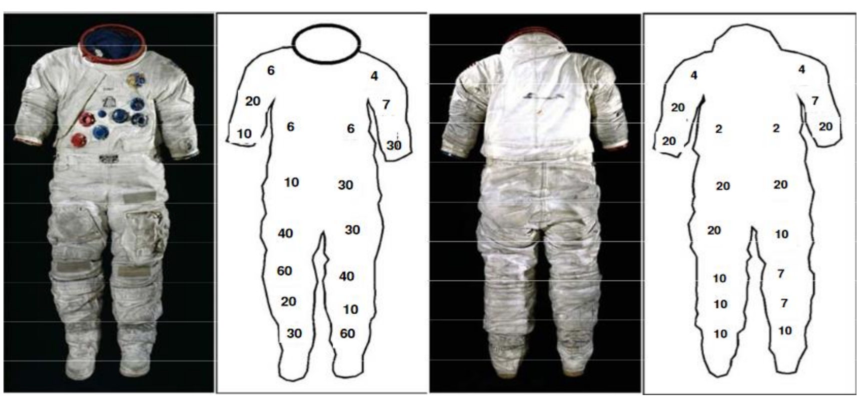


Figure 69: Concentrations of titanium (in units of 100 ppm) in the suit fabric for Jack Schmitt’s suit for Apollo 17. This seems to be a good tracer of the lunar dust.

Mars has worse dust problems than the Moon. For instance the Moon doesn’t have dust storms. Several times a decade dust storms on Mars block out the sunlight for weeks making surface conditions as dark as night.

Our rovers on Mars haven't yet travelled fast enough to create their own dust clouds but fast moving rovers or even people walking would throw the dust up into the air, as fine as cigarette ash, similarly to the arcs of dust thrown up by rovers on the moon.

Figure 70: Clouds of lunar dust thrown up by the rover, during Apollo 16. On Mars the dust is as fine as this, but it would linger in the air and not fall back in ballistic arcs as it did on the Moon (NASA, 1972).

Lunar dust falls back in ballistic arcs, in an atmosphere so thin it is classified as an exosphere rather than an atmosphere.

However, on Mars, some of the finest dust kicked up by astronauts or rovers would linger in the air for some time even in calm weather without winds.

Ignoring atmospheric drag, the ballistic arcs would be the same shape and height if the dust was kicked up one and a half times faster (calculated as sqrt(3.72/1.62) where 3.72 m/s^2 and 1.62 m/s^2 are for Mars gravity and the lunar gravity respectively) (Hsu et al, 2012:455). The lunar rover was driving at around 10 km / hour (Hsu et al, 2012), so as a rough estimate, a Mars rover driving at around 15 km / hour in similar conditions would produce similar height ballistic arcs to the lunar rover, though only for large particles.

Dust of 1 mm diameter settles at a speed of about 5 meters per second (Fuerstenau, 2006:Figure 1) so wouldn’t linger in the atmosphere for long in calm weather. However, a one micron particle will take half an hour to fall a meter in Martian conditions (Fuerstenau, 2006:Figure 1), though it wouldn’t be thrown up so high as it would on the Moon.

The perchlorates in Martian dust, although useful as a resource for colonists to make fuel and oxygen, would also be potentially harmful. Perchlorates interfere with regulation of the thyroid gland (by impairing uptake of iodine).

Inhaling a few milligrams of Martian dust could exceed the recommended maximum daily dose for perchlorates (Reference dose or RfD) (Davila et al, 2013). When the perchlorates are activated by ionizing radiation they may change to the more deadly chlorates and chlorites with some potential for more serious and immediate effects such as respiratory difficulties, headaches, skin burns, loss of consciousness and vomiting.

There are methods for dealing with this, used for dust suppression when mining uranium, lead or other heavy metal contaminated areas. But it adds to the complexity of Mars colonization (Davila et al, 2013).

The challenges to keep the dust out impact on most aspects of a mission to the surface (Rucker, 2017). It can be done. The suitport may be a solution for the problem of dust inside habitats (Boyle et al, 2013).

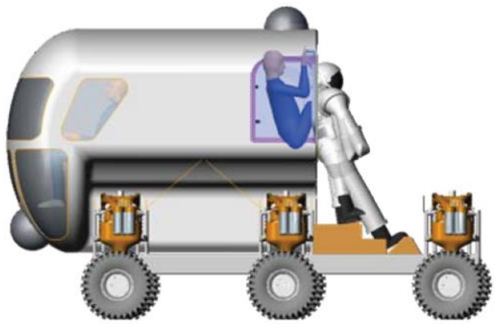


Figure 71: Suitport illustration from (Gernhard et al, 2008) reduces loss of air when exiting or entering the rover and greatly reduces the dust problem.

None of this makes Mars settlement impossible, but Mars does not seem to be an optimal place to colonize for its own sake. There has to be another reason to take the tremendous efforts needed to colonize a place like this.

Planetary protection as an essential part of an ambitious, vigorous approach to human exploration - starting with exploration and settlement experiments on the Moon

One way to explore the solar system in an ambitious, and yet responsible way is to focus human efforts on the moon to start with. The spacesuits and habitats designed for Mars also work on the Moon. Some of the challenges are similar, such as the dust, which may be easier to deal with on the Moon. Injured astronauts remain only a couple of days MEDEVAC from Earth and replaced damaged equipment can get to the explorers again in a matter of days.

Whatever we do further afield, the Moon is bound to be the main place tourists visit outside of Earth for the foreseeable future. It will be a science hub, it may become a hub for the maintenance and manufacture of spacecraft in the natural vacuum conditions and the low gravity for launch to orbit (Schrunk et al, 2007), and we saw that it is of astrobiological interest too in **Potential for early discoveries of Martian life from samples of Martian meteorites preserved in ice at the lunar poles - likely pre-sterilised by natural processes sufficiently to protect Earth**

Once there are human settlers anywhere in our solar system, some will surely settle the Moon, either the vast lunar caves, or the peaks of almost eternal light at the lunar poles with near constant temperatures and nearby accessible lunar ice. There would be hotels on the Moon, tourists visiting, scientific bases and perhaps commercial exports from the Moon.

So why not start our settlement experiments on the Moon? Sending humans into space is hard. The Apollo astronauts were test pilots that made decisions in seconds with a cool head, in situations that would have swiftly killed less able astronauts. One example of this is when Apollo 10 tumbled during the test of the ascent stage from just above the lunar surface and they had seconds to act to identify and resolve the problem (Evans, 2014) (Woods et al, 2018). Returning to the Moon is dangerous enough at present. We don't need to look for other missions that are more exciting and dangerous than this.

The retired Canadian astronaut Chris Hadfield, former commander of the ISS, interviewed by New Scientist, put it like this (Klein, 2017)

> ***"I think ultimately we'll be living on the moon for a generation before we get to Mars. If the world and the moon were threatened and the only way to preserve our species was to launch from Earth, we could go to Mars with yesterday's technology, but we would probably kill just about everybody on the way."***
>
> ***"It's as if you and I were in Paris, paddling around in the Seine in little canoes saying, 'We've got boats, we've got paddles, let's go to Australia!' Australia? We can barely cross the English Channel. We're sort of in that boat in space exploration right now. A journey to Mars is conceivable but it's still a lot further away than most people think."***

The Moon is not only safer, it's also a natural place to test and develop reliable technology that can later be used for multi-year missions throughout the solar system. A habitat that is self-sufficient for multiple years on the Moon, similarly to an interplanetary mission, would eliminate all the costs of re-supply from Earth. This is worth developing for its own sake as it would be a major saving for lunar exploration compared to one that needs to be supplied every three months like the ISS. In the process of learning to live on the Moon we learn to live on a voyage to another planet in a small spacecraft.

The Moon is of astrobiological interest too. It has the same meteorite flux as Earth, but in the conditions of the Moon we can recognize meteorites that fell billions of years ago. It should have meteorites from early Earth, Mars, Ceres and possibly Venus too. The amounts may be substantial.

According to one estimate, 7 ppm of the lunar surface materials are from Earth. A 10 km by 10 km region of the lunar surface may have 20 metric tons of materials from early Earth, including any fossils of early life. The materials transferred from Venus over the same area may amount to 1-30 kg and from Mars as much as 180 kg (Armstrong et al, 2002).

Some of these meteorites would still contain organics, preserved on the Moon at liquid nitrogen temperatures in the permanently shaded craters at its poles, a storehouse of cryopreserved samples of ancient chemistry and biology from the inner solar system. In this way we can study organics and perhaps even cryopreserved life from the early solar system, with no risk of forward or backwards contamination. The lunar caves are also of great interest and the Moon may have many surprises in store for us that we can't yet anticipate.

Once our spacecraft are proven to be safe for long duration missions, this can then be followed by orbital missions to Mars and the telerobotic exploration of Mars in the context of a vigorous program of human exploration.

Perhaps the result of our astrobiological surveys is that we find early Martian life or even protocells, that mean we need to preserve Mars free from terrestrial life, for decades, to give enough time to understand what is there and study it from orbit, or indefinitely. Or perhaps we find mirror life or some other life form that is hazardous to return to Earth or our habitats.

If so, it's a discovery to treasure, not a reason to despair. As Carl Sagan put it in "Cosmos", (Sagan, 1980)

> ***The existence of an independent biology on a nearby planet is a treasure beyond assessing, and the preservation of that life must, I think, supersede any other possible use of Mars.***

Race and Randolph suggest that human space explorers may have a moral obligation to respect the integrity of extraterrestrial ecosystems just as they do for terrestrial ecosystems we value (Race, 2002).

> *While pragmatic concerns are important in maintaining the opportunity for future science study, a more fundamental and central rationale for a policy of non-interference or non-disturbance of an extraterrestrial ecosystem arises if ET life is found. Put simply, human space explorers may have a moral obligation to respect the integrity of extraterrestrial ecosystems just as they do those on Earth. It can be argued that extraterrestrial ecosystems should continue to function essentially the same as they did before their discovery by space explorers, following their natural evolutionary or development trajectory, whatever that might entail.*

Depending what we find, Martian life might be as valuable and interesting on the microbial scale as our tropical reefs or rainforests, and as we find out more about them and how their ecosystems work, even though only microbial, or maybe primitive multicellular life, we might value them in the same way that we value unique ecosystems on Earth.

If we do find mirror life on Mars, say, risking our astronauts and Earth's biosphere, or the treasure of an independently originated life that we could easily have made extinct by mistake, we will know that it was a wise decision to do the telerobotic study from orbit first. There are many other places in our solar system for humans to explore in person, and perhaps eventually to settle.

As T Heppenheimer put it (Heppenheimer, 1977:chapter 2):

> ***"Mars, the focus of so many hopeful dreams, might be bypassed. It will see its research centers for geology and other studies, but it appears to have few resources which cannot be had elsewhere. Even if it did, its gravity would make it costly to lift them out. Its atmosphere is just thick enough to prevent the use of a mass-driver. Yet the atmosphere is too thin to screen the solar ultraviolet or permit the use of aircraft for transportation.***
>
> ***Mars of the great volcanoes, Mars of the deserts, of the frosty nights and the whistling winds in the canyons—if it is to be colonized, it will be done as an afterthought in the history of the human reach into space. It may remain a vast dry land, far from the major centers of commerce or population, thinly populated and of interest mainly to the people that live there. Mars may be the Australia of future centuries."***

The Moon has some potential for commercial exports – while there is no convincing case for commercial exports for Mars - and extant life on Mars, especially of novel biochemistry, could potentially be of great commercial value

The Moon has some potential for viable commercial exports including possibly platinum and ice for spacecraft, and could have a viable tourist industry (Wingo, 2004) (Wingo, 2016) (Spudis, 2016) (Schrunk et al, 2007).

It is not easy to find similar exports for Mars because of the deeper gravity well than the Moon, and the distance, too far, at present for easy establishment of a viable tourist industry or other industries that require easy and safe transit within days instead of months.

The discovery of unfamiliar life on Mars could give the planet this commercial interest needed for long term settlement. This could be a reason to explore Mars telerobotically from orbit and start the first settlements in orbit.

Intellectual understanding of unfamiliar life on Mars could be the most valuable export from the planet, commercially as well as intellectually. Whether Mars has early life or advanced but different life like mirror life, or related life that has evolved for a few hundred million years on a different planet, it would be the only terrestrial planet within light years of travel that has life on it apart from Earth. Studying that life from orbit could be a motivation for space settlement in orbit around Mars.

Study of terrestrial extremophiles has led to enzymes that are widely used and are the basis of a billion dollar industry in extremophile enzymes (Sarmiento et al, 2015). Study of life from Mars could lead to many discoveries of a similar nature.

Discovery of extant life on Mars could lead to long term interest in the planet, including orbiting colonies using sterile robots as our mobile eyes and hands to explore the planet from orbit via telepresence, and perhaps develop it commercially too, making it more habitable for Martian life

It's impossible to know yet what we find on Mars. Perhaps we do find mirror life that's hazardous to humans or to Earth's biosphere. Or perhaps we find early life on Mars or some other form of life that's vulnerable to extinction from competition with terrestrial life (similarly to the Arthur C. Clarke story for Venus). See

- Planetary protection as an essential part of an ambitious, vigorous approach to human exploration - starting with exploration and settlement experiments on the Moon
- Arthur C. Clarke's story "Before Eden" exploring the theme of accidental extinction of extraterrestrial life in the forwards direction (above)

If we do find hazardous life that can never be returned to Earth, or early life that we value as a treasure, or for some other reasons astronauts need to remain in orbit around Mars, it would still be possible to develop the surface of Mars commercially, and with commercial exports. Martian commerce could be done telerobotically too, using the methods of autonomous mining (Mueller et al, 2012) (Laguna, 2021).

Several authors have suggested bootstrapping space exploration with seed factories that makes more equipment on the surface of the Moon or other destinations, and even a copy of itself (Freitas et al, 1981) (Kalil, 2014) (Metzger et al., 2013).

This would also help with keeping the equipment on Mars sterile of terrestrial life, if this is needed. So long as the original seed factory is sterile, any equipment made on Mars made by the small seed factory of 100% sterile manufacturing robots and 3D printers would itself also be sterile. This could be a way to accelerate exploration or commercial use of Mars.

Chris McKay has discussed an approach he calls planetary ecosynthesis. If we do find a second genesis of life on Mars, biologically different from terrestrial life – it could be that the life still lingers in isolated refugia, but is headed towards future extinction.

In this scenario, we might decide not just to preserve it but to enhance conditions for growth and make Mars more habitable for Martian life, to whatever extent we can. He suggests this could greatly benefit humans, and more than outweigh any utilitarian value from attempts to make Mars itself more habitable for terrestrial life (McKay, 2009).

> *Perhaps the most interesting and challenging case is that in which Mars has, or had, life and this life represents a distinct and second genesis.*
>
> *I would argue that if there is a second genesis of life on Mars, its enormous potential for practical benefit to humans in terms of knowledge should motivate us to preserve it and to enhance conditions for its growth. Observations of Mars show that currently there is no global biosphere on that planet and if life is present it is in isolated refugia or dormant. It is possible that life present on Mars today is at risk of extinction if we do not alter the Martian environment so as to enhance its global habitability.*
>
> *An appreciation for the potential utility and value of the restoration of a Martian biota does not depend on the assignment of intrinsic value to alternative lifeforms. The creation of a second biosphere using a second genesis of life could be of great utilitarian value for humans in terms of the knowledge derived ranging from basic biology to global ecology. And a case can be made that its' value exceeds the opportunity cost of not establishing human settlements on Mars.*

The discovery of extant life could then, indirectly, lead to a long term interest in the planet and settlement of the Martian moons as well as settlements in orbits around Mars that are of especial value for studying the surface such as the orbit proposed for HERRO (Oleson et al, 2013) (Valinia, 2012). These would be a few of the many settlements in an expanding exploration throughout the solar system. Humans and robots would work together, the sterile robots on the surface as our mobile eyes and hands and the humans in orbit operating them for the decision making.

This could be a stepping stone to human outposts or colonies further afield such as Jupiter’s Callisto or Saturn’s Titan, and settlements in self contained habitats throughout the solar system, spinning slowly for artificial gravity and built from materials from asteroids and comets

We could set our sights on eventually sending humans to Callisto in the Jupiter system. Callisto, the outermost of the larger moons of Jupiter has abundant resources of ice, organics from carbonaceous meteorites, and other resources, Callisto also has lower radiation levels than Mars and the same planetary protection classification as the Moon (Adams et al, 2003) (Kerwick 2012) (McGuire et al, 2003)

Once we have faster interplanetary transport, Saturn’s moon Titan is an attractive target for a human base, since it is one of the few places in the solar system where a spacesuit is not needed. The temperatures are extremely low but this needs only thermal protection, essentially, high tech drysuits with the thermal protection only 7.5 cm thick, and batteries to heat a visor and gloves (Nott 2009), due to its greater than Earth pressure atmosphere. Thermal protection is far easier to make and maintain than protection from vacuum conditions.

Titan has abundant wind and hydro power (Hendrix et al, 2017), organics for making plastics, a stable environment and complete protection from ionizing radiation and large meteorites. Titan’s planetary protection status needs to be confirmed but forward and backward contamination of any native Titan life seems unlikely at such low temperatures. The main contamination risk would be of flows of liquid water (cryovolcanism). If liquid water is present, it would need careful study first, as this would provide a possible habitat for Earth life as well as a habitat that could have alien life that our astronauts and Earth need protection from (Wohlforth et al, 2016a) (Wohlforth et al, 2016b).

Whether any of these are easy places to live long term may depend on the gravity requirements for human health which are not yet known. However it is not yet known if the gravity on the Moon or Mars is suitable for human health long term either. It’s possible that they all need to be supplemented with the use of slow centrifuges spinning for artificial gravity during sleep, exercise etc.

If our aim is space settlement, planets may not necessarily be the obvious choice they seem to be. There is enough material in the asteroid belt to eventually build habitats with area the equivalent of a thousand times the surface land area of the Earth or more in the form of Stanford Torus type habitats and such habitats are more customizable to match human requirements. This is an observation that goes back to O'Neil in 1969 when he was teaching freshman physics, (Heppenheimer, 1977:chapter 2)

The first answers they came up with indicated there was more than a thousand times the land area of Earth as the potential room for expansion. They concluded that the surface of a planet was not the best place for a technical civilization. The best places looked like new, artificial bodies in space, or inside-out planets.

The classical science-fiction idea, of course, is to settle on the surface of the moon or Mars, changing the conditions there as desired. It turned out that there were several things wrong with this, however. First, the solar system doesn't really provide all that much area on the planets—a few times the surface area of Earth, at most. And in almost all cases the conditions on these planets are very hard to work with.

There's far more potential for settlement in the asteroid belt than there is on either Earth or Mars, measured according to the available land area. In addition, settlers can choose whatever climate and even atmosphere, and gravity level that they like for the habitats.

Over the centuries, and millennia, settlers may be able to set up colonies out to Pluto and beyond, using thin film mirrors to concentrate the sunlight (Johnson et al, 1977).

"At all distances out to the orbit of Pluto and beyond, it is possible to obtain Earth-normal solar intensity with a concentrating mirror whose mass is small compared to that of the habitat."

Conclusion - legal process is both understandable and necessary

I hope the considerations presented above show that we do need to take extreme care with a sample returned from Mars, at least until it is better characterized. This is not just bureaucratic red tape. There are sound reasons that require it, grounded in the need to protect human health, and the proper functioning of Earth's biosphere.

The legal ramifications are far greater once we consider possibilities for extraterrestrial life, such as mirror life, to disrupt the entire biosphere of Earth. Almost all aspects of human life become relevant to the legal process.

We saw that it is possible that some formulation of Sagan's criterion becomes legally required as a result of expert discussion of the worst case scenarios in the process of public scrutiny of the sample return plans.

If a version of Sagan's criterion is applied, mission planners will need to be able to guarantee that the mission is risk free, at least to the level where there is no appreciable risk of large scale modification of the biosphere or harm to numbers of the order of a billion people or more. At our present state of knowledge we don't know enough to give that assurance.

We saw that if any of the current published designs is used as a basis, relying on HEPA or ULPA filters, the design of the facility seems certain to be changed during the legal process. We saw that the technology to build the filters that are likely to be legally required doesn't exist yet. If such a radical redesign is needed at the end of the legal process this pushes the sample return into the 2040s and NASA is not permitted to take the level of risk with public funding.

The proposed solution to return samples to an extraterrestrial location such as above GEO avoids all these issues and has maximum flexibility. If there is no life in the sample or the life is soon shown to be harmless or easy to handle, it can be returned to Earth with only a few months extra delay compared to a sterilized return, with some sterilized samples returned immediately. If there is life and it is hazardous or potentially so, it can be studied in orbit around Earth

This could become the norm for any return of extraterrestrial biochemistry, whether from Mars, Europa, Enceladus, Ceres, or any other location. In this way the sample holding satellite above GEO could become the nucleus of a future extraterrestrial life receiving laboratory for our samples returned from anywhere in the solar system. This might become a general requirement for all missions returning samples that could contain life.

They would be returned to the orbital laboratory and studied telerobotically, until the capabilities of any life in the sample are well enough characterized to evaluate the risks. They can then be returned to Earth as soon as it is possible to demonstrate without reasonable doubt that they can be handled safely in a terrestrial facility, while any radically different biology such as mirror life is likely to be studied telerobotically in the orbital laboratory for the foreseeable future, requiring significant advances in technology before it can be studied safely directly on Earth.

This solution, to return unsterilized samples to above GEO, is not only a way to bypass legal complexities. This can be part of a measured step by step process that keeps Earth protected at all stages, while optimizing the science return and reducing the cost. This process also lets us protect our planet using the far more secure Prohibitory Precautionary Principle rather than the Best Available Technology Precautionary Principle.

This mission has an ethical dimension which requires wider participation in decision making than is usual for a space mission. We conclude that the complex legal process is both understandable and necessary.

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[Uses Harvard reference style, but in this draft, instead of a, b, c etc., I use unique ids like (NASA, 2020tesgs) - the idea is to search / replace these ids with a, b, c etc once the list is complete, after peer review]

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> *…*
> *Skin is an active immunological organ, and dysfunctional innate defenses have serious clinical implications. Products of the stratum corneum, including free fatty acids, polar lipids, and glycosphingolipids accumulate in the intercellular spaces and horny layer, exhibiting antimicrobial properties, and functioning as a first line of defense. Antimicrobial peptides (AMPs) exhibit potent and targeted resistance against a wide spectrum of common pathogens. When this barrier is breached, second lines of protection are provided by inflammatory cascades in the subepithelial tissue. Approximately sixteen AMPs have been shown to be expressed in the skin (Table 1)*

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On Earth, photosynthesis and respiration by living things cause tiny fluctuations in our planet's otherwise steady oxygen concentration. We shouldn't expect this on Mars, though. "That's far out," Telling says: Mars appears too inhospitable for a critical mass of life capable of sustaining either process. "It's almost certainly going to be a nonbiological chemical reaction."

Trainer herself does not rule out a biological explanation, but nevertheless underscores its unlikeliness. "People in the community like to say that it will be the explanation of last resort, because that would be so monumental," she says. There are abiotic mechanisms aplenty, both known and unknown, to rule out first before leaping to any more sensational claims.

Andrews, R.G., 2020, Rocks, Rockets and Robots: The Plan to Bring Mars Down to Earth, Scientific American

A single U.S. facility ticking all of these boxes could cost around $500 million, Dreier says. And it is not yet clear if others will be built in Europe

...

MSR's masters are foregoing parachutes because the devices cannot be guaranteed to work, Vijendran says—something immortalized in 2004 by the solar-wind-particle-gathering Genesis mission, whose sample capsule broke open after an unintentional hard landing. In this case, it is simpler to build a rigid capsule that can withstand such a landing. "It just comes in, and, wham, it hits the ground," Vago says. "That's going to be an interesting one."

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>
> *"In this White Paper we argue that it is not yet time to start down the MSR path. We have by no means exhausted our quiver of tools, and we do not yet know enough to intelligently select samples for possible return. In the best possible scenario, advanced instrumentation would identify biomarkers and define for us the nature of potential sample to be returned. In the worst scenario, we would mortgage the exploration program to return an arbitrary sample that proves to be as ambiguous with respect to the search for life as ALH84001."*

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> *Photosynthesis is primarily useful for providing energy for the reduction of environmental carbon ...*
>
> *There are six known pathways for fixing carbon dioxide, of which the Calvin Cycle used in oxygenic phototrophs is the least efficient in terms of the energy and the reducing equivalents (electrons) required per mole of fixed CO_2 ...*
>
> *However, the great advantage provided by oxygenesis was its capacity to liberate life from the need to find rare electron donors such as sulphide, hydrogen or Fe(II) to support the reduction of carbon dioxide, giving oxygenic photosynthesisers an advantage over all other forms of life ...*
>
> *There are six known pathways for fixing atmospheric carbon, of which the Calvin Cycle used in oxygenic phototrophs is the least efficient in terms of the energy and the reducing equivalents (electrons)required per mole of fixed CO_2. Rubisco has a very low turnover for fixing carbon, and its carboxylase activity is compromised by opposing oxygenase activity that uses molecular oxygen to break down Ribulose-1,5-bisphosphate rather than fix CO_2 into it. Despite this, the first inventor of water-splitting was successful, and filled the niche ...*
>
> *Oxygenesis evolved only once. There are two possible explanations for this. One is that it is a Random Walk process, requiring a sequence of unlikely evolutionary steps, which would not have evolved elsewhere. The hypotheses on the origins of oxygenesis above hint this may not be the case, but do not prove it. The other explanation is that the evolution of oxygenesis is a Many Pathsprocess, one which has a high probability of occurring, but is also a Pulling Up the Ladder event, such that once oxygenesis evolved once that evolution removed the preconditions for its evolution again, in this case filling the niche of a photosynthesiser freed from limitation of an electron donor supply. The biochemistry of oxygenic photosynthesis points toward this second explanation.*

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>
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archaea and eukaryotes, and include genomic, plasmid and viral DNA (Dorward and Garon, 1990; Klieve et al., 2005; Soler et al., 2008; Biller et al., 2014; Gaudin et al., 2014; Jiang et al., 2014; Grande et al., 2015; Yáñez-Mó et al., 2015). As such, vesicles can function as vehicles of horizontal gene exchange (Yaron et al., 2000; Renelli et al., 2004; Klieve et al., 2005). Shotgun sequencing of vesicle-associated DNA from ocean samples has revealed sequences from diverse bacteria, archaea and eukaryotes (Biller et al., 2014), suggesting that vesicles could be an important mechanism mediating gene transfer among marine microbes.

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Our results suggest that bacteria might indeed survive on Mars if shielded from UV, for instance by martian dust, since it is known that a few millimeters of soil is enough for UV protection (Mancinelli and Klovstad, 2000; Cockell and Raven, 2004). In view of the resistance of desert strain of Chroococcidiopsis to ionizing radiation (Billi et al., 2000; Verseux et al., 2017), the exposure in LEO to a total dose of 0.5 Gy of ionizing radiation did not affect biofilm survival. Hence, based on the dose of 76 mGy/year measured by the Curiosity rover at Gale Crater's surface (Hassler et al., 2013), dried biofilms would survive on Mars more than half a decade. In addition, since the UV dose received in LEO corresponds to approximately 8 h under a Mars UV flux at the equator (Cockell et al., 2000), the speculated biofilm survival supports the possible dissemination of viable organisms. If carried, for instance, by winds at 5 m/sec (Gomez-Elvira et al., 2014) with the average flux mentioned above, they could travel more than 100km without dying. However, other factors found on Mars need to be taken into account so as to reduce the planetary protection risk, such as the presence of perchlorates that have been shown to be highly damaging to life (Wadsworth and Cockell, 2017)

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… Our finding suggests that a putative microbial life-form at least as resistant to desiccation and radiation as the investigated desert cyanobacterium could withstand some exposure to UV on the martian surface.

… Our findings support the hypothesis that opportunistic colonization of protected niches on Mars, such as in fissures, cracks, and microcaves in rocks or soil, could have enabled life to remain viable while being transported to a new habitat

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> 10: *“**SR-SAG2 Finding 3-1:** Cell division by Earth microbes has not been reported below –18°C (255K).*
>
> ***“Revised Finding 3-1:** Cell division by Earth microbes has not been reported below –18°C (255K). The very low rate of metabolic reactions at low temperature result in doubling times ranging from several months to year(s). Current experiments have not been conducted on sufficiently long timescales to study extremely slow-growing microorganisms.”*

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>
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>
> ***Section 3.3:*** *In the Moon's formation temperatures of proto-Earth and progenitor impactor material in simulations grow to thousands of Kelvins, sufficient to drive off the great majority of all volatiles, but these are not necessarily the only masses in the system. Either body might have been orbited by satellites containing appreciable volatiles, which would likely not be heated to a great degree and which would have had a significant probability of being incorporated into the final moon. Furthermore, there is recent discussion of significant water being delivered to Earth/Moon distances from the Sun in the minerals themselves (Lunine et al. 2007, Drake & Stimpfl 2007), and these remaining mineral-bound even at high temperatures up to 1000K (Stimpfl et al. 2007). The volume of surface water on Earth is at least 1.4 × 109 km3, so even if the specific abundance of lunar water is depleted to 10−6 terrestrial, one should still expect over 1010 tonnes endogenous to the Moon, and it is unclear that later differentiation would eliminate this. This residual quantity of water would be more than sufficient to concern us with the regolith seepage processes outlined above.*

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>
> *The U.S. takes a different approach for filter classification of HEPA filters. The mother of all test procedures for these filters in the U.S. is MIL-STD-282, which was introduced in 1956. Other test procedures include e.g. IEST-RP-CC001 and IEST-RP-CC007. Each test procedure specifies certain particle sizes at which efficiency is evaluated. Depending on the filter class evaluated, this is done at 0.3 µm, 0.1 - 0.2 µm or 0.2 - 0.3 µm.*

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> *"Only recently, with the reality of a Mars Sample Return project, have we started to revisit and think in depth about implementation of backwards planetary protection," said Lisa Pratt, NASA's planetary protection officer. The last time NASA seriously thought about backwards planetary protection, she noted at the MEPAG meeting, was during the Apollo program a half century ago.*
>
> *...*
>
> *A concept review and development milestone known as Key Decision Point (KDP) A are scheduled before the end of the current fiscal year.*
>
> *"We're not prepared to discuss that at this point in time," Watzin said when asked at the MEPAG meeting for the cost of the overall program. "As we go forward into KDP-A, we'll have to start talking about that. Towards the end of this fiscal year is when we'll be ready to have that conversation."*

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> *That schedule was too aggressive for the independent panel. "The schedules required to support launches in 2026 were substantially shorter than the actual experience from recent, somewhat similar programs," like Mars 2020 and Curiosity, Thompson said.*
>
> *Under a revised schedule recommended by the panel, the lander mission would launch in 2028. The orbiter could launch in either 2027 or 2028, since its use of electric*

propulsion gives it the flexibility to pursue alternative trajectories. That revised schedule would delay the return of the samples until 2033.

At the same time, the study warned about delaying the missions beyond 2028 [because of risk of landing before a global dust storm with solar power issues]
...
It also recommended looking at adding a radioisotope thermoelectric generator (RTG) to the lander, or at least the lander with the MAV, to ensure sufficient power and to keep the rocket's propulsion system from getting too cold.

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Examples of such "molecular fossils" are 1.64 Ga old carotenoid derivatives (Lee and Brocks, 2011) and degradation products of chlorophylls and hemes (geoporphyrins; Callot and Ocampo, 2000) which have been reported, for example, from ~500 Ma old oil shales (Serebrennikova and Mozzhelina, 1994). Hence, the extraordinary stability of certain molecular fossils opens the prospect of detecting chemical traces of life on other planets and moons even if it became extinct a long time ago.

It is highly unlikely that a natural abiotic process generates long chain molecules that have precisely defined lengths, ordered sequences, and homochiral building blocks. Therefore, proteins and nucleic acids can certainly be regarded as strong chemical biosignatures.

Low to moderate enantiomeric excesses, as they occur, for example, in meteoritic α,α-dialkyl amino acids (Pizzarello and Cronin, 2000), are definitely not indicative of a biological origin.

On the other hand, a lack of enantiopurity can be a false-negative result because the initial enantiopurity could have been lost by racemization, a process well-known for the proteinogenic L-amino acids (Bada and Schroeder, 1975; Bada, 1985). Furthermore, one should not discard the possibility that an extraterrestrial organism synthesizes both enantiomers. In fact, terrestrial bacteria produce diverse D-amino acids (e.g., D-Ala, D-Glu, D-Leu, D-Met, D-Phe, and D-Tyr) which have effects on the peptidoglycan of the cell wall, both directly by incorporation into the polymer and indirectly by regulating enzymes that synthesize and modify peptidoglycan (Höltje, 1998; Lam et al., 2009). Another intriguing example from terrestrial life is the simultaneous presence of L- and D-isovaline in some fungal peptides (Degenkolb et al., 2007).

No natural non-biological processes that generate them have been observed in nature, but there are some indications that, at least in rare instances, natural abiotic compounds might be enantiopure. For example, there is a single case where, under laboratory *conditions, a small enantiomeric excess of an amino acid was amplified to near enantiopurity (>99%; Klussmann et al., 2006). This amino acid was serine under solid–liquid equilibrium conditions in water at the eutectic point. However, for all other amino acids tested, enantiopurity was not achieved. Also, this mechanism will not work with chiral compounds that crystallize as conglomerates (i.e., mixtures of pure L and pure D crystals). Because of the special conditions and compounds necessary, it is unclear if this physical process is relevant to the generation of enantiopurity (i.e., enantiomeric excesses near 100%) in extraterrestrial environments.*

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> *“RNA is the key to the ribosome, which is what makes proteins. There’s almost no question that RNA, which is a molecule involved in catalysis, arose before proteins arose,” Benner explains. The difficulty is that for RNA to assemble into long strands– which is needed for genetics – you can’t have the assembly taking place in water. “Most people think that water is essential for life. Very few people understand how corrosive water is,” Benner says. For RNA, water is extremely corrosive – bonds cannot be made within water, preventing long-strands from forming.*
>
> *However, Benner says that these paradoxes can be resolved with the help of two very important groups of minerals. The first are borate minerals. Borate minerals–which contain the element boron–prevent life’s building blocks from devolving into tar if incorporated into organic compounds. Boron, as an element, is seeking electrons to make itself stable. It finds these in oxygen, and together the oxygen and boron form the mineral borate. But if the oxygen boron finds is already bonded to carbohydrates, the carbohydrates linked with boron form a complex organic molecule dotted with borate that’s less resistant to decomposition.*
>
> *The second group of minerals that come into play involve those that contain molybdate, a compound that consists of molybdenum and oxygen. Molybdenum, more famous for its conspiratorial relation to the Douglas Adams classic A Hitchhiker’s Guide to the Galaxy than for its other properties, is crucial, because it takes the carbohydrates that borate stabilized, bonds to them and catalyzes a reaction which rearranges them into ribose: the R in RNA.*

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> *Soils from the hyper-arid core of the Atacama Desert have cell numbers and culturable counts similar to University Valley permafrost (Supplementary Table S3), but small, viable microbial communities are activated and detected when Atacama soils are wetted (Navarro-González et al., 2003; Crits-Christoph et al., 2013). Our results suggest that microorganisms in the University Valley permafrost soils analysed here are not exposed to sufficiently long and frequent clement conditions to allow for metabolism or growth. Instead, our results suggest that a fundamental threshold may be crossed in some University Valley permafrost soils, where the combination of permanently subfreezing temperatures, low water activity, oligotrophy and age are severely constraining the evolution of functional cold-adapted organisms*
>
> *Very low microbial biomass was found by direct microscopic cell counts (1.4−5.7 × 10^3 cells per g soil) in both the dry and ice-cemented permafrost using DTAF stain as described by Steven et al. (2008). Comparatively, 2 orders of magnitude higher cell counts (1.2−4.5 × 10^5 cells per g soil) were detected in the active layer and permafrost soils from the Antarctic Peninsula.*

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The MSR mission currently being planned will return limited amounts of sample, mainly rocks. It is not scheduled to collect airfall dust, which is the material required in relatively large quantities for testing. However, the returned tubes, which will have been exposed on the Martian surface for around 10 years,will almost certainly be covered in dust - and it is possible that this material might be suitable for the abrasion testing. What is likely to be more useful, though, is that collection and characterization of the airfall dust from the exterior surfaces of the sample tubes will help in production of a high-quality dust simulant. The grain size, shape, angularity, composition and density of the airfall dust will be replicated and large quantities synthesised, enabling large-scale testing of engineering systems to be undertaken.

...

The disadvantages of removing a sample from its environment prior to analysis revolve around changes that might occur because the sample is no longer in thermal or redox equilibrium with its surroundings.

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"Although some observers see value in "bright-line" levels of acceptable risk, history suggests that acceptable risk will ultimately be defined on a case-by-case basis. Key decision factors such as the risk of he exposed population, the resource cost of meeting risk targets, and the scientific quality of risk assessments vary enormously from one decision context to another.

Administrative discretion is necessary to weight these factors on a case-by-case basis. No magic risk number can substitute for informed and thoughtful consideration by accountable officials who work with the public to make balanced decisions.

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“How likely is it then, that ‘non-self’ recognition will work also for alien microbes?"

"Here we presume, that general evolutionary principles hold. Namely, that biological defense mechanisms evolve only when the threat is actually present and not just a theoretical possibility. Under this assumption the outlook for two clashing complex biospheres becomes quite dire."

"In the best case scenario the microbes of one of the biospheres will eat at first through the higher multicellular organisms of the other biosphere. Primitive multicellular organisms may however survive the onslaught through a strategy involving rapid reproduction and adaption. The overall extinction rates could then be kept, together with the respective recovery times, 1–10 Ma, to levels comparable to that of terrestrial mass extinction events."

"In the worst case scenario more or less all multicellular organism of the planet targeted for human settlement would be eradicated. The host planet would then be reduced to a microbial slush in a pre-cambrian state, with considerably prolonged recovery times. The leftovers of the terrestrial and the indigenous biospheres may coexist in the end in terms of ‘shadow biospheres’ "

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Improved instrumentation on rovers that might detect and identify a diversity of potential in situ biosignatures, including ancient organic molecular biosignatures, designed with the ability to differentiate biotic and abiotic signals in micro- or macrostructures. Instrumentation could also be better attuned to the unique complications of biosignature preservation on Mars (e.g., deeper drilling to access potentially better preserved organics)

The fluorescence spectrometers on SHERLOC can detect condensed carbon and aromatic organics by deep UV-induced fluorescence, and SHERLOC's Raman spectrometer will allow classification of aromatic and aliphatic organics. Raman spectrometry can also be used to detect minerals relevant to aqueous chemistry. While these measurements would allow us to identify reduced carbon compounds, there may not be sufficient structural information to distinguish between a biological signal and extraterrestrial organic input.

A major knowledge gap that will directly impact our ability to choose an appropriate landing site is what terrestrial analog environments might look like—what the biosignature signals might be—if photosynthetic microorganisms had not evolved and instead the environments were only inhabited by chemosynthetic microorganisms

4.4. Strategies and priorities

In many of the environments discussed, there is a dichotomy between habitability and preservation—many of the conditions that make an environment more habitable are destructive to one or more of the biosignatures of interest. For example, fluid flow in the subsurface of hydrothermal environments helps create the redox gradients that support communities that inhabit the outflow channel. Fluids are also essential for lithification and the associated decrease in permeability essential for long-term preservation. Preservation is enhanced by rapid burial and mineral precipitation that encases and lithifies biological materials in less permeable matrices—in these cases, silica from hydrothermal environments, or silica-enriched aqueous environments, is an important material for preservation. However, these same fluids can degrade biosignatures such as mineralogy, chemistry, and micro- and macrostructures. One strategy for astrobiological exploration has to be to seek out a "sweet spot" where these two balance each other so that long-term preservation is possible. This sweet spot may occur as conditions change through time.

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As shown in Table 2, when a sample containing 100,000 spores was analyzed, either Bleach Rite® or 10% bleach was able to dramatically reduce (<0.0001% remaining) the number of viable spores at the earliest time point, and no viable spores were detected after 20 minutes of treatment. Complete sterilization was not attained until 20 minutes post-inoculation due to 1 cfu being present at 10 minutes in the 10% bleach-treated groups.

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5W) developed for Mars under SBIR. The compressor could be mounted on the lander and not be a part of sample-canister mass that is returned to Earth; for example, it could utilize a solenoid release/separation mechanism, with Schrader-like input valve in series with microvalve seal. Airborne dust also could be collected with addition of 3 valves and a dust filter (Figure 6). After gas reservoir is filled and reservoir valves closed, large volumes of Mars air would be pumped through filter to collect and trap dust and its valves closed.

With consideration of upcoming Mars-targeted missions, we conclude that gas collected in a newly designed and purpose-built valved sample-tube sized vessel, which could be flown on either SFR or SRL, would be considered of higher priority than either the head space gas or a sealed M2020 sample tube. Conceptually, this vessel would require no more physical space to return than a sealed empty sample tube and alleviate concerns about the manufacturing and history of a non-purpose-built vessel, and the valving would provide a more robust mechanism for sealing the vessel and testing the seal upon return.

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chapter 7

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On Spirit there is a yearly pattern with steady dust deposition throughout roughly the colder half year from late southern summer to late southern winter, which encompasses the Martian aphelion, and overall dust removal during the warmer and windier perihelion season from late southern winter to late southern summer.

On Opportunity ... the overall variation between highs and lows is smaller, and there are two periods of overall dust deposition and two periods of overall dust removal every year. The deposited dust thickness peaks once in the middle of the northern hemisphere spring. This peak recurs very regularly 6 times. ... There is also a peak roughly in the middle of the southern spring. This peak is clear in the first year, but the pattern becomes more irregular later in the mission and is entirely absent in the last year.

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PM: I disagree with Laura on this. Article VI of the Outer Space Treaty provides that all state parties to the treaty are responsible for their activities in outer space, whether they're carried out by government agencies or private companies. Countries are required to subject private companies within their jurisdiction that engage in space activities to an authorization requirement and continuing supervision. So, the United States is responsible for compliance with the Outer Space Treaty by our private companies or entities that go into space.

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>
> *The resistance of D. radiodurans is not exclusive to radiation and desiccation but extends also to many toxic chemicals and conditions. Therefore, Dra is called a polyextremophile, a robust "generalist," to be distinguished from specialized extremophiles with an evolutionary redesign of their proteome (e.g., proteins purified from thermophiles are thermostable in vitro). Unlike specialized extremophiles, Dra does not thrive on extreme conditions—indeed, it does not grow while desiccated or when heavily* irradiated—but it can reproduce under standard growth conditions after recovering from damage inflicted by chronic moderate, or acute intense, exposures to cytotoxic conditions.

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> *Hydrated sulfates may form through notable acid weathering of dust and sand inside the ice deposit when volcanic aerosols dissolve in the thin films of water to create acidic solutions (36); however, this process has difficulty explaining the duricrust features. Therefore, one scenario that we prefer is that the predepositional regolith underwent cementation and lithification during the rising or infiltration of briny groundwater to form the observed platy rocks (Fig. 5). The salt cements (e.g., sulfates or opaline silica) precipitate from the groundwater in the capillary fringe zone, where active evaporation and accumulation can occur (37). Episodic fluctuation of the groundwater table may further thicken the indurated section and result in a fine-layered structure. After evaporation, the regolith overlying the duricrust is subject to deflation and erosion, while the duricrusts are resistant to aeolian erosion (38). In this scenario, kilometer-scale briny groundwater may have been episodically active and interacting with the colluvium at the landing site. Alternatively, aqueous minerals such as hydrated silica have been observed to be associated with flow features and pitted cones elsewhere in the northern plains (12), and the observed mineralogy and duricrust in this work may have some generic link with the pitted cones in the vicinity of the rover (Fig. 1), which requires further investigation by the Tianwen-1 orbiter and Zhurong rover*
>
> *The hydrated minerals and widespread salt cementations imply the presence of briny liquid water in the subsurface, which may have been generated by melting the ground ice during temporary climate perturbations (e.g., volcanism and impacts).*
>
> *Specifically, possible dike swarms responsible for landform formation or recent volcanism from the Elysium region could have been a heat source for maintaining the groundwater system or melting the ice. Alternatively, local transient liquid water under current climate condition may be responsible for local melting of subsurface ground ice, forming indurated duricrust, in which case the water-rock interaction and the spatial extent would be limited.*
>
> *Determining the mineralogy and spatial extent of the platy rocks in future traverse would provide clues to distinguish different climate conditions for these water activities. Regardless of the potential heat source, the in situ observations manifest recent aqueous activities on Mars, suggesting that the cold and dry late Amazonian epoch may have been episodically punctuated by short-duration climatic warming events that result in melting of ground ice at latitude less than 30°N. The in situ identification of such environments points to a more active Amazonian surface hydrosphere for Mars than previously considered.*

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have liquid water, quotes Morten Bo Madsen, associate professor and head of the Mars Group at the Niels Bohr Institute at the University of Copenhagen. :

> *"We have discovered the substance calcium perchlorate in the soil and, under the right conditions, it absorbs water vapour from the atmosphere. Our measurements from the Curiosity rover's weather monitoring station show that these conditions exist at night and just after sunrise in the winter. Based on measurements of humidity and the temperature at a height of 1.6 meters and at the surface of the planet, we can estimate the amount of water that is absorbed. When night falls, some of the water vapour in the atmosphere condenses on the planet surface as frost, but calcium perchlorate is very absorbent and it forms a brine with the water, so the freezing point is lowered and the frost can turn into a liquid. The soil is porous, so what we are seeing is that the water seeps down through the soil. Over time, other salts may also dissolve in the soil and now that they are liquid, they can move and precipitate elsewhere under the surface," explains Morten Bo Madsen, associate professor and head of the Mars Group at the Niels Bohr Institute at the University of Copenhagen.*

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> *page 419: Category 1: Small and medium-sized meteoroids (with radii from 2 to 80 cm and masses from 0.1 kg to~6:5 tons (if ρ≈3g/cm3). These meteoroids provide no shielding against the galactic cosmic rays, on the contrary they increase the dose rates caused by unshielded GCR by the creation of more particles in-side the meteoroids. However, they could still serve as vehicles for viable transfers from Mars to Earth lasting 1 million years* for D. radiodurans R1 and 0.3 million years for B. subtilis (wild type) if DNA decay is not limiting.

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> *A temperature-regulated change in CO_2 solubility could at least partially account for the amplitude of the LR oscillation. However, the HT oscillation phase leads the LR oscillation by as much as two hours, an unusual circumstance if this were simply a chemical oscillation driven by thermal fluctuation.*
>
> *(Admittedly there is uncertainty concerning the delay between change in temperature at the head end assembly, perhaps one inch over the 0.5 cc soil sample, and soil sample temperature per se. However, a two-hour lag seems quite long for what is presumably a convective and radiative process. Similarly, thermal-induced movement of gas between the soil sample and the beta detector requires only about 20 minutes.)*
>
> *Furthermore, the LR oscillation does not slavishly follow the thermal variation; rather, it seems that the LR rhythm is extracted from the HT oscillation, while high frequency noise is not. This is very common in terrestrial organisms in which a low frequency periodic stimulus (i.e., a zeitgeber) such as a 12:12 light/dark cycle can entrain a circadian rhythm, while high frequency transients in the same stimulus are ignored (e.g.,*

turning on the light in the bathroom at night for a minute or two does not alter normal entrainment to the light/dark cycle).

Furthermore, there is abundant evidence that as little as a 2º C temperature cycle can entrain circadian rhythms in terrestrial organisms such as lizards, fruit flies, and bread molds and entrainment can be preferential to the diminution phase of the temperature cycle, in analogy to the temperature fall that occurs at sunset on Mars).

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Dried-rewetted biofilms and dried-UV-irradiated-rewetted biofilms were tested for respiration by monitoring the INT reduction by dehydrogenases after 72 h of rehydration. The INT staining revealed 30 and 10% of alive cells with insoluble red formazan spots in the cytoplasm of dried-rewetted biofilms and dried-UV-irradiated-rewetted biofilms, respectively,

After 7 years of air-drying, Chroococcidiopsis not only avoided genome degradation but preserved at least a sub-set of mRNAs and 16S ribosomal RNA.

... In the present work, the occurrence of survivors in dried biofilms and dried-UV-irradiated biofilms was proved by growth after transfer into liquid BG-11 medium (not shown) and by INT reduction after 72 h of rewetting.

Reshaping the boundaries of Chroococcidiopsis desiccation and UV tolerance has implications in the search for extra-terrestrial life since it contributes to defining the habitability of Mars and planets orbiting other stars. In fact, the UV dose used here corresponds to that of a few hours at Mars's equator (Cockell et al., 2000). Hence, considering that survivors occurred in the bottom layers of the biofilms (Baqué et al., 2013), it might be hypothesized that if a biofilm life form ever appeared during Mars's climatic history, it might have been transported in a dried state under UV radiation, from niches that had become unfavorable to niches that were inhabitable (Westall et al., 2013). The reported survival also suggests that intense UV radiation fluxes would not prevent the presence of phototrophic biofilms or their colonizing of the landmass of other planets.

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> *D-1: NASA and ESA should replan the baseline MSR program for SRL and ERO launches in 2028, with the potential of a 2027 ERO launch continuing to be studied for feasibility and potential benefits.*
>
> *NASA Response: NASA partially concurs with this recommendation. The MSR team will continue to examine the 2026, 2027 and 2028 launch opportunities during Phase A, while working to maintain current schedules to mature the design and retire risk as quickly as possible during Phase A, while also working to minimize program impacts due to COVID.*
>
> *C-3:This study should be augmented to include a strong focus on potential Radioisotope Thermoelectric Generator(RTG)incorporation on either a single-lander or two-lander approach, to achieve the following benefits:Type1 launch option in 2028 Possible longer surface timeline RTG-sourced heating of the MAV*
> *NASA Response: NASA concurs with this recommendation*

NASA, 2020tesgs, The Extraordinary Sample-Gathering System of NASA's Perseverance Mars Rover.

NASA, 2021nmttm, NASA Moves to the Next Phase in a Campaign to Return Mars Samples to Earth, SciTechDaily

NASA, 2021mpb, Marscopter press briefings.

Marscopter altitude: **Bob Balaram** says looking at 10 meters, limited by the range of the laser altimeter. MiMi Aung says 600 to 700 meters.

1:01:32 from: After NASA's Historic First Flight: Ingenuity Mars Helicopter Update Streamed live on 19 Apr 2021

Maximum separation: **MiMi Aung:** "The vehicles can be apart up to a kilometer or further ... The signal to noise ratio is extremely good. We can go beyond 1 kilometer distance."
33:41 from: NASA’s Ingenuity Mars Helicopter’s Next Steps (Media Briefing) Streamed live on 30 Apr 2021

Distance for a single flight: **Bob Balaram:** “I think a total of 600 meters is not unreasonable. 2 minutes flight at 5 meters per second is a possibility. That’s probably where we'll see how well it does, and if there is more margin we can use for the flights. That's probably a good place to think of the limit.”
1:01:20 from: NASA’s Ingenuity Mars Helicopter’s Next Steps (Media Briefing) Streamed live on 30 Apr 2021

NASA, 2021prmtl, Perseverance Rover, Mission Timeline › Landing

NASA, 2021wnpr, Watch NASA’s Perseverance Rover Land on Mars, Thomas Zurbukin at 14:45

Macy Ragsdale: *Is anything alive on Mars?*

Thomas Zurbuchen (NASA associate administrator): *That's a question i ask myself, is anything alive there, and frankly at the surface where we're going right now with Perseverance we do not believe there's anything alive right there, because of the radiation that's there, it's chilling cold and there's really no water there. But guess what we think that three billion years ago this looked like a stream that you may see on earth and frankly a lot more similar than Earth but water with a magnetic field just like the earth with an atmosphere and the question is at that time three billion years ago were there single cell organisms just off the type that developed on earth so is there life on on Mars overall we don't know but where we're going right now we're really looking for ancient life and that's what we're so excited about.*

NASA, 2022mpfs, Fact Sheet Proposed Action, MSR PEIS Fact Sheets

NASA, 2022msr, public comments, MSR, PEIS

NASA, 2022smsr The Safety of Mars Sample Return, MSR PEIS Fact Sheets

Such a Mars sample receiving facility would have design and sample handling requirements equivalent to those of biological safety laboratories used for research studies of infectious diseases. The well-established safety protocols and engineering controls used to isolate hazardous biological materials in such laboratories address issues that are very similar to those involved in Mars sample return. At this time, there are several options under study for implementing a Mars sample receiving facility.

NASA, 2022nepa, National Environmental Policy Act; Mars Sample Return Campaign Federal Register / Vol. 87, No. 73 / Friday, April 15, 2022 / Notices

The general scientific consensus is that the Martian surface is too inhospitable for life to survive there today. It is a freezing landscape with no liquid water that is continually bombarded with harsh radiation.

Scientists are interested in returning samples that may reveal what the Martian environment was like billions of years ago, when the planet was wetter and may have supported microbial life.

There is no current evidence that the samples collected by the Mars 2020 mission from the first few inches of the Martian surface could contain microorganisms that would be harmful to Earth's environment.

Nevertheless, out of an abundance of caution and in accordance with NASA policy and regulations, NASA would implement measures to ensure that the Mars samples are contained (with redundant layers of containment) so that they could not impact humans or Earth's environment, and the samples would remain contained until they are examined and confirmed safe for distribution to terrestrial science laboratories. NASA and its partners would use many of the basic principles that biological laboratories use today to contain, handle, and study materials that are known or suspected to be dangerous.

NASA, 2022nic, NASA Invites Comment on Initial Plans for Mars Sample Return Program

NASA will consider all comments received during the scoping process in the subsequent development of the MSR Draft Environmental Impact Statement, which is currently scheduled to be released for public comment later this year.

NASA, 2022wip, *"Where is Perseverance?". Mars 2020 Mission Perseverance Rover*

NASA, n.d.ame, Atmosphere, Mars Education

NASA, n.d.cls, Curiosity's Landing Site: Gale Crater

NASA, n.d.cm, Chris McKay, at NASA Ames

NASA, n.d.dan, Dynamic Albedo of Neutrons (DAN), see also archived page for scientists: Dynamic Albedo of Neutrons (DAN)

NASA, n.d.ecilm, Eugene Cernan in Lunar Module

NASA, n.d.hsp, Health Stabilization Program

NASA, n.d. mbtn, Mars, by the numbers surface area 144,371,391km2. This seems to be based on the volumetric mean radius of 3389.5 kilometers as 4*pi*3389.5^2. See NASA n.d. Mars Fact Sheet/ Since a sphere has the minimum surface area to volume ratio of any spheroid then the Martian area is at least this much.

NASA, n.d.mfs, Mars Fact sheet

NASA, n.d.monm, Map of NASA's Mars Landing Sites

NASA, n.d.MOXIE MOXIE, and MOXIE for scientists.

NASA, n.d. MSASL, Martian seasons and solar longitude at: http://www-mars.lmd.jussieu.fr/mars/time/solar_longitude.html accessed on July 17, 202

NASA, n.d. PRLS, Perseverance Rover's Landing Site: Jezero Crater, accessed at https://mars.nasa.gov/mars2020/mission/science/landing-site/, accessed on 17 July 2020.

NASA, n.d.sd, Shield Development

NASA, n.d.,SEH,, System Engineering Handbook, see particularly
2.5 Cost Effectiveness considerations
3.3 Project Pre-Phase A: Concept Studies
3.4 Project Phase A: Concept and Technology Development
3.5 Project Phase B: Preliminary Design and Technology Completion

NASA, n.d.WiC, Curiosity: Mission: Where is the rover?

Curiosity landing site: 137.44°E, 4.589°S

NASA, n.d.WiP, Where is Perseverance?

Perseverance landing site: 18.45°N 77.45°E, NASA, n.d. WISO, What is Surface Operations?

drills core samples from about 30 promising rock and “soil” (regolith) targets and caches them on the Martian surface (Objective C)

Naseem, M., Osmanoglu, Ö. and Dandekar, T., 2020. Synthetic Rewiring of Plant CO_2 Sequestration Galvanizes Plant Biomass Production. *Trends in Biotechnology*, *38*(4), pp.354-359.

The CETCH cycle requires less energy to operate than other aerobic CO_2 -fixation pathways. One limitation of CETCH is the production of glyoxylate, a less active metabolic intermediate that requires acetyl-CoA (AcCoA) or propanoyl-CoA [3] for conversion into other metabolites. Also, glyoxylate is not well connected to other metabolic pathways. Despite functional impediments associated with any synthetically designed pathway, CETCH is the most efficient artificial cycle that fixes (in vitro) several-fold more CO_2 than does the natural CBB. The incorporation of CETCH-based enoyl-CoA carboxylase/reductases (ECRs) should be an excellent alternative to the native Calvin cycle. It can sequester approximately 80 CO_2 molecules per second (in vitro) compared with RuBisCO, which fixes two to five CO_2 molecules per second in plants.

National Research Council. 2009. Assessment of Planetary Protection Requirements for Mars Sample Return Missions (Report). p. 59.

"*It has been estimated that the planning, design, site selection, environmental reviews, approvals, construction, commissioning, and pre-testing of a proposed SRF will occur 7 to 10 years before actual operations begin. In addition, 5 to 6 years will likely be required for refinement and maturation of SRF-associated technologies for safely containing and handling samples to avoid contamination and to further develop and refine biohazard-test protocols. Many of the capabilities and technologies will either be entirely new or will be required to meet the unusual challenges of integration into an overall (end-to-end) Mars sample return program.*"

National Center for Biotechnology Information, 2022g, PubChem Compound Summary for CID 750 ,Glycine

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> *page 15: The NC-77 transgenic line, however, had a three-fold increase in bio-mass yield compared with wild-type. This increased bio-mass production in NC transgenics with adjustable light harvesting antenna sizes, however, raises the question why have algae and plants evolved large, less effi-cient, fixed light-harvesting antenna systems that oversaturate downstream electron transfer processes during most (80%) of the day. In mixed species environments, the abil-ity to shade or reduce the light available to competing spe-cies may offer a selective advantage, because limiting light availability to other species would reduce their growth rates and presumably their fitness (Zhuet al., 2008; Ortet al., 2015). Species competing for light are clearly impacted by shading as plant canopies close or as algal cultures reach high cell densities. Thus, having large light-harvesting antenna systems may reduce light availability for competitors and enhance fitness for plants or algae thatshade competitors as is the case in high-density algal cul-tures. In addition, plants living lower in the canopy or algae growing deeper in the water column often experi-ence very low light conditions.*
>
> *Having a large light-harvesting antenna would allow photosynthesis and growth at light intensities that could not support the growth of algae with smaller antenna sizes optimized for growth at higher light intensities. In fact, algae that grow at extreme depths in the oceans have among the largest light-harvesting antenna sizes known in photosynthetic organisms (Yamazakiet al., 2005).*

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NOAA, n.d.cwcu, Can we clean up, stop, or end harmful algal blooms?

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> A critical nutrient to the expansion of both subsurface and surface life on any planet is the availability of nitrogen as an aqueous species. On Earth, microorganisms evolved the ability to fix N2 into ammonia with the development of nitrogenase to overcome this constraint. Nitrogenases, Nif proteins, are complex enzymes, utilizing iron, molybdenum, and/or vanadium, that exist in both bacterial and archaeal domains. Phylogenetic comparison of genes that comprise nitrogenases and a complement of proteins required for their regulation indicate that nitrogenases emerged in anoxic sulfidic environments on Earth within obligate anaerobic thermophilic methanogens and were transferred to obligate anaerobic clostridia (Boyd et al., 2015), both common subsurface microorganisms. As Nif proteins were adopted first by the aerobic diazotrophic lineage Actinobacteria and then by the more recently evolved aerobic Proteobacterial and Cyanobacterial lineages, the Nif protein suite became more complex to protect the core MoFe-bearing proteins from O2 (Boyd et al., 2015). Although it is not clear whether the emergence of the more complex protein occurred prior to or after the Great Oxidation Event, it is certain that the ancestral protein emerged in an anoxic environment when the demands for aqueous nitrogen species exceeded the abiotic supply. The implications for martian ecosystems are that nitrogenase would have also likely emerged within an anaerobic subsurface environment, not in the oxic surface environment.

Experiments on the effects of low pN2 on diazotrophic nitrogen-fixing soil bacteria have shown that they could grow in N2 partial pressures of 5 mbar but not 1 mbar (Klingler et al., 1989). This result suggests that further experiments on wild-type species are required to determine whether the evolution of pN2 in the martian atmosphere was a significant deterrent to the expansion of early life, especially after Mars lost most of its atmosphere. Analyses of the nitrogen budget and of nitrogen cycling from deep subsurface environments in South Africa indicate that the pN2 is higher at depth than on the surface, that most of this N2 originates from the rock formations through nitrogen cycling, and that N2 is being actively fixed in the subsurface by microbial communities (Silver et al., 2012; Lau et al., 2016b). Given the presence of a cryosphere barrier to diffusion on Mars, the nitrogen availability and perhaps even the pN2 of subsurface brines are likely to be higher there than on the martian surface.

…

Meter-sized Fe(II)-rich carbonate/iron oxide concretions (Fig. 4) are found in Jurassic sandstone deposits of southwest Colorado that were formed at hundreds of meters' depth between 2 and 0.5 Ma as the Colorado River Basin was uplifted (McBride et al., 2003; Loope et al., 2010). Similar-sized ferroan calcite and siderite concretions occur in Late Paleocene/Early Eocene Wasatch Group sandstones, and siderite nodule-bearing cores from the formation (Lorenz et al., 1996) yielded thermophilic Fe(III)-reducing bacteria that were capable of producing prodigious quantities of siderite (Roh et al., 2002). In subaqueous systems unconstrained by rock matrix, authigenic carbonate mounds at CH4 and hydrocarbon seeps, formed from carbon mobilized by methane- and alkane-oxidizing microorganisms (Greinert et al., 2001; Formolo et al., 2004; Ussler and Paull, 2008), can be hundreds of meters tall and more than a kilometer wide (Klaucke et al., 2008).

OpenClipArt, n.d., Etiquette CD rom

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The message to both clinicians and clinical microbiologists is that there are no uniformly nonpathogenic fungi: any fungus can cause a lethal infection in a sufficiently immunocompromised host and should never be dismissed out of hand as a contaminant.

Phillips, C.R., 1974. The planetary quarantine program: Origins and achievements, 1956-1973 (Vol. 4902). Scientific and Technical Information Office, National Aeronautics and Space Administration.

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Preva, n.d., Preva Dental X-ray System

> *The maximum momentary line current (less than 5 s) of the Preva is 10 A when operated on 120 V (1.2 kW). Operation at higher input voltage will reduce the maximum current (5 A at 240 V). The technique factors producing the maximum momentary line current are 65 kV, 7 mA, 2 s*

PubChem, n.d., Bisphenol A, Retrieved October 15, 2020 from https://pubchem.ncbi.nlm.nih.gov/compound/Bisphenol-A#datasheet=LCSS.

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> *An extraterrestrial pathogen lacks existing diagnostic testing and medical management protocols. Future health emergency response measures may need to incorporate knowledge deficits into plans and exercises, and all those responding, including healthcare workers and first responders, will need education and training in advance of the spacecraft's return.*
>
> *The lack of knowledge surrounding extraterrestrial pathogens, from disinfection to incubation periods, presents a novel situation for which current public health and healthcare emergency preparedness efforts have not been developed. The spectrum of biological threats (natural outbreak, intentional attack, and laboratory accident) does not include a novel pathogen of unknown biological makeup.*

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193:

> *Meteorite analysis has detected boron in Martian clays, important for abiogenesis since borate minerals can stabilize ribose and catalyze other prebiotic chemistry reactions (see Stephenson et al. 2013 and sources therein). Mars may also have enjoyed greater availability of prebiotically important phosphate than Earth (Adcock et al. 2013). Climate models suggest liquid water was transient on Mars (Wordsworth et al. 2013b), which suggests the evidence of wet/dry cycles. Such cycles are useful for prebiotic chemistry: aqueous eras are beneficial for the formation of biotic monomers, while dry eras tend to concentrate feedstock molecules and aid monomer polymerization (Benner & Kim 2015), relevant to the formation of nucleotides and amino acids (Patel et al. 2015). Finally, the putative dryness of Mars and the potential acidity of its early aqueous environment*

owing to dissolved carbonic acid from a CO_2 -dominated atmosphere, suggest molybdate, which is suggested to catalyze formation of prebiotically important sugars such as ribose, may have been stable on Mars (Benner & Kim 2015; Benner et al. 2010). Hence, there is growing interest in the possibility that prebiotically important molecules may have been produced on Mars(Benner 2013), and even the hypothesis that life may have originated on Mars and been seeded to Earth (Kirschvink & Weiss 2002; Gollihar et al. 2014; Benner & Kim 2015)

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Renno, N., 2014, How liquid water forms on Mars, YouTube video, University of Michigan Engineering (transcript from 1:48 onwards)

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> *...*
>
> *Evaluations of the proposal should be conducted both internal and external to NASA and Centre National d’Etudes Spatiale (CNES) and the space research communities in the nations participating in the mission. An ethical review should be conducted at least at the level of the Agencies participating and these reviews made public early in the process (in France, the national bioethics committee, Comité Consultatif National d'Ethique pour les Sciences de la Vie et de la Santé, CCNE, is the appropriate organization). The final protocol should be announced broadly to the scientific community with a request for comments and input from scientific societies and other interested organizations. Broad acceptance at both lay public and scientific levels is essential to the overall success of this research effort.*

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> …
> *"A single terrestrial microorganism reproducing as slowly as once a month on Mars would, in the absence of other ecological limitations, result in less than a decade in a microbial population of the Martian soil comparable to that of the Earth's. This is an example of heuristic interest only, but it does indicate that the errors in problems of planetary contamination may be extremely serious."*

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>
> *There are so many examples of human misuse of the Earth that even phrasing this question chills me. If there is life on Mars, I believe we should do nothing with Mars. Mars then belongs to the Martians, even if the Martians are only microbes. The existence of an independent biology on a nearby planet is a treasure beyond assessing, and the preservation of that life must, I think, supersede any other possible use of Mars.*

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As a result the wild-type cells located deeper in the culture are exposed to ever decreasing levels of light the further they are from the illuminated surface (see“Open PondSystems”section). These shaded cells are prevented from capturing enough solar *energy to drive photosynthesis efficiently. This in turn drastically reduces the efficiency of the overall culture.In contrast, small antenna cell lines with reduced LHCIIlevels have the advantage that they improve the light penetration into the bioreactor (Fig.7a) and better match itto the energy requirements of each photosynthesizing cell. Thus small antenna cells at the bioreactor surface absorb only the light that they need, largely eliminating fluores-cence of excess energy. This in turn allows more light (i.e.the light wasted in wild-type as fluorescence and heat) to penetrate into the bioreactor so that even cells deeper in the culture have a near optimal exposure to light*

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>
> *July, 1976, the month that VL1 [Viking Lander 1] landed on theMartian surface, was also the month of the outbreak of Legionnaires' disease at the American Legion convention in Philadelphia.*
>
> *The cause, Legionella pneumophila, is a facultative, Gram-negative rod that is one of several human pathogens now known to be carried in the intracellular environments of protozoan hosts. L. pneumophila can also persist, even outside of any host, as part of biofilms.*
>
> *In essence, all that a potentially infectious human pathogen needs to emerge and persist is to grow and live naturally under conditions that are similar to those that it might later encounter in a human host. On Mars, these conditions might be met in a particular niche within the extracellular environment of a biofilm, or within the intracellular environment of another single-celled Martian organism. It is important to note the numerous biofilms observed aboard the Mir space station, which were found on surfaces and within water*

plumbing. These films were often multi-species and included bacteria, fungi, and protozoa.

To be sure, the genetic similarity between humans and protozoa is much greater than could be expected between humans and the Martian host of a Martian microbe.

However, the L. pneumophila example does bring into question the rationale of the need for host-pathogen coevolution. Even in the context of a planetary bio-sphere that is limited to single-celled life, and even where there is unlikely to have been a co-evolution between agent and host organism, the possibility of infectious agents, even an invasive type, cannot be ruled out.

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> *There are different ways of looking at such a community of progenotes. On the one hand, it could have been the loose-knit evolutionary (genetic) community just discussed. On the other, it could have been more like a modern bacterial consortium, with cells*

cross-feeding one another not only genetically but also metabolically. Cell–cell contacts would have facilitated both processes. In both views of the community, the latter in particular, it is not individual cell lines but the community of progenotes as a whole that survives and evolves. It was such a community of progenotes, not any specific organism, any single lineage, that was our universal ancestor—a genetically rich, distributed, communal ancestor.

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Highlights of what's new in this article

[needs updating]

The planetary protection literature already covers the issue of the length of quarantine period for an astronaut or technician (Carl Sagan observed that the latency period of leprosy is measured in decades), and the ethical issue of keeping an astronaut or technician in quarantine when they have a sudden life threatening condition potentially caused by extraterrestrial materials.

However I found no previous mention of asymptomatic carriers, like typhoid Mary (Korr, 2020) as an issue for the use of human quarantine to protect Earth from extraterrestrial life. Also I found no mention of the need for quarantine to contain life that is not pathogenic of humans but could cause problems for other terrestrial lifeforms, or the need for quarantine to contain unfamiliar biology such as mirror life that could have adverse effects on the terrestrial ecosystems. This article concludes that there seems to be no way to contain such hazards using human quarantine, unless we know what is in the sample and what its capabilities are. This conclusion seems to be new.

See

- Complexities of quarantine for technicians accidentally exposed to sample materials

In discussions of worst case scenarios for return of extraterrestrial life, I have seen no previous study of the effects of returning mirror life.

See

- Example of mirror life nanobacteria spreading through terrestrial ecosystems

Several of the other worst case scenarios I look at here seem to be new to the planetary protection literature. See for instance:

- Possibility of extraterrestrial Martian life setting up a “Diminished Gaia” on Earth
- Worst case scenario where terrestrial life has no defences to an alien biology - humans survive by ‘paraterraforming’ a severely diminished Gaia

I found no previous mention of the observation that NASA would need to know what they need to build before they can start the build process, and that since they won’t be able to overrule objections as they did for Apollo, this won’t be known until they complete the legal process. As a result the timescale for a sample return in this article is longer than in previous studies.
See

- NASA procedural requirements for mission planners - they need to have a clear vision of the problems and how they can be solved before key point A

Previous work has proposed that the reason that Mars is close to the cold arid limit of life with its atmosphere close to the triple point of water could be due to processes such as abiotic photosynthesis with up to several bars of CO_2 sequestered in the Martian dust alone. However I found no previous mention of the idea that this could be the result of biology and biotic photosynthesis.

Previous work has looked at the possibility of swansong biospheres, also at an anti-gaia where life makes a planet gradually less inhabitable until it makes itself extinct. However I found no previous mention of the idea of combining both of those in a swansong gaia, where life maintains the atmosphere at close to the triple point of water, never making itself extinct, for billions of years. and can do so over a wide range of emissions scenarios for the volcanoes.

See

- Suggestion of a self perpetuating “Swansong Gaia” maintaining conditions slightly above minimal habitability for billions of years - as a way for early life to continue through to present day Mars

This is relevant to the topic of this article because processes to keep the atmosphere at slightly above minimal habitability for billions of years add to the possibilities for returning viable life in the sample.

Previous articles have suggested returning extraterrestrial life to low Earth orbit or to the Moon, but I found no previous discussion of returning it to the Laplace “ring” plane above GEO.

See

- **Recommendation** to return a sample for teleoperated 'in situ' study above Geosynchronous Equatorial Orbit (GEO)

Previous articles suggest sterilizing extraterrestrial samples with gamma rays. However I found no previous discussion of using nanoscale X-ray emitters for sterilization during the six months return flight from Mars to Earth.

See:

- Suggestion to use nanoscale X-ray emitters for sterilization

There are many proposals for sample receiving facilities. However I wasn't able to find any that correctly cited the ESF 2012 requirement that release of a single particle of 0.05 microns is not permitted under any circumstances. The EURO CARES design cites this study but due to an unfortunate typo, the design is for a one in a million chance of release of a sample of 0.1 microns, per particle, which would not comply.

See:

- Order of magnitude typo in cite for EURO-CARES sample return facility design - ESF study's probability $< 10^{-6}$ is for unsterilised particles of 0.01 µm not 0.1 µm

Previous studies all look at HEPA and ULPA filters. This seems to be the first to notice that these filters don't comply with the ESF recommendations, and that new technology is needed if these recommendations become legal requirements

See

- Filter technology innovations needed for 0.05 µm standard - HEPA and ULPA filters are not adequate

This seems to be the first study to consider the available technology and observe that even the best experimental filters in laboratories such as an experimental filter to attempt to contain the smallest droplets with individual SARS - CoV2 viruses don't yet comply with the ESF recommendation

See

- Example of best available nanofilter technology from 2020, not yet commercially available, filters out 88% of ambient aerosol particles at 0.05 microns - far short of the ESF requirement to filter out 100% at this size – though the ESF requirement at 0.05 microns can be met with nanoparticles in water under high pressure

Previously Chris McKay suggested grabbing a sample of dirt as a low cost sample return mission. However I found no previous suggestion to modify the ESA sample fetch rover to add a sample of dirt on top of the rock samples from Perseverance.

- Possible use of Perseverance - or modification of ESA's Sample Fetch Rover to return samples from shallow sand dune subsurface

Previous articles have looked at the effect of UV on transfer of life in the dust, however I found no discussion of the possibility that native Martian life could evolve to cover itself in nanoparticles of iron oxides to protect its propagules from UV light, in a process similar to the agglutinated external sediment cysts built by some foraminifera in the sea.

See

- Could Martian life be transported in dust storms or dust devils, and if so, could any of it still be viable when it reaches Perseverance?

I found no previous suggestion to add empty sample tubes with magnets in the neck to the ESA fetch rover, to be left on the surface to collect dust from dust storms and dust devils while the rover fetches the Perseverance samples..

See

- **Recommendation:** Extra sample of air and airfall dust to search for Martian life, assess forward contamination issues for terrestrial microbes, dust dangers for astronauts, and to return a random sample of wind-eroded rock from distant parts of Mars
- **Proposal:** magnets could be used to enhance dust collection
- **Proposal:** to use the sample return capsule as a dust collector – keep it open to the atmosphere before adding the sample tubes

I have found no previous papers on sampling the dust in dust storms to search for traces of distant inhabited habitats perhaps thousands of kilometers away, such as happens on Earth with terrestrial transfer of spores in dust storms from deserts. Also I can't find any suggestion that spores from such habitats could explain the Viking results. See:

- Searching for distant inhabited habitats on Mars through presence or absence of spores in the dust

The idea of using the Marscopter or the Perseverance rover itself to look for young craters within reach of Perseverance excavated to a depth of several meters in the last few thousand years seems to be new to this article.

See

- Proposal to use Marscopter or observations by Perseverance from a high elevation to search for recently excavated small craters for less degraded organics from early Mars

Carl Sagan said of a Mars sample return:

> *The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives.*

However I can't find previous studies that elaborate on this and connect it with insights from synthetic biology to suggest that the legal process of a sample return would be likely to consider the need for higher standards of containment than for a normal biosafety laboratory.

See

- Formulating Sagan's criterion and variations on the precautionary principle - which one is appropriate for a Mars sample return?

The suggestion that one possible outcome of the legal process is that the mission can't go ahead seems to be new to this article.

See

- A requirement for similar levels of safety to those used for experiments with synthetic life would lead to the Prohibitory version of the Precautionary Principle and make unsterilized sample return impossible with current technology and current understanding of Mars

The suggestion that Mars could have life that can never make safe contact with Earth's biosphere is an unstated background to the planetary protection literature, but this article may be first to state this clearly.

I think this may be the first paper to say explicitly that the worst case scenarios include situations where we can never return life from Mars to Earth, and where quarantine of astronauts can't protect Earth, for instance if Mars has mirror life. At present we have no way to prove that any unfamiliar biology on Mars would be safe for Earth's biosphere.

See

- Similar considerations apply to astronauts returning from Mars - in some scenarios such as mirror Martian life, astronaut quarantine would be insufficient to protect Earth's biosphere

There have been several proposals to study Mars telerobotically from orbit, but the more detailed suggestion that we need to complete a rapid preliminary astrobiological survey of Mars from orbit first before we can make properly informed decisions about sending humans to the surface seems to be new.

See

- Resolving these issues with a rapid astrobiological survey of Mars, tele-operating rovers from orbit

The proposal to use the technology for a Venus lander to construct heat sterilized 100% sterile rovers to explore Mars is not new to this article but the detailed discussion is new, especially the proposal that we need to develop a specification for 100% sterile rovers before we start large scale exploration of Mars from orbit. Such a specification and designs based on it will greatly simplify planetary protection for exploring Mars and other potential locations for life like Europa and Enceladus.

See

- Design specifications for 100% sterile rovers for fast safe astrobiological surveys throughout the solar system

The aim in this article is to try to anticipate some of the issues that will be raised in the future, as experts from disciplines like epidemiology, synthetic biology, the engineering of filters, and ethicists and lawyers examine NASA's recommendation:s. Many new points in this article come as a result of widening the literature examined to cover these fields.

For details about some of the other points that may be new, see:

- What is new to the planetary protection literature in this article below.

Do please contact me if you know of previous work on these topics, thanks!

Outline - and what's new in this article

[needs updating]

Much of this paper is an application of material from the wider scientific literature that so far hasn't been applied to the problems involved in planetary protection.

"New presentation" means a new take on the literature, organized to make comparisons easier, shed light on connections.

"Summarizes:" means it summarizes the literature

"Expands" means it summarizes the literature but with extra details or suggestions

"Recommendation" means a recommendation for future missions.

"New" means to the best of my knowledge, the material is new to the published literature about planetary protection. To give one example, to my knowledge, the issue of symptomless superspreaders has never previously been discussed in the context of planetary protection but is widely discussed in epidemiology.

“original” means original research, such as the proposal of a Martian swansong Gaia.

Many of these matters may have been discussed informally and not published previously. If the reader knows of any previous publication of any of these points - including preprints, or video presentations or discussions - do say and I will cite it. Thanks!

This section is primarily to assist reviewers. However it may also be of interest to readers too, as an outline and to show what is new in the article.

[Mid edit, sections marked (...) need to be written]

No legal precedent for a restricted sample return with “potential for adverse changes to the environment of Earth” (Apollo guidelines had no peer review)

Summarizes: Perseverance has landed on Mars, the legal process hasn’t started yet, and there is no legal precedent.

Apollo procedures didn’t protect Earth even according to the Interagency Committee on Back Contamination (ICBC) that advised NASA A

Summarizes:NASA was able to overrule objections in 1969, but would not be able to do so today.

Comet and asteroid sample returns are straightforward - but are unrestricted sample returns - sterilized during collection - or Earth has a similar natural influx

Summarizes: we have already done comet and asteroid sample returns but they were straightforward because there was no back contamination risk.

Controversial 2019 recommendation to classify parts of Mars as category II, similar to the Moon, in forward direction

Summarizes: Stern et al.’s recommendation to classify regions of Mars as Category II like the Moon in the forwards direction, yet restricted category V in the backward direction, similarly to the situation for the Moon in 1969. They base this on the report by Rummel et al. from 2014.

- 2015 review: maps can only represent the current incomplete state of knowledge for a specific time – with knowledge gaps on survival of terrestrial life in dust storms and potential for life to survive in habitats hard to detect from orbit - so can’t yet be used to identify which areas of Mars are of planetary protection concern in the forwards direction

Summarizes: Board (2015) which was not cited by Stern et al. criticises Rummel et al. and concludes that before a Category II classification of parts of Mars we need to complete knowledge gaps on transfer of terrestrial life in the dust storms, and potential for life to survive in microhabitats on Mars not easily detected from orbit.

2015 review: maps can only represent the current incomplete state of knowledge for a specific time – with knowledge gaps on survival of terrestrial life in dust storms and potential for life to survive in habitats hard to detect from orbit - so can't yet be used to identify which areas of Mars are of planetary protection concern in the forwards direction

Summarizes: NAS 2020 review says the category system applies to a mission not a target and makes criticisms similar to Board et al in 2015 of attempts to categorize regions of Mars using maps.

Why we may need to protect Earth from backward contamination even if it turns out that forward contamination is unlikely or impossible - example of Sagan's proposed habitat on the Moon

New presentation: The Apollo missions attempted to protect Earth from backwards contamination of and didn't protect the Moon from forward contamination. It's interesting to look into how this was possible and ask whether such a situation could arise on Mars for future missions

This is possible if

1. Native habitats for Martian life can't be colonized with terrestrial life (e.g. too cold for terrestrial life), OR
2. Contamination with terrestrial life spreads only with great difficulty over thousands of years from one part to another of the Martian surface.

Comparison of the Moon as understood in 1969 with Mars as we understand it today

New presentation - the idea of comparing the situation in 1969 with Mars as we understand it today in this direct point by point way may be new.

First restricted sample return since Apollo - with proposed microhabitats and no natural way for any life from surface layers to reach Earth

Summarizes: some of the proposed microhabitats such as the RSLs, the puzzling Viking results and discusses Greenberg's natural contamination principle.

First restricted (potentially life bearing) sample return since Apollo, however, science reviews in 2009 and 2012 have lead to increasing requirements on such a mission – especially as the result of discovery of the very small starvation mode nanobacteria

Summarizes: ESF study found a theoretical minimum size for terrestrial life which also matched the scanning electron micrographs of nanobacteria that passed through a 0.1 micron nanofilter

By European Space Foundation study (2012), particles larger than 0.05 μm in diameter are not to be released under any circumstances

Summarizes: requirements from the 2012 ESF study

The three proposed methods of containing samples in a Mars sample receiving facility, BSL-4 in a clean room, clean room in a BSL-4 and triple wall - with examples for each design

Summarizes: some of the proposed sample receiving facility designs

Order of magnitude typo in cite for EURO-CARES sample return facility design - ESF study's probability < 10^{-6} is for unsterilised particles of 0.01 μm not 0.1 μm while 100% containment is required for particles of 0.05 μm

New: Order of magnitude typo in the EURO-CARES cite of the ESF report - they cite the requirement as a one in a million chance of containing a particle with diameter greater than 0.1 μm. The ESF report discusses a one in a million chance for a particle with diameter greater than 0.01 μm.

This one in a million figure also refers to the chance of a release of a single particle at this size over the lifetime of the facility, and EURO-CARES treats it as a probability per particle.

The ESF study has a requirement of 100% containment at 0.05 μm.

The papers on sample receiving facility designs don't seem to have picked up on this requirement, but it would be brought up in the legal process.

Filter technology innovations needed for 0.05 μm standard - HEPA and ULPA filters are not adequate

New: HEPA and ULPA filters used for biosafety level IV facilities don't comply with the requirements of the ESF sample return study.

Example of best available nanofilter technology from 2020, not yet commercially available, filters out 88% of ambient aerosol particles at 0.05 microns - far short of the ESF requirement to filter out 100% at this size – though the ESF requirement at 0.05 microns can be met with nanoparticles in water under high pressure

New: discussion of whether a suitable filter can be made with current technology. Experimental nanofilters can filter out 100% of particles at this size for water treatment but have significant maintenance challenges.

For aerosols the best available technology seems to be represented by a proposed filter in development in 2020 capable of filtering out individual SARS-Cov2 particles as small as 0.06

µm. This filter, which is “state of the art” and not yet available commercially, is only able to filter out 88% of particles at 0.05 µm, far short of the 100% requirement of the ESF study.

The technology doesn’t seem to exist yet.

Need for maintenance for future 0.05 µm compliant filters

Summarizes: Equipment needs to be maintained and filters need regular replacement.

New: nanofilters for removing 0.05 µm aerosol particles are likely to be challenging to maintain and replace safely.

ESF study’s recommendation for regular review of the size limits

Expands: - the ESF study said regular review is needed. By 2020, eight years later, another review is certainly required since the minimum size was reduced from 250 nm to 50 nm / 10 nm in just three years from 2009 to 2012.

Scientific developments since 2012 relevant to review of 0.05 µm / 0.01 µm size limits

Expands: Discussion of the possibility of an RNA world microbe with a diameter of only 0.014 µm. This is mentioned in the 1999 limitations of size report as a possible interpretation of the ALH84001 meteorite nanoscale features, but got little attention since then.

The new suggestion in this article is that small microbes with a novel biochemistry such as mirror life could be better able to compete with larger terrestrial organisms. New research into synthetic life since the ESF study in 2012 might lead a review to reconsider the possibility of small RNA world microbes in the sample.

Priority early on in legal process to decide on filter requirements and to outline future technology to achieve this standard

(...)

Need for advanced planning and oversight agency set up before start of the legal process

(...)

NASA procedural requirements for mission planners to develop a clear vision of the problems and show how the solution will be feasible and cost-effective before key decision point A because of significant costs involved in modifying designs after the build starts

Summarizes existing literature

Potential changes in requirements as a result of the legal process

(...)

Minimum timeline: 2 years to develop consensus legal position, 6-7 years to file Environmental Impact Statement, 11 years to build sample return facility

(...)

Need for legal clarity before build starts - NASA has reached keypoint A for the budget for entire program, but can't know what they will be legally required to build for the facility.

(...)

Need for legal clarity before launch of ESA's Earth Return Orbiter, Earth Entry Vehicle, and NASA's Mars Ascent Vehicle.

(...)

Likely legal requirement for facility to be ready to receive samples well before unsterilized samples return to Earth

(...)

Legal process likely to extend well beyond 6 years with involvement of CDC, DOA , NOAA, OSHA etc, legislation of EU and members of ESA, international treaties, and international organizations like the World Health Organization

(...)

Public health challenges responding to release of an extraterrestrial pathogen of unfamiliar biology

Summarizes existing literature

Complexities of quarantine for technicians accidentally exposed to sample materials

(...)

Vexing issue of authorizations to remove technicians from quarantine to treat life threatening medical incidents in hospital

Expands: covers the ethical issues for human quarantine mentioned in Meltzer et al of balancing an uncertain risk of planetary protection against a certainty that an individual's life can be saved. Gives examples and discusses how it is ethically understandable that preventing risk of death or serious injury to an individual has the highest priority - but it then negates most of the value of quarantine.

Example of technician in quarantine with acute respiratory distress and symptoms similar to Legionnaires' disease

New: This is already in planetary protection discussions - but the suggestion that a Martian pathogen of human lungs could also be symptomless in some individuals like some cases of Legionnaires' disease may be new.

Arbitrariness of technician's quarantine period for an unknown pathogen

(...)

How do you quarantine a technician who could be a symptomless super-spreader of an unknown Martian pathogen?

New: Symptomless super-spreader carriers (like Typhoid Mary). This doesn't seem to be discussed previously in the planetary protection literature.

Martian microbes could participate harmlessly or even beneficially in the human microbiome but harm other terrestrial organisms when the technician exits quarantine - example of wilting Zinnia on the ISS

New: That alien life could become part of the human microbiome, and remain harmless to humans then harm other creatures or the biosphere on leaving quarantine.
The idea of alien life becoming part of the human microbiome in quarantine may be new.

What if mirror life becomes part of the technician's microbiome?

New: That the human microbiome could support mirror-life nanobacteria - especially if it is pre-adapted to use non mirror organics on Mars.

Survival advantages of mirror life competing with terrestrial life that can't metabolize mirror organics

New: That mirror-life nanobacteria from Mars may be preadapted to metabolize non mirror organics

This could be from adaptation to use racemic mixtures of organics from meteoritic and comet infall, or the result of co-existing with non mirror life on Mars.

Examining advantages of a mirror-life nanobacteria to survive in the wild on Earth even if its biochemistry is simpler than any terrestrial life (similarly to the arguments used in favour of nanobes in a shadow biosphere).

Similar considerations apply to astronauts returning from Mars - in some scenarios such as mirror Martian life, astronaut quarantine would be insufficient to protect Earth's biosphere

(...)

Telerobotics as a solution to all these human quarantine issues

(...)

Zubrin's arguments in: "Contamination from Mars: No Threat" and the response of planetary protection experts in "No Threat? No Way":

(...)

These complexities arise due to need to contain almost any conceivable exobiology

(...)

Sterilized sample return as aspirational technology demonstration for a future astrobiology mission

(...)

Level of sterilization needed similar to ~100 million years of Martian surface ionizing radiation - and would leave present day life and past life still recognizable - if recognizable without sterilization

s

(...)

Suggestion to use nanoscale X-ray emitters for sterilization

(...)

Effects of gamma radiation on rock samples - and need to test X-rays

(...)

Past and present day life in Jezero crater expected to be patchy, and hard to detect, amongst a masking signal of abiotic organics

(...)

Perseverance's target, an ancient delta in Jezero crater - high potential - but need to manage expectations - with limited in situ biosignature detection, samples not likely to resolve central questions in astrobiology

(...)
Limitations on cleanliness of the Mars sample tubes with estimated 0.7 nanograms contamination per tube each for DNA and other biosignatures and a roughly 0.02% possibility of a viable microbe in at least one of the tubes

(...)

Modern miniaturized instruments designed to detect life in situ on Mars - could also be used to examine returned samples in an orbital telerobotic laboratory

(...)

Could Perseverance's samples from Jezero crater in the equatorial regions of Mars contain viable or well preserved present day life?

(...)

Detection by Curiosity rover of liquid water as perchlorate brines in Gale crater sand dunes and similar conditions are predicted in Jezero crater dunes

Expands: Discussion of the brine layer found in sand dunes by Curiosity. Although too cold for terrestrial life, I argue that it is potentially habitable by martian life with lower temperature limits than terrestrial life using chaotropic agents, or biofilms or both. Nilton Renno briefly mentioned the idea of a biofilm making this layer habitable in an interview but it hasn't had much attention. This increases the potential for returning native life from Jezero crater.

Experiments with black yeasts, fungi and lichens in Mars simulation conditions suggest life could use the night time humidity directly without liquid water

Summarizes: Discussion of experiments in the ability of some fungi and lichens to metabolize in the presence of the high night time humidity but without liquid water, in Mars surface conditions of high UV and low atmospheric pressure and extreme variations in temperature. This possibility again can't be ruled out, and more experiments are needed.

Not much seems to have been done by way of published research in the last few years

Surface conditions of ionizing radiation, UV radiation, cold and chemical conditions don't rule out the presence of life

(...)

Sources of nitrogen on Mars as potential limiting factor – unless Martian life can fix nitrogen at 0.2 mbar

Expands: Discussion of the possibility of nitrogen fixation in the present day Martian atmosphere even with its low levels of nitrogen - which could contribute to habitability for present day life. Experiments so far have shown that this is possible at terrestrial atmospheric pressure and Martian partial pressures of nitrogen. If this is possible it expands habitability of the Martian near subsurface. This possibility can't be ruled out and needs experiments on low pressure nitrogen fixation in Mars simulation chambers.

Could Martian life be transported in dust storms or dust devils, and if so, could any of it still be viable when it reaches Perseverance?

(...)

Native Martian propagules (spore aggregates or hyphal fragments) could be up to half a millimeter in diameter, and evolve extra protection such as a shell of agglutinated iron oxide particles or chitin

(...)

Potential for spores and other propagules from nearby or distant regions of Mars similarly to transfer of spores from the Gobi desert to Japan

New: Suggestion that if Martian life is wind dispersed, it may be dispersed seasonally during the dust storm seasons. Spore formation may also be triggered by the low light levels of a dust storm.

Suggestion for year round sample collection of the dust to search for seasonal wind dispersed spores, e.g. with one sample tube left open to the dust for a Martian year. A null result here would also be significant and it would also help with studies of the survivability of terrestrial microbes as the composition and chemical composition of the dust is also likely to vary seasonally and in dependence on storms.

Searching for distant inhabited habitats on Mars through presence or absence of spores in the dust

(...)

Could local RSL's be habitable and a source of wind dispersed microbial spores? Both dry and wet mechanisms leave unanswered questions - may be a combination of both or some wet and some dry

Summarizes: Discussion of the Recurring Slope Lineae (RSLs). Though the dry formation model gets most publicity neither the dry nor the wet models are able to explain all the features, for instance the dry formation model is currently unable to explain seasonality and resupply. There may be elements of both models or some may be formed in one way and some in the other. This makes it an open question whether the RSLs are potentially habitable to present day life.

Could Perseverance find well preserved past life? Knoll criterion and difficulties of recognizing life by its structures

New: Suggestion that early Martian life might lack nitrogen fixation may be new (an obvious suggestion but not mentioned before AFAIK). Previous studies have already suggested it might lack photosynthesis, and might never have evolved it.

This is relevant for the search for past life in Jezero crater as it would be much less common if there is no nitrogen fixation as well as no photosynthesis

Summarizes:

- **issues with recognizing past life as life**
- **likely ambiguity of returned samples of past life**
- **abiotic chiral imbalances in some meteorites**

• **abiotic C13 depletion**
• **likely presence in returned samples of micron and nanoscale features** that resemble microbes and may be associated with organics
• **Infall of organics from meteorites, comets and interplanetary dust and indigenous processes** such as abiotic photosynthesis making it hard to distinguish abiotic and biotic organics
• **Degradation of past life by racemization, reactive chemicals, etc.**
Perseverance could detect distinctive biosignatures like chlorophyll and carotene - but only for exceptionally well preserved life
(...)

Recommendations to increase the chance of returning present day life, unambiguous past life, and other samples of astrobiological interest by adapting ESA's Sample Fetch Rover and Perseverance caching strategies

New: Specific recommendations for additional samples that Perseverance and the ESA fetch rover could take, that could increase the astrobiological interest.

Young craters within 90 days travel of the landing site - to search for past life less damaged by cosmic radiation - near certainty of a crater of 16 to 32 meters in diameter less than 50,000 years old

New: Recommendation to search for young (< 10 million years old) craters in Jezero crater more than 2 meters deep within the region accessible by Perseverance.

Original research: calculation of the probability of craters of various sizes and ages within reach of Perseverance for drives of various lengths in days

Probability of a new crater within reach of Perseverance forming during the mission

Original research: calculation of probability of craters of various sizes forming in the next 4 or 10 years within reach of Perseverance.

Dating young craters from orbit through fresh appearance with sharp rim
Summarizes: research into how the appearance of a crater changes due to erosion

Original research: there aren't enough craterlets of 10 cm upwards to use to identify the youngest craters of up to 32 meters.

Recommendation to use Marscopter or observations by Perseverance from a high elevation to search for recently excavated small craters for less degraded organics from early Mars
(...)
Exposure of organics through wind erosion - for samples of less degraded past life
(...)

- **Recommendation:** Extra sample of air and airfall dust to search for Martian life, assess forward contamination issues for terrestrial microbes, dust dangers for astronauts, and to return a random sample of wind-eroded rock from distant parts of Mars

New: Leaving a sample tube uncapped during a dust storm or indeed for an entire season before adding rock samples on top, or collect samples using vacuum spore collectors

Value to astrobiology of samples of the brine layers found by Curiosity in sand dunes at depths of 0 to 15 cms - to search for present day life

(...)

Possible use of Perseverance - or modification of ESA's Sample Fetch Rover to return samples from shallow sand dune subsurface

New: Suggestion to attempt to sample the brine layer found by Curiosity

Placing a pile of regolith or sand dune dust in the sample return capsule on top of a plate placed over the sample tubes before sealing it for return to Earth or in the base of the capsule - which might help resolve the controversies about the Viking labelled release experiment and give us a sample of regolith

Suggestion of a self perpetuating "Swansong Gaia" maintaining conditions slightly above minimal habitability for billions of years - as a way for early life to continue through to present day Mars

-

New: Suggestion of a self perpetuating Martian "Swansong Gaia" where feedback processes, unlike those on Earth, keep the planet perpetually at a very low level of habitability, but not quite sterile

Such feedbacks would include photosynthetic life cooling down the planet by removing CO_2 in balance with volcanic emissions keeping it at close to the triple point for water.

Previous explanations for the atmospheric pressure so close to the triple point have involved abiotic processes for maintaining pressure close to the triple point, including abiotic photosynthesis.

The new suggestion is that it could also be caused by biology. This suggestion, if true, increases the potential for present day life at low levels, barely detectable but still present. If true it also increases the possibility of present day surface or near surface life that has remained there since life first evolved on Mars

Methanogens as part of the cycle with a warming effect limited by the response of photosynthesis in a Swansong Gaia

(...)

Self limiting methanogens, methanotrophs, and Fe(III)-reducing bacteria maintaining a subsurface Swansong Gaia hydrology

(...)

Could seasonal oxygen be a possible signal of photosynthesis maintaining a Swansong Gaia homeostasis on Mars?

(...)

How does this Swansong Gaia compare with the original "Gaia hypothesis?".
(...)
Potential limits on the biomass of a Swansong Gaia on Mars using the amounts of free CO and H_2 in the atmosphere
(...)
Testing the "Swansong Gaia" hypothesis
(...)
Recommendation to return a sample for teleoperated 'in situ' study above Geosynchronous Equatorial Orbit (GEO)

New: Recommendation to return unsterilized samples to above GEO.
Advantages include

- that it is far in delta v from either Earth or the Moon,
- that it is suitable for low latency telepresence from Earth.
- The article suggests using the Laplace plane at an inclination of 7.2 degrees to the equatorial plane, a similar point of equilibrium to Saturn's ring plane. This minimizes dispersion of any high area to mass ratio (HAMR) materials shed by the sample containing satellite.

The Moon, and LEO have been suggested before but above GEO seems to be a new suggestion

Return to within the Laplace plane above GEO to contain debris in event of an off nominal explosion or other events
(...)
Low energy transfer of an Earth Return Vehicle from Mars to above GEO
(...)
Preliminary study of the returned sample above GEO
(...)
Studying life telerobotically in orbit above GEO
(...)
Possibility of early discovery of extraterrestrial microbes of no risk to Earth
(...)
Early discovery of a familiar terrestrial microbe on Mars is not enough to prove sample is safe without more research
New: Warning that the presence of closely related terrestrial life in the sample does not rule out the possibility of simultaneous presence of novel biology.

As a specific example, a mirror cyanobacteria might co-habit the same microbiomes with non-mirror cyanobacteria, perhaps with both descended from a chirality indifferent early form of life based on something similar to Joyce's enzyme.

This may be especially likely with Benner's hypothesis that life originated on Mars. Mars might have greater biodiversity than terrestrial life, including perhaps multiple independently evolved life chemistries, and branches of life, only some of which have got to Earth via panspermia.

Possibility of discovery of high risk extraterrestrial microbes needing extreme caution
(...)

Could Martian life have got to Earth on meteorites? Our Martian meteorites come from at least 3 m below the surface in high altitude regions of Mars
Expands: surface layers of dust, salt, and brines, which are most habitable and most likely to have life, are not able to get into the ejecta after a typical asteroid impact. This point is not original but it is seldom emphasized. This greatly reduces the possibility of meteorite transfer of life during the three billion years of the present drier Amazonian period on Mars.

Has life from Mars caused mass extinctions on Earth in the past?
Expands: Discussion of whether Martian life could have caused the Great Oxygenation event on Earth. This expands on a statement in the 2009 NRC study that the possibility of past life from Mars causing mass extinctions on Earth can't be ruled out. They don't give an example; an example helps make the discussion more concrete.

Potential diversity of extraterrestrial life based on alternatives to DNA such as RNA, PNA, TNA, additional bases and an additional or different set of amino acids
(...)

Could present day Martian life harm terrestrial organisms?

New: Detailed discussion of example worst case scenarios, such as sequestration of CO_2 by a mirror life ocean dwelling photoautotroph that has no secondary consumers. Although such worst case scenarios have had some discussion for laboratory safety for synthetic biology, such example worst case scenarios don't seem to be mentioned in planetary protection discussions yet.

New: Possibility that extraterrestrial fungi could infect humans with invasive biofilms. Opportunistic fungi kill an estimated 1.5 million people worldwide every year.

New: Possibility that antifungals and antibiotics have no effect on a novel biochemistry.

Could a Martian originated pathogen be airborne or otherwise spread human to human?
(...)

Microplastics and nanoplastics as an analogue for cells of alien life entering our bodies unrecognized by the immune system.
New: Discussion of permeability of the human body to microplastics and nanoplastics and exploration of whether it could be similarly permeable to alien live with a novel biochemistry not recognized by the immune system

Exotoxins, protoxins, allergens and opportunistic infection
New: Possibility that extraterrestrial fungi and other microbes could also be allergens

New: Suggestion that novel amino acids may be misincorporated similarly to BMAA and may be neurotoxins for Earth life by causing protein folding anomalies. The article proposes as a hypothesis that this might be a common occurrence for a biosphere collision with a biochemistry that has a radically different vocabulary of amino acids,

New: Discussion of impact on our biosphere of cyanobacteria more efficient at photosynthesis than terrestrial life

(...)

(...)

(...)

New: Discussion of the effects on Earth’s biosphere if a novel biochemistry becomes established, to the point where the number of microbes in an ecosystem of the novel biochemistry are the same as for terrestrial biochemistry, within orders of magnitude

New: Discussion of paraterraforming a degraded biosphere in worst case scenario

New: Discussion of the possibility of novel introduced life evolving or changing gene expression after release from a sample handling facility
New: Discussion of the possibility of life that is initially maladapted developing the capabilities to spread widely, after first establishing small populations on Earth

(...)

New: Discussion of potential beneficial effects of introducing extraterrestrial biology - most discussion focuses only on the negative effects and the potential for beneficial effects needs to be mentioned.

(...)

(...)

New: Suggestion that the use of the Best Available Technology version of the Precautionary Principle in the ESF study could be challenged in a legal review. Formulation of Sagan's statement that “we cannot take even a small risk with a billion lives” - as a criterion that if the potential worst case scenario impacts on the lives or livelihoods of of the order of a billion people or more, we should always use the Prohibitory version of the Precautionary principle rather than the Best Available Technology version

New: Suggestion that the legal review may lead to more stringent requirements than anticipated by mission planners, and that it is not guaranteed that a legal review would approve any unsterilized return. If something resembling Sagan’s criterion becomes established in law as a requirement, then we can’t currently provide this certainty. A return of an unsterilized sample to a non terrestrial facility would then be the legally required standard for future sample return of extraterrestrial biology at the early stages when we are not yet able to prove it is safe for Earth.

New: One possible outcome of the legal process is a decision that an unsterilized sample can’t be returned until it can be handled in such a way that there is no appreciable risk of adverse effects on Earth’s environment - i.e. that it is required to use the Prohibitory rather than the Best Available Technology version of the Precautionary Principle in this situation.

New: The conclusion might also be that even a minute risk of severe impact on the lives or livelihoods of a billion people always counts as “appreciable risk”. This is referred to as “Sagan’s Criterion” as it is based on a statement he made.

The authors of the 2012 ESF study say “*It is not possible to demonstrate that the return of a Mars sample presents no appreciable risk of harm.”*

If this is the outcome of the legal process, the sample would need to be sterilized or returned to some other location not connected to Earth’s biosphere to fulfill the legal requirements.

(...)

Resolving these issues with a rapid astrobiological survey, with astronauts teleoperating rovers from orbit around Mars

(...)

Value of telerobotic exploration for a planet with complex chemistry developed over billions of years, but no life

(...)

Design specifications for 100% sterile rovers for fast safe astrobiological surveys throughout the solar system

(...)

Mars less habitable than a plateau higher than Mount Everest, so high our lungs need a pressure suit to function

(...)

Dust as one of the greatest inhibitors to nominal operation on the Moon - and likely on Mars too

(...)

Planetary protection as an essential part of an ambitious, vigorous approach to human exploration

(...)

Conclusion - legal process is both understandable and necessary

(...)