A sterilized sample return would achieve virtually the same science return as an unsterilized mission and keep Earth 100% safe

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[MID EDIT TO TAKE ACCOUNT OF THE CHANGE IN THE EIS APPROVAL PROCESS WHICH CAN NOW BE COMPLETED IN LESS THAN A YEAR - SOME SECTIONS STILL REFER TO THE OLDER 6-7 YEARS PROCESS]

For NASA’s draft EIS see my preprint: [Such serious flaws in NASA's Environmental Impact Statement for a Mars Sample Return - omits major impacts – uses old science later overturned – statements cited to sources that say the opposite – no response to significant public concerns - and haven’t done the update for size limits recommended by the ESF in 2012 after they reduced it from 0.2 to 0.05 microns in just 3 years](https://osf.io/2jfnv)

For higher resolution graphics download original [Word document](http://robertinventor.com/booklets/NASA_expected_sterilize_Mars_samples_before_2039.docx) or [high resolution pdf](http://robertinventor.com/booklets/NASA_expected_sterilize_Mars_samples_before_2039.pdf). [Some figures omitted - need permission]

Please note this preprint is not yet peer reviewed and is currently in the process of development with frequent updates.

Section titles are written like mini-abstracts. For a fast overview, read the headings, and drill down into sections of interest for more details

Contents

# Introduction

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)](#kix.jbi8mnfxz305), [(Bada et al, 2009)](#kix.b77th2810md), [(Davila et al, 2010](#kix.ngzkl9svh8bg)).

Perseverance lacks these capabilities. They will be important for future astrobiological missions to Mars [(Hays et al, 2017)](#kix.p2ms9qhyq6kd) 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)](#kix.wcfa2l3gtnu). See

* [[Several studies by astrobiologists concluded we need capabilities to identify life in situ, for a reasonable chance to resolve central questions of astrobiology](#h_several_studies_in_situ)](#_kfeh6gdu853u)

It is especially important to try to return samples with clear biosignatures because Perseverance’s sample tubes are not 100% clean.

However sadly Perseverance can’t do this. In later papers we will recommend that the ESA fetch rover adds bonus samples of dirt, dust, atmosphere, and perhaps also some Martian pebbles picked up by a pre-sterilized Marscopter for astrobiologists that may be of more interest. Even if Mars has abundant present day life, as much as 1000 ultramicrobacteria per gram or more, we wouldn’t be able to spot it amongst the biosignatures unless it has very radically different biology., We can never prove the samples are safe for Earth. We can only detect life that is provably unsafe for Earth and only if it is in very large quantities in the samples.

As a result the samples will go to indefinite hold and critical review and will need to be sterilized. We will argue in other papers that the geological sample returns should be sterilized to keep Earth 100% safe and that NASA’s EIS should include a sterilized sample return as a “reasonable alternative”

# **[Mars sample tubes weren’t sterilized 100% out of concern by engineers that a sterile container might not be able to open on Mars - higher levels of sterilization needed to detect life unless Perseverance returns life with recognizably different biology or abundant exceptionally well preserved life](#h_lim_cleannliness)**

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)](#kix.uym5qp28t6e6).

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.

Perseverance could still detect Martian life if it is recognizably very different from terrestrial life, for instance, mirror biology, or if there are very large quantities of exceptionally well preserved life. But as we’ll see, the achieved levels of sterilization would not be enough to prove presence of life if it occurs in small quantities and is similar to terrestrial biology. Sadly also, the achieved levels of sterilization will likely make it hard to impossible to prove that there is no Martian life in the sample leading to risk of a false positive that will mean that samples have to be contained indefinitely as they can’t be proved to be safe to distribute unsterilized.

## Achieved levels of sterilization yield a 0.02% probability of a viable cell in at least one sample tube, so if a single viable microbe is found in one of the tubes, proof of detection of Martian life can only achieve 3.09 sigma

For living cells, the requirement is no more than a 0.1% chance of a single live terrestrial organism. This is per tube rather than per gram of sample. They estimate that they achieved a much more stringent 0.00048% [(Boeder et al, 2020: table 6)](#kix.cp59c0yrj4zr).

If their estimate is accurate, this makes it no more than a [0.02%](https://www.google.com/search?q=100*(1-(1-4.8+*10%5E-6)%5E38)) chance of finding a single live terrestrial organism in at least one of the 38 tubes [(NASA, 2020prst)](#kix.rb6yo0gof6do).

This makes the significance level for a viable microbe about [3.09 sigma](https://www.wolframalpha.com/input/?i=Prob+x+%3E+3.09+or+x+%3C+-3.09+if+x+is+standard+normal). 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 or closely resembles terrestrial life.

If a microbe is found which uses what seems to be 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)](#kix.41z9kctjpkl7). Clean room samples usually have numerous unrecognized microbial species [(Weinmaier et al, 2015)](#kix.kd26vdz6pu1l).

However, if a viable microbe is found with a clearly non terrestrial biology that would be a sure sign that it came from Mars.

## Estimated achieved level of maximum 0.7 nanograms for each tested biosignature and 8.1 nanograms total organic contamination in every gram of returned rock sample – with no tests for chlorophyll or carotenoids, amongst the most robust biomarkers for ancient life on Mars, which could also get into the tubes, for instance through the cyanobacteria found in clean room samples

As well as that small chance of a viable microbe, the sample tubes are 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[(](#kix.cp59c0yrj4zr) [Boeder et al, 2020: table 1)](#kix.cp59c0yrj4zr) [(Summons et al, 2014:991)](#kix.1hbhfaqalc2).:

* 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 per gram of returned rock sample after modelling for the effects of the tubes, contamination on the rock boring instruments and so on [(Boeder et al, 2020: table 6)](#kix.cp59c0yrj4zr).

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)](#kix.1hbhfaqalc2)

This limit of 1 nanogram per gram of returned rock sample is for the particular most abundant organic measured [(Summons et al, 2014:991)](#kix.1hbhfaqalc2). 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)](#kix.1hbhfaqalc2) and the final list of organics measured in [(Boeder et al, 2020: table 1)](#kix.cp59c0yrj4zr)

They also have a limit on the total organic carbon per sample tube.

The requirement for total organics was 10 nanograms per gram of returned rock sample. Their best estimate of the level of total organics achieved was 8.1 nanograms per gram [(Boeder et al, 2020: table 6)](#kix.cp59c0yrj4zr),

This means each gram of returned rock sample could have 8.1 nanograms of organics and 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)](#59niufgu58xk) and hard to distinguish from organics from infall from meteorites, comets, and interplanetary dust ([Goetz et al, 2016:247](#kix.5ee0degz9iqz)) [(Frantseva et al, 2018)](#kix.43cshwr9iept) as we saw in:

* [The processes on Mars expected to destroy most surface organics from past life](#h_processes_destroy_organics)

Chlorophyll and carotenoids are amongst the top candidates of the more complex molecules that could be preserved on Mars if it has life similar to terrestrial biology, because they are so resistant to ionizing radiation.

See:

* [Perseverance could detect distinctive biosignatures like chlorophyll and carotenoids - but only for exceptionally well preserved present day life and chiral excesses and C12 / 13 ratios also occur in meteorites](#h_perseverance_could_detect_chlorophyll)

Clean room samples include cyanobacteria ([Hendrickson et al, 2021](#H_Hendrickson_2021)), so the sample tubes are likely to have chlorophyll and carotenoids in them

Since they didn’t test for these biosignatures, we can't give achieved levels of organics**,** but if either carotene or chlorophyll is found in minute quantities of a few molecules, it would again be hard to rule out terrestrial contamination.

## [Perseverance’s estimated achieved levels of 8.1 nanograms of organic contamination per gram of returned rock sample is more than the amount of organics in 81,000 ultramicrobacteria, or 160 million hypothetical minimal volume RNA world nanobes and is equivalent to the organics found in trillions of terrestrial amino acids](#h_perseverance_organics_contamination) – life detection instruments that astrobiologists hope to send to Mars can detect a single amino acid in a gram of sample

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 similar volume to an ultramicrobacteria that grows to normal sized cells when cultivated, found in old or starved cultures of normal bacteria ([Duda et al, 2012](#b_Duda_2012)) ([Nakai, 2020](#b_Nakai_2020)). Some ultramicrobacteria or ultracells may be much smaller down to a volume of 0.02 cubic microns ([Duda et al, 2012](#b_Duda_2012)).

There are 10,000 microns in a centimeter so one gram (cubic centimeter) of water has a volume of a trillion cubic microns.

So a nanogram (billionth of a gram) of water has the same volume as 1000 cubic microns, or 10,000 to 50,000 ultramicrobacteria or ultramicrocells. This isn’t taking account of the water content which would likely multiply these figures by 3 or 4, as water content is typically 70% or more of the cell’s mass ([Cooper et al, 2007](#b_Cooper_2007)).

Assuming Martian microbes have a density similar to water. the estimated level of 8.1 nanograms per gram of returned rock sample for total organics is enough mass for 81,000 ultramicrobacteria at 0.1 cubic microns.

As for the hypothetical minimal volume RNA world nanobes, now referred to as ribocells, we can use an estimated volume of 50,000 nm3 ([Board et al, 1999](#kix.onye7oc8xdfg): [117](https://www.nap.edu/read/9638/chapter/6#117)), or [0.00005 cubic microns](https://www.google.com/search?q=50000+cubic+nanometers+in+cubic+microns). Even a picogram, the mass of a cubic micron of water, is enough mass for 20,000 of those hypothetical ribocells and the 8.1 nanograms are the same mass as 162 million of those hypothetical ribocells.

The 0.7 nanograms per biosignature is enough for 7,000 ultramicrobacteria and 14 million hypothetical minimal volume ribocells. That is per biosignature. The sample could contain thousands of ultramicrobacteria and millions of ribocells and they wouldn’t be detected as contributed unusual levels of biosignatures.

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 Perseverance’s samples indefinitely](#h_permitted_contamination)

The largest amino acid by mass is Tryptophan W with a molecular mass of 204.22 g/mol ([NCBI, 2022t](#b_NCBI_2022)) and the smallest is Gycine with a molecular mass of 75.07 g/mol ([NCBI, 2022g](#b_NCBI_2022_glycine)), So the estimated 0.7 nanograms per organic per gram of returned rock sample, if applied to amino acids would permit between [2 trillion](https://www.google.com/search?q=6.0221408*10%5E23+*0.7%2F%2810%5E9*204.23%29) and [5.6 trillion](https://www.google.com/search?q=6.0221408*10%5E23+*0.7%252F(10%5E9*75.07)) 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](#b_Schirber_2013)).[(Noell et al, 2016)](#kix.p67mg41cwbkw). This reflects the expected difficulty of finding the signature of degraded past life in samples.

## We can expect to find novel species and genera from terrestrial contamination in the sample tubes – in a ribosomal survey of samples taken from the clean room used to assemble Perseverance, 4 species were found that didn’t closely resemble any previously detected terrestrial ribosome – and 41 species only detected through their small ribosomal subunit and example of the genus Tersicoccus first found in clean room samples

Many microbial species are only known from the rRNA in the small 16 S subunit of their ribosomes, the factory that a cell uses to make proteins from the mRNA instructions.



[Figure ??](#figur_viking_circadian_rhythms): Messenger RNA enters through this hole which opens like a latch to let it in when it is translated into a protein.

Small subunit of the ribosome protein factory.

.RNA strand in orange. - many microbial phyla are only known through this RNA sequence

This rRNA strand is very stable. It is used to study microbial diversity, because it’s conserved with less variability from species to species than most sequences [(Goodsell, 2000)](#b_Goodsell_2000).

As of 2016 there were at least 89 phyla of bacteria and 20 of archaea that were recognized only by these RNA databases of the small ribosome subunit [,](#b_Goodsell_2000) though the true count of phylae for bacteria could be far higher with estimates of up to 1,500 bacteria phylae [(Solden et al, 2016)](#h_Solden_2016).

In a study of the rRNA 16S subunit of ribosomes in the clean room used to assemble Perseverance, researchers found 49 identified species using 16S mRNA sequencing. Four of them were novel, differing by more than 98.7% from any previously sequenced ribosome ([Hendrickson et al, 2021](#H_Hendrickson_2021)).

It seems likely that this level of diversity extends to the residual organics in the sample tubes. Astrobiologists would also be able to use detection methods such as Astrobionibbler able to detect a single amino acid in a gram of sample ([Schirber, 2013](#b_Schirber_2013)).[(Noell et al, 2016)](#kix.p67mg41cwbkw), never mind a ribosome which is made up of numerous amino acids and nucleotides.

If so, with the high level of scrutiny of the samples, astrobiologists may well identify ribosomes in all the tubes.

If each tube is analysed until ribosomes are found we can perhaps expect that most of the tubes will contain at least one ribosome unique to that tube, and that perhaps three or four of the terrestrial ribosomes found may be sufficiently different to be unlike any previously known species.

Astrobiologists might then be able to isolate new species or a new genus from the sample tubes, which in reality comes from Earth. For an example of a novel genus found in clean rooms, to illustrate how this might play out, the genus Tersicoccus was discovered in two clean room samples in 2013 (Kennedy Space Center, Florida, USA and Centre Spatial Guyanais, Kourou, French Guiana) [(Vaishampayan et al, 2013).](#b_Vaishampayan_2013) Three years later, another species in this same genus, Tersicoccus solisilvae sp, was later isolated from forest soil in Munnar, India [(Sultanpuram et al, 2016),](#b_Sultanpuram_2016) then a year after that, a new strain of the original species was later isolated from Lake Biwa, the largest lake in Japan [(Nakajima et al, 2017).](#b_Nakajima_2017) There are no reports yet of the original strain in Florida or French Guiana, even though it was found in both places, but presumably got into those clean rooms from nearby locations.

A similar scenario could play out with the Perseverance samples, that the astrobiologists find the genetic sequence of a new genus, and hypothesize it could be a Martian organism – but a few years later find it in terrestrial soil, a lake, ocean, desert etc.

## The permitted contamination will make it challenging to prove Perseverance’s samples do NOT have Martian life in them and make it harder to spot genuine Martian microbes that closely resemble terrestrial biology – they will need to contain exceptionally well preserved past or present day life - or we need to collect additional samples in more sterile containers with the sample fetch lander

This will make it challenging to prove that the samples do NOT have Martian life in them which could lead to an unnecessary requirement to sterilize the samples indefinitely.

* [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 Perseverance’s samples indefinitely](#h_permitted_contamination)

This also makes it harder to spot microbes from Mars that closely resemble terrestrial biology.

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.

We can’t do anything about this for Perseverance but this paper suggests that we can add extra sample tubes or containers to the ESA lander to collect samples similar to Viking’s scoop of dirt, and dust samples. Sample containers that target present day life clearly have to be 100% sterile. Sample containers for past life also should be 100% sterile, if detection of life is the main objective.

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 significant amounts of viable or well preserved present day life. A single viable cell with biology resembling terrestrial life would not necessarily count as a proof of life from Mars but a sample containing many viable cells could count as proof, or indeed any proof of non terrestrial biology.

# References (some quotations included to assist verification)

[Uses Harvard reference style, but in this draft, instead of a, b, c etc., I use unique ids like [(NASA, 2020tesgs](#kix.76qmy7dxcqdq)[)](#kix.6aisxl7zz0qc) - the idea is to search / replace these ids with a, b, c etc once the list is complete, after peer review]

## A

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*Approximately every 28 days, fully differentiated cuboidal basal keratinocytes with large nuclei, abundant organelles, and a phospholipid membrane migrate apically from the basal layer through the spinous and granular layers [4]. During this turnover process, an accumulation of keratin and lipids ensues which then undergoes terminal differentiation to form the stratum corneum
…
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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*"A future direction, if this phenomenon exists in nature, would be to bioprospect for these organisms and put them to use,"*

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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](https://www.scientificamerican.com/article/rocks-rockets-and-robots-the-plan-to-bring-mars-down-to-earth1/), 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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## B

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*"Two strategies have been suggested for seeking signs of life on Mars: The aggressive robotic pursuit of biosignatures with increasingly sophisticated instrumentation vs. the return of samples to Earth (MSR). While the former strategy, typified by the Mars Science Laboratory (MSL), has proven to be painfully expensive, the latter is likely to cripple all other activities within the Mars program, adversely impact the entire Planetary Science program, and discourage young researchers from entering the field."*

*"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."*

Bada, J.L., Ehrenfreund, P., Grunthaner, F., Blaney, D., Coleman, M., Farrington, A., Yen, A., Mathies, R., Amudson, R., Quinn, R. and Zent, A., 2008. [Urey: Mars organic and oxidant detector](http://astrobiology.berkeley.edu/PDFs_articles/08UreySpaceSciRev.pdf). *Strategies of Life Detection*, pp.269-279.

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*Spores in cavities will only be subjected to abrasion when the cavities crack open and the spores can get hit upon by a mineral particle. This process may be slow and explain the long tail of the number of surviving spores.The grain size of the regolith will likely affect the above-mentioned mechanisms and thus would have influence on the survival time of present microorganisms. We will address the effect of grain size in more detail in coming experiments.*

Bains, W. and Schulze-Makuch, D., 2016. [The cosmic zoo: the (near) inevitability of the evolution of complex, macroscopic life](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5041001/). Life, 6(3), p.25.

*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 CO₂ ...*

*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 CO₂. 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 CO₂ 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 Paths process, 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.*

Bandfield, J.L., Glotch, T.D. and Christensen, P.R., 2003. [Spectroscopic identification of carbonate minerals in the Martian dust](http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.720.8096&rep=rep1&type=pdf). Science, 301(5636), pp.1084-1087.

Baqué, M., Napoli, A., Fagliarone, C., Moeller, R., de Vera, J.P. and Billi, D., 2020. [Carotenoid Raman Signatures Are Better Preserved in Dried Cells of the Desert Cyanobacterium Chroococcidiopsis than in Hydrated Counterparts after High-Dose Gamma Irradiation](https://www.mdpi.com/2075-1729/10/6/83/htm). *Life*, *10*(6), p.83.

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One such cycle, which is predicted to be two to three times faster than the Calvin–Benson cycle, employs the most effective carboxylating enzyme, phosphoenolpyruvate carboxylase, using the core of the naturally evolved C4 cycle. Although implementing such alternative cycles presents daunting challenges related to expression levels, activity, stability, localization, and regulation, we believe our findings suggest exciting avenues of exploration in the grand challenge of enhancing food and renewable fuel production via metabolic engineering and synthetic biology.

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Beaty, D.W., Allen, C.C., Bass, D.S., Buxbaum, K.L., Campbell, J.K., Lindstrom, D.J., Miller, S.L. and Papanastassiou, D.A., 2009. [Planning considerations for a Mars sample receiving facility: Summary and interpretation of three design studies](https://authors.library.caltech.edu/53810/1/ast.2009.0339.pdf). Astrobiology, 9(8), pp.745-758.

Beaty, D.W., Grady, M.M., McSween, H.Y., Sefton‐Nash, E., Carrier, B.L., Altieri, F., Amelin, Y., Ammannito, E., Anand, M., Benning, L.G. and Bishop, J.L., 2019. [The potential science and engineering value of samples delivered to Earth by Mars sample return](https://onlinelibrary.wiley.com/doi/full/10.1111/maps.13232): International MSR Objectives and Samples Team (iMOST). *Meteoritics & Planetary Science*, *54*, pp.S3-S152.

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*Cyanobacteria are known to promote the precipitation of Ca-carbonate minerals by the photosynthetic uptake of inorganic carbon. This process has resulted in the formation of carbonate deposits and a fossil record of importance for deciphering the evolution of cyanobacteria and their impact on the global carbon cycle. Though the mechanisms of cyanobacterial calcification remain poorly understood, this process is invariably thought of as extracellular and the indirect by-product of metabolic activity. Here, we show that contrary to common belief, several cyanobacterial species perform Ca-carbonate biomineralization intracellularly.*

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*These small, spherical, lipid membrane-bound structures typically range in size from ~20 to 200 nm diameter and provide a means for cells to interact with their environment over both spatial and temporal scales*

*Perhaps one of the most striking features of extracellular vesicles is that they can contain nucleic acids (Dorward et al., 1989; Valadi et al., 2007; Rumbo et al., 2011; Biller et al., 2014). DNA fragments of diverse sizes, ranging from hundreds of bp to >20 kb have been reported in vesicles from Gram-negative bacteria, Gram-positive bacteria, 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.*

Billi, D., Staibano, C., Verseux, C., Fagliarone, C., Mosca, C., Baqué, M., Rabbow, E. and Rettberg, P., 2019a. [Dried biofilms of desert strains of Chroococcidiopsis survived prolonged exposure to space and Mars-like conditions in low Earth orbit](https://www.researchgate.net/profile/Cyprien-Verseux/publication/331027480_Dried_Biofilms_of_Desert_Strains_of_Chroococcidiopsis_Survived_Prolonged_Exposure_to_Space_and_Mars-like_Conditions_in_Low_Earth_Orbit/links/5ee9e56d299bf1faac5c948f/Dried-Biofilms-of-Desert-Strains-of-Chroococcidiopsis-Survived-Prolonged-Exposure-to-Space-and-Mars-like-Conditions-in-Low-Earth-Orbit.pdf). Astrobiology, 19(8), pp.1008-1017.

*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)*

Billi, D., Verseux, C., Fagliarone, C., Napoli, A., Baqué, M. and de Vera, J.P., 2019b. [A desert cyanobacterium under simulated Mars-like conditions in low Earth orbit: implications for the habitability of Mars](https://www.researchgate.net/profile/Daniela-Billi/publication/331027480_Dried_Biofilms_of_Desert_Strains_of_Chroococcidiopsis_Survived_Prolonged_Exposure_to_Space_and_Mars-like_Conditions_in_Low_Earth_Orbit/links/5ca21364a6fdcc1ab5ba0613/Dried-Biofilms-of-Desert-Strains-of-Chroococcidiopsis-Survived-Prolonged-Exposure-to-Space-and-Mars-like-Conditions-in-Low-Earth-Orbit.pdf). *Astrobiology*, *19*(2), pp.158-169.

*In this experiment, survival of the Chroococcidiopsis strain occurred only with those cells that were mixed with martian regolith simulant and plated as thin layers (about 15–30 μm, corresponding to 4–5 cell layers).*

*… 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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Blackmond, D.G., 2010. [The origin of biological homochirality](https://cshperspectives.cshlp.org/content/2/5/a002147.full.pdf). *Cold Spring Harbor perspectives in biology*, *2*(5), p.a002147.

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 Board, S.S. and National Research Council, 2009. *Assessment of planetary protection requirements for Mars sample return missions*. National Academies Press. [page 48](https://www.nap.edu/read/12576/chapter/7#48)

[5, Potential for Large Scale Effects](https://nap.nationalacademies.org/read/12576/chapter/7#48)

*"Despite suggestions to the contrary, it is simply not possible, on the basis of current knowledge, to determine whether viable Martian life forms have already been delivered to Earth. Certainly in the modern era, there is no evidence for large-scale or other negative effects that are attributable to the frequent deliveries to Earth of essentially unaltered Martian rocks. However the possibility that such effects occurred in the distant past cannot be discounted.”*

Board, S.S. and National Research Council, 2012. [*Vision and voyages for planetary science in the decade 2013-2022*](https://solarsystem.nasa.gov/resources/598/vision-and-voyages-for-planetary-science-in-the-decade-2013-2022/). National Academies Press.

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[10](https://www.nap.edu/read/21816/chapter/4#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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*Kasting: “After doing some rough calculations on the effects of a mirror cyanobacteria invasion, Jim Kasting isn’t sure which would kill us first—the global famine or the ice age. “It would quickly consume all the available nutrients,” he says. “This would leave fewer or perhaps no nutrients for normal organisms.” That would wipe out the global ocean ecology and starve a significant portion of the human population. As the CO₂ in the ocean was incorporated into inedible mirror cells, they would “draw down” CO₂ from the atmosphere, Kasting says. For a decade or two, you would have a cure for global warming. But Kasting predicts that in about 300 years the bugs would suck down half of Earth’s atmospheric CO₂. Photosynthesis of most land plants would fail. “All agricultural crops other than corn and sugar cane would die,” he says. (They do photosynthesis a little differently.) “People might be able to subsist for a few hundred years, but things would be getting pretty grim much more quickly than that.” After 600 years, we’d be in the midst of a global ice age. It would be a total evolutionary reboot—both Kasting and Church think mirror predators would evolve, but whatever life existed on Earth by that point wouldn’t include us..*

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Boyle, R., Rodriggs, L.M., Allton, C., Jennings, M. and Aitchison, L.T., 2013. [Suitport feasibility-human pressurized space suit donning tests with the marman clamp and pneumatic flipper suitport concepts](https://core.ac.uk/download/pdf/42736689.pdf). In *43rd International Conference on Environmental Systems* (p. 3399).

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*Even in this dessicated place, she found life: photosynthetic bacteria that had made a home in tiny chambers within halite salt crystals. There’s a small amount of water retained inside the halite and, at night, it cools down and condenses both on the walls of the chambers and on the surface of the organisms that are sitting there.*

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***Section 3.1 final para:*** *Simply scaling by the time between molecular collisions, corresponding to a 125 m diameter ice patch at φ = 0, we find at the base of the regolith a 160 m patch at φ = 26◦ (Aristarchus Plateau), 580 m at φ = 51◦.6 (Plato), 2.3 km at φ = 65◦ (10% polar cap), and an essentially divergent value, 522 km at φ = 82◦ (1% polar cap). If in fact the regolith layer is much deeper than suspected, the added depth of low diffusivity dust significantly increases the patch area: 170 m at φ = 26◦, 830 m at φ = 51◦.6, and 4 km at φ = 65◦.*

***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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*In 1998* [*EN 1822*](https://www.emw.de/en/filter-campus/filter-classes.html) *came into effect. This was the first standard, which established a filter classification system for* [*HEPA filters*](https://www.emw.de/en/products/hepa-air-filters/) *based on filtration process theory. EN 1822 also introduced the evaluation criterion MPPS (Most Penetrating Particle Size). MPPS is the particle size at which the air filter has its lowest arrestance. Not just a whim of nature, MPPS relates directly to physical mechanisms in the* [*filtration process*](https://www.emw.de/en/filter-campus/theory-of-particle-filtration.html)*.*

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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.”*

Foust, J., 2021, [The multi-decade challenge of Mars Sample Return](https://spacenews.com/the-multi-decade-challenge-of-mars-sample-return/), Space News

*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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*"The method originates from the USA, and is used to remove paint from aircraft fuselage. A powerful jet of frozen carbon dioxide (CO₂) crystals, about the size of a rice kernel, blasts the paint right off the metal. The researchers made this crude instrument substantially more refined. Instead of CO₂ pellets, they use carbon dioxide snow to work on each individual component – from the highly sophisticated aluminum workbench to the ring washers. Here’s the rub: the beam that the jet emits is additionally accelerated with a blast of CDA (clean dry air) that encases it. This is how it penetrates into every nook and cranny, removing even the minuscule pollutant. As soon as the tiny snowflakes hit the relatively hot surface, they become gaseous, causing their volume to explosively expand 800-fold. The detonation pressure completely sweeps away every single bit of dust, even fingerprints which the cold gas had just turned brittle. “This approach involves a dry process that does not warp surfaces. When cleaning, these can be gently treated with CO₂. That makes it unnecessary to apply heat or chemicals,” Gommel says when explaining the advantages of this method.* "

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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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*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 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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*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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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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*“What are the mechanisms by which HGT occurs? Currently these include: transduction, a process whereby a viral capsule is used to transfer genetic material from one cell to another; conjugation, a process exhibited by microbes during which a plasmid or a small piece of a plasmid from one donor cell is transferred to another recipient cell (Prof. Matxalen Llosa—see summary report); transformation, which occurs when a competent cell takes up a “naked” strands of nucleic acid from the environment—such strands of nucleic acids may not necessarily have been exuded by living entities (e.g., mitochondrion genes transferred to eukaryote chromosomes), they could also be from recently dead cells, as well as from long extinct organisms; gene transfer agents (GTA), which are bacteriophage-like particles containing random cellular genomic segments intended for transduction to another living recipient cell; and membrane vesicle transfer (MVT), in which small membrane sacs emanating from the surface of a cell contain genetic material for transfer to another living recipient cell.”*

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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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**LM:** Pamela and I disagree on this, but there’s a provision in the Outer Space Treaty, Article VI, which says that each country must supervise and authorize the activities of its nongovernmental entities. This is not a self-executing provision, and the U.S. Supreme Court has held that a non-self-executing treaty is not domestically enforceable. ...

**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 desiccated bacteria are constituents of the dust occasionally blown up by the winds into the atmosphere and stratosphere. where bacteria from different geographic origins mix while being exposed to UVC light 100 to 1000 times more intense than on Earth’s surface. They eventually rehydrate when falling back on Earth with the rain and snow (this is how Francois-Xavier Pellay in our laboratory collects robust bacteria) and—depending on their genomic constitution—develop, or not, in the ecological niches into which they happen to fall. Indeed, the most efficient cellulose degraders are deinococci found growing in the waist of the wood-sawing industry (Deinove, pers. comm.).*

*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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my visit to you November 6, 1957….*

*“It must have been around this time surely that I began to think of the scientific consequences of lunar and planetary probes. … I have in mind the quite tangible possibility of contamination by terrestrial organisms of the surfaces of Mars and Venus, unless stringent precautions are taken to sterilize any vehicles sent there...."*

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*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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*“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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*(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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*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](https://mars.nasa.gov/news/8682/the-extraordinary-sample-gathering-system-of-nasas-perseverance-mars-rover/).

NASA, 2021nmttm, [NASA Moves to the Next Phase in a Campaign to Return Mars Samples to Earth](https://scitechdaily.com/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](https://youtu.be/JM_2hmdRnfQ?t=3692) from: [After NASA's Historic First Flight: Ingenuity Mars Helicopter Update Streamed live on 19 Apr 2021](https://youtu.be/JM_2hmdRnfQ)

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](https://www.youtube.com/watch?v=BAlXe-U0ws4&t=2021) from: [NASA’s Ingenuity Mars Helicopter’s Next Steps (Media Briefing) Streamed live on 30 Apr 2021](https://youtu.be/BAlXe-U0ws4)

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](https://youtu.be/BAlXe-U0ws4?t=3680) from: [NASA’s Ingenuity Mars Helicopter’s Next Steps (Media Briefing) Streamed live on 30 Apr 2021](https://youtu.be/BAlXe-U0ws4)

NASA, 2021prmtl, [Perseverance Rover, Mission Timeline › Landing](https://mars.nasa.gov/mars2020/timeline/landing/)

NASA, 2021wnpr, Watch NASA’s Perseverance Rover Land on Mars, Thomas Zurbukin at [14:45](https://youtu.be/gm0b_ijaYMQ?t=885)

***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](https://downloads.regulations.gov/NASA-2022-0002-0002/attachment_5.pdf), [MSR PEIS Fact Sheets](https://www.regulations.gov/document/NASA-2022-0002-0002)

NASA, 2022msr, [public comments](https://www.regulations.gov/document/NASA-2022-0002-0001), MSR, PEIS

NASA, 2022smsr [The Safety of Mars Sample Return](https://downloads.regulations.gov/NASA-2022-0002-0002/attachment_7.pdf), [MSR PEIS Fact Sheets](https://www.regulations.gov/document/NASA-2022-0002-0002)

*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](https://downloads.regulations.gov/NASA-2022-0002-0001/content.pdf) 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](https://www.nasa.gov/press-release/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?"*](https://mars.nasa.gov/mars2020/mission/where-is-the-rover/). Mars 2020 Mission Perseverance Rover

NASA, n.d.ame, [Atmosphere](https://marsed.asu.edu/mep/atmosphere), Mars Education

NASA, n.d.cls, [Curiosity's Landing Site: Gale Crater](https://mars.nasa.gov/msl/timeline/prelaunch/gale-crater/)

NASA, n.d.cm, [Chris McKay, at NASA Ames](https://www.nasa.gov/content/chris-mckay/)

NASA, n.d.dan, [Dynamic Albedo of Neutrons (DAN)](https://mars.nasa.gov/msl/spacecraft/instruments/dan/), see also archived page for scientists: [Dynamic Albedo of Neutrons (DAN)](https://web.archive.org/web/20210224030220/https%3A//mars.nasa.gov/msl/spacecraft/instruments/dan/for-scientists/)

NASA, n.d.ecilm, [Eugene Cernan in Lunar Module](https://www.nasa.gov/content/images-of-astronaut-gene-cernan)

NASA, n.d.hsp, [Health Stabilization Program](https://www.nasa.gov/sites/default/files/atoms/files/health_stabilization_program_technical_brief_ochmo_021020.pdf)

NASA, n.d. mbtn, [Mars, by the numbers](https://solarsystem.nasa.gov/planets/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](https://nssdc.gsfc.nasa.gov/planetary/factsheet/marsfact.html)/ 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](https://nssdc.gsfc.nasa.gov/planetary/factsheet/marsfact.html)

NASA, n.d.monm, [Map of NASA's Mars Landing Sites](https://mars.nasa.gov/resources/24729/map-of-nasas-mars-landing-sites/)

NASA, n.d.MOXIE [MOXIE](https://mars.nasa.gov/mars2020/spacecraft/instruments/moxie/), and [MOXIE for scientists](https://mars.nasa.gov/mars2020/spacecraft/instruments/moxie/for-scientists/).

NASA, n.d. MSASL, [Martian seasons and solar longitude](http://www-mars.lmd.jussieu.fr/mars/time/solar_longitude.html) 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](https://mars.nasa.gov/mars2020/mission/science/landing-site/), accessed at <https://mars.nasa.gov/mars2020/mission/science/landing-site/>, accessed on 17 July 2020.

NASA, n.d.sd, [Shield Development](https://hvit.jsc.nasa.gov/shield-development/)

NASA, n.d.,SEH,, [System Engineering Handbook](https://www.nasa.gov/seh/index.html), see particularly

2.5 [Cost Effectiveness considerations](https://www.nasa.gov/seh/2-5-cost-effectiveness-considerations#Fig2-5-1https://www.nasa.gov/seh/2-5-cost-effectiveness-considerations)

3.3 [Project Pre-Phase A: Concept Studies](https://www.nasa.gov/seh/3-3-project-pre-phase-a-concept-studies)

3.4 [Project Phase A: Concept and Technology Development](https://www.nasa.gov/seh/3-4-project-phase-a-concept-and-technology-development)

3.5 [Project Phase B: Preliminary Design and Technology Completion](https://www.nasa.gov/seh/3-5-project-phase-b-preliminary-design-and-technology-completion)

NASA, n.d.WiC, [Curiosity: Mission: Where is the rover?](https://mars.nasa.gov/msl/mission/where-is-the-rover/)

Curiosity landing site: 137.44°E, 4.589°S

NASA, n.d.WiP, [Where is Perseverance?](https://mars.nasa.gov/mars2020/mission/where-is-the-rover/)

Perseverance landing site: 18.45°N 77.45°E,

NASA, n.d. WISO, [What is Surface Operations?](https://mars.nasa.gov/mars2020/timeline/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 CO₂ Sequestration Galvanizes Plant Biomass Production](https://www.sciencedirect.com/science/article/pii/S0167779919303142). *Trends in Biotechnology*, *38*(4), pp.354-359.

*The CETCH cycle requires less energy to operate than other aerobic CO₂ -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*](https://www.sciencedirect.com/science/article/pii/S0167779919303142#bb0015)*] 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 CO₂ 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 CO₂ molecules per second (in vitro) compared with RuBisCO, which fixes two to five CO₂ molecules per second in plants.*

 National Research Council. 2009. [Assessment of Planetary Protection Requirements for Mars Sample Return Missions (Report)](http://www.nap.edu/openbook.php?record_id=12576&page=28). 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](https://pubchem.ncbi.nlm.nih.gov/compound/Glycine)

National Center for Biotechnology Information, 2022t, [PubChem Compound Summary for CID 6305, Tryptophan](https://pubchem.ncbi.nlm.nih.gov/compound/Tryptophan). Retrieved May 20, 2022 from <https://pubchem.ncbi.nlm.nih.gov/compound/Tryptophan>.

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Negi, S., Perrine, Z., Friedland, N., Kumar, A., Tokutsu, R., Minagawa, J., Berg, H., Barry, A.N., Govindjee, G. and Sayre, R., 2020. [Light regulation of light‐harvesting antenna size substantially enhances photosynthetic efficiency and biomass yield in green algae](https://lodgbot.com/wp-content/uploads/2020/05/13May20-The-Plant-Journal-Algae-photosynthesis-improvement.pdf). *The Plant Journal*.

*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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Nicholson, W.L., Krivushin, K., Gilichinsky, D. and Schuerger, A.C., 2013. [Growth of Carnobacterium spp. from permafrost under low pressure, temperature, and anoxic atmosphere has implications for Earth microbes on Mars](https://www.pnas.org/content/110/2/666.short). Proceedings of the National Academy of Sciences, 110(2), pp.666-671.

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Niles, P.B., Catling, D.C., Berger, G., Chassefière, E., Ehlmann, B.L., Michalski, J.R., Morris, R., Ruff, S.W. and Sutter, B., 2013. [Geochemistry of carbonates on Mars: implications for climate history and nature of aqueous environments](http://faculty.washington.edu/dcatling/Niles2012_CarbonatesOnMarsReview.pdf). Space Science Reviews, 174(1), pp.301-328.

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*anets*, *106*(E10), pp.23317-23326.

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## O

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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](https://commons.wikimedia.org/wiki/File%3AEtiquette_cd-rom_01.svg)

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*page 8530: A principal limitation of efficient photosynthesis is that organisms absorb more light in full sunlight than they can use productively. The reason seems clear: high absorptivity provides effective capture at low light intensities, such as at dawn and dusk and on cloudy days, and it obviates competition from other phototrophs by absorbing the light before they do.*

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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 CO₂ -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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Contains:

* ***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.

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*Pages 94-5: Questions about the adequacy of the SRF to maintain the new life form must also be addressed, including the possible need to add equipment, change operations, review emergency plans, or upgrade the facilities because of what has been found.*

*Concerns about security should also be reconsidered, especially in view of the potential disruptive activities of any terrorists or ‘radical’ groups that may be opposed to sample return. The advisability of allowing distribution of untested sample material outside the SRF2684* may need to be reconsidered, as well.

*Plans should be developed well in advance in order to avoid a frenzied, reactive mode of communications between government officials, the scientific community, the mass media, and the public. Any plan that is developed should avoid a NASA-centric focus by including linkages with other government agencies, international partners, and external organizations, as appropriate. It will also be advisable to anticipate the kinds of questions the public might ask, and to disclose information early and often to address their concerns, whether scientific or non-scientific.
...*

*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.*

In the long term, the discovery of extraterrestrial life, whether extant or extinct, in situ or within returned sample materials, will also have implications beyond science and the SRF per se. Such a discovery would likely trigger a review of sample return missions, and plans for both robotic and human missions. Legal questions could arise about ownership of the data, or of the entity itself, potentially compounded by differences in laws between the United States and the countries of international partners. In any event, ethical, legal and social issues should be considered seriously. Expertise in these areas should be reflected in the membership on appropriate oversight committee(s).

Page 101: **Communications** Unusual or unprecedented scientific activities are often subject to extreme scrutiny at both the scientific and political levels. Therefore, a communication plan must be developed as early as possible to ensure timely, and accurate dissemination of information to the public about the sample return mission, and to address concerns and perceptions about associated risks. The communication plan should be pro-active and designed in a manner that allows the public and stakeholders to participate in an open, honest dialogue about all phases of the mission with NASA, policy makers, and international partners. Risk management and planetary protection information should be balanced with education/outreach from the scientific perspective about the anticipated benefits and uncertainties associated with Mars exploration and sample return.

The communication plan should also address how the public and scientific community will be informed of results and findings during Life Detection and Biohazard testing, including the potential discovery of extraterrestrial life. Because of the intense interest likely during initial sample receipt, containment, and testing, procedures and criteria should be developed in advance for determining when and how observations or data may be designated as “results suitable for formal announcement.” Details about the release of SRF information, the management of the communication plan, and its relationship to the overall communications effort of the international Mars exploration program should be decided well in advance of the implementation of this protocol.

Rummel, J.D., Beaty, D.W., Jones, M.A., Bakermans, C., Barlow, N.G., Boston, P.J., Chevrier, V.F., Clark, B.C., de Vera, J.P.P., Gough, R.V. and Hallsworth, J.E., 2014. [A new analysis of Mars “special regions”: findings of the second MEPAG Special Regions Science Analysis Group (SR-SAG2)](https://www.researchgate.net/profile/David_Beaty/publication/268444482_A_new_analysis_of_Mars_Special_Regions_findings_of_the_second_MEPAG_Special_Regions_Science_Analysis_Group_SR-SAG2/links/547c9b0b0cf27ed9786229dd.pdf)

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## S

Sagan, C, 1961. [Organic matter and the Moon](https://www.nap.edu/download/18476)., National Academy of Sciences.

[*Page 23*](https://www.nap.edu/read/18476/chapter/5#23)*: It is remarkable that the depth at which surviving lunar organic matter is expected to be localized (section II) is just the depth at which temperatures appear to be optimum for familiar organisms (section IV). At such temperatures and depths, some moisture should be expected, arising from meteoritic and organic bound water. Watson, Murray and Brown (1961) have recently pointed out that ice could have been retained on permanently shaded areas of the Moon. These circumstances provide all the survival requirements of many terrestrial organisms - water and their metabolites, appropriate temperature, and negligible radiation. That autochthons evolving with the changing environment could also survive under these conditions is far from inconceivable.*

 Sagan, C., Levinthal, E.C. and Lederberg, J., 1968. [Contamination of Mars](https://profiles.nlm.nih.gov/ps/access/BBABJH.ocr). *Science*, *159*(3820), pp.1191-1196.

*"The prominent dust storms and high wind velocities previously referred to imply that aerial transport of contaminants will occur on Mars. While it is probably true that a single unshielded terrestrial microorganism on the Martian surface ... would rapidly be enervated and killed by the ultraviolet flux, ... The Martian surface material certainly contains a substantial fraction of ferric oxides, which are extremely strongly absorbing in the near ultraviolet. ... A terrestrial microorganism imbedded in such a particle can be shielded from ultraviolet light and still be transported about the planet."*

*…*

*"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."*

Sagan, C., 1973, [*The Cosmic Connection - an Extraterrestrial Perspective*](https://www.e-reading.life/bookreader.php/148581/Sagan_-_The_Cosmic_Connection___An_Extraterrestrial_Perspective.pdf)

*I reach this conclusion reluctantly. I, myself, would love to be involved in the first manned expedition to Mars. But an exhaustive program of unmanned biological exploration of Mars is necessary first****. The likelihood that such pathogens exist is probably small, but we cannot take even a small risk with a billion lives.*** *Nevertheless, I believe that people will be treading the Martian surface near the beginning of the twenty-first century.*

Sagan, C., 1977. [Reducing greenhouses and the temperature history of Earth and Mars](https://www.nature.com/articles/269224a0). *Nature*, *269*(5625), pp.224-226.

Sagan, C., 1980., *Cosmos: The Story of Cosmic Evolution, Science and Civilisation*

full quote:

*The surface area of Mars is exactly as large as the land area of the Earth. A thorough reconnaissance will clearly occupy us for centuries. But there will be a time when Mars is all explored; a time after robot aircraft have mapped it from aloft, a time after rovers have combed the surface, a time after samples have been returned safely to Earth, a time after human beings have walked the sands of Mars. What then? What shall we do with Mars?*

*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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***(2) Collecting gas in a newly-designed, valved, sample-tube-sized vessel that is flown on either the Sample Fetch Rover (SFR) or the Sample Retrieval Lander (SRL)***

***...***

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*… Legionella pneumophila enters mononuclear phagocytes by depositing complement C3b on its surfaces and using that host protein to serve as a ligand for binding to macrophage cell surfaces. After ingestion, the bacteria remain in vacuoles that do not fuse with lysosomes, apparently due to the influence of soluble substances produced by the bacteria.*

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*Surprisingly, however, we have found that O₂ does not demonstrate the predictable seasonal behavior of the other major components. Surface O₂ measurements by SAM yield abundances that vary between 1300 and 2200 ppmv; when corrected for the annual global mean pressure, O₂ varies from 1300 to 1900 ppmv. Despite large instrument backgrounds, these are the first precise in situ measurements of O2, revealing a surprising seasonal and interannual variation that cannot be accounted for in current chemical models. Though Mars has the potential to generate significant O₂ release due to abundances of oxidants in/at its surface, the mechanisms by which O₂ 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.*

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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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*Abstract: Surface seawater was collected in four different seasons in the coastal East China Sea adjacent to the Yangtze River Estuary and phytoplankton community diversity was analysed using rbc L genetic markers.*
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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)](#kix.i3u68r56h0sy) 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](#h_complexities_of_quarantine)](#h_complexities_of_quarantine)

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](#h_example_of_mirror_life)

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](#_kuefq6e917l6)
* [Worst case scenario where terrestrial life has no defences to an alien biology - humans survive by ‘paraterraforming’ a severely diminished Gaia](#_p8mguenm8mn)

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](#_xla39n7rhqn1)

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 CO₂ 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](#h_suggestion_swansong_Gaia)

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)](#h_Recommendation_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](#_qmz83mcmqrxs)

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](#_mdfjnmunzqky)

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](#_f0mc527l4mw4)

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](#_wxhe1kvuyput)](#h_nanofilter)

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](#_9e0adi3zubdd)

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?](#h_transported_dust_storms_dust_devils)

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](#h_rec_air)
* [**Proposal:** magnets could be used to enhance dust collection](#h_proposal_magnets)
* [**Proposal:** to use the sample return capsule as a dust collector – keep it open to the atmosphere before adding the sample tubes](#h_proposal_sample_returjn_capsule_dust)

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 one originally living cell per gram – a rough first estimate assuming uniform mixing throughout Mars for a first estimate requires life to cover between 114,000 and 1,140 square kilometers with densities of life in the dust similar to an Antarctic RSL analogue in cell count, but less than a tenth of a square kilometer if any reach a billion cells per gram – these figures can be higher if any source habitats with high densities of cells are closer to the rover with uneven mixing](#h_Searching_for_distant_habitats_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](#_2bzb19kzv667)

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?](#_fp1s7mhe7n2d)

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](#h_any_requirement_similar_synthetic_life)

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](#h_astronaut_mirror_life)

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](#_k25tnylg2ekh)

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](#_4f9mmbuo6v1t)

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](#_v3mk32itl19x) 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)**](#_xkd93xv99557)

**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](#_tj6p37ygj9f7)

**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](#h_Comet_and_asteroid)

**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](#_jc8iush5q1j7)

**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](#h_2015_maps)

**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](#h_2015_maps)

**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](#_on4osbov63dz)

**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](#_9nd42ridv6j1)

**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**](#_kiftltovmy6p)

**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](#h_increasing_requirements)

**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](#_3js02w62qkf0)

**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](#_mdfjnmunzqky)

**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](#_f0mc527l4mw4)

**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](#_wxhe1kvuyput)](#_wxhe1kvuyput)

**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](#_aufdkljm0dkh)

**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](#h_ESF_regular_review)

**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](#_4kt6j3orab8b)

**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](#_93ji66cqejk)

(...)

[Need for advanced planning and oversight agency set up before start of the legal process](#_v23hp14dpnnx)

(...)

[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](#_xla39n7rhqn1)

Summarizes existing literature

[Potential changes in requirements as a result of the legal process](#_8v7pce832u7s)

(...)

[Minimum timeline: 2 years to develop consensus legal position, 6-7 years to file Environmental Impact Statement, 11 years to build sample return facility](#h_MinimumTimeline)

(...)

[[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.](#h_need_for_legal_clarity)](#_pdvvj8y2sqjl)

(...)

[[Need for legal clarity before launch of ESA’s Earth Return Orbiter, Earth Entry Vehicle, and NASA’s Mars Ascent Vehicle.](#h_need_for_legal_clarity_earth_return)](#_3ku6cvvx8agc)

(...)

[Likely legal requirement for facility to be ready to receive samples well before unsterilized samples return to Earth](#_2ixeoo5rp3q7)

(...)

[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](#h_legal_process_likely)

(...)

[**Public health challenges responding to release of an extraterrestrial pathogen of unfamiliar biology**](#_h3xlihwvoz0)

Summarizes existing literature

[**Complexities of quarantine for technicians accidentally exposed to sample materials**](#h_complexities_of_quarantine)

(...)

[Vexing issue of authorizations to remove technicians from quarantine to treat life threatening medical incidents in hospital](#_i5u77k7ag6jk)

**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](#_yga1rhqn8rpx)

**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](#_tzbdyck03mo4)

(...)

[How do you quarantine a technician who could be a symptomless super-spreader of an unknown Martian pathogen?](#_tu1phnn2a9h)

**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](#_dwfiy8grncyn)

**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?](#_c4x8f4hxlkj0)

**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](#_27aq7y25n4kx)

**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](#_287ajdtckwy)

(...)

[Telerobotics as a solution to all these human quarantine issues](#_hq85xokbn43c)

(...)

[Zubrin's arguments in: "Contamination from Mars: No Threat" and the response of planetary protection experts in "No Threat? No Way":](#_46cr20rbmp4w)

(...)

[**These complexities arise due to need to contain almost any conceivable exobiology**](#_se5oz9sa1i6j)

(...)

[**Sterilized sample return as aspirational technology demonstration for a future astrobiology mission**](#h_Sterilized_sample_return)

 (...)

[[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](#_2oqtgaj3wac7)](#h_level_sterilization)

[s](#_2oqtgaj3wac7)

(...)

[Suggestion to use nanoscale X-ray emitters for sterilization](#_qmz83mcmqrxs)

(...)

[Effects of gamma radiation on rock samples - and need to test X-rays](#_dkbd7p7vhaw5)

(...)

**Why it’s a major challenge to find samples from Jezero crater to help decide central questions in astrobiology until we can send in situ life detection instruments - most past biosignatures will be degraded beyond recognition – nearly all organics on Mars are expected to be abiotic - past and present day life is expected to be low in concentration and patchy in distribution – and all this is especially challenging if Martian life never developed photosynthesis or nitrogen fixation**

(...)

[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](#_c06p2zao6zvk)

(...)

[Limitations on cleanliness of the Mars sample tubes with estimated 0.7 nanograms contamination each for DNA and other biosignatures per gram of returned rock sample, and a roughly 0.02% possibility of a viable microbe in at least one of the tubes](#h_lim_cleannliness)

(...)

 [Modern miniaturized instruments designed to detect life in situ on Mars - could also be used to examine returned samples in an orbital telerobotic laboratory](#h_Modern_miniaturized_instruments)

(...)

[**Could Perseverance’s samples from Jezero crater in the equatorial regions of Mars contain viable or well preserved present day life?**](#h_could_samples_contain_viable_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](#h_detection_liquid_water)

**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](#h_experiments_black_yeasts)

**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](#_qbomwrudqbj7)

(...)

[Sources of nitrogen on Mars as potential limiting factor – unless Martian life can fix nitrogen at 0.2 mbar](#h_sources_of_nitrogen)

**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?](#h_transported_dust_storms_dust_devils)

(...)

[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](#h_Native_Martian_propagules)

(...)

[Potential for spores and other propagules from nearby or distant regions of Mars similarly to transfer of spores from the Gobi desert to Japan](#_kdyk9up638hw)

**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 one originally living cell per gram – a rough first estimate assuming uniform mixing throughout Mars for a first estimate requires life to cover between 114,000 and 1,140 square kilometers with densities of life in the dust similar to an Antarctic RSL analogue in cell count, but less than a tenth of a square kilometer if any reach a billion cells per gram – these figures can be higher if any source habitats with high densities of cells are closer to the rover with uneven mixing](#h_Searching_for_distant_habitats_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](#h_could_local_RSL)

**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**](#_brwfiu3mn2bf)

**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](#h_perseverance_could_detect_chlorophyll)

(...)

[**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**](#_drq89oose4bw)

**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](#_vctrprx8w5u1)

**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](#_w4m4h8ozphlp)

**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](#_1l0srgglbe91)

**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](#_2bzb19kzv667) early Mars

(...)

[Exposure of organics through wind erosion - for samples of less degraded past life](#h_exposure_organics_wind_erosion)

(...)

* [**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](#h_rec_air)

**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](#_2iwhmajunqhu)

(...)

[Possible use of Perseverance - or modification of ESA’s Sample Fetch Rover to return samples from shallow sand dune subsurface](#_9e0adi3zubdd)

**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**](#_md8f75hyofko)

**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 CO₂ 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](#_w000n5uck2n9)

(...)

[Self limiting methanogens, methanotrophs, and Fe(III)-reducing bacteria maintaining a subsurface Swansong Gaia hydrology](#_ew8jyqninat5)

(...)

[Could seasonal oxygen be a possible signal of photosynthesis maintaining a Swansong Gaia homeostasis on Mars?](#_lzmkpbjprkhu)

(...)

[How does this Swansong Gaia compare with the original “Gaia hypothesis?”.](#_xnzrvmigmtfd)

(...)

[Potential limits on the biomass of a Swansong Gaia on Mars using the amounts of free CO and H₂ in the atmosphere](#_xnkj4yfjaylg)

(...)

[Testing the “Swansong Gaia” hypothesis](#_y8zjv32xbzy)

(...)

[**Recommendation to return a sample for teleoperated ‘in situ’ study above Geosynchronous Equatorial Orbit (GEO)**](#h_Recommendation_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](#_u6rda2g1xrg9)

(...)

[Low energy transfer of an Earth Return Vehicle from Mars to above GEO](#_flib5ihr0y4g)

(...)

[Preliminary study of the returned sample above GEO](#_6doc0sfjbdcc)

(...)

[Studying life telerobotically in orbit above GEO](#_7tytnuk25o44)

(...)

[Possibility of early discovery of extraterrestrial microbes of no risk to Earth](#h_poss_early_discovery)

(...)

[Early discovery of a familiar terrestrial microbe on Mars is not enough to prove sample is safe without more research](#_95b5ooobkldt)

**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](#_d765msr1bx3c)

(...)

[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](#h_could_Martian_life_have_got_to_Earth)

**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?](#_w18lxh14foya)

**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](#h_potential_diversity_et_life)

(...)

[**Could present day Martian life harm terrestrial organisms?**](#_90r679e00r6y)

**New:** Detailed discussion of example worst case scenarios, such as sequestration of CO₂ 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?](#_mjy48ylbinho)

(...)

[Microplastics and nanoplastics as an analogue for cells of alien life entering our bodies unrecognized by the immune system.](#_c04xtjg7bmog)

**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](#_nv8m95nwa6eh)

**New:** Possibility that extraterrestrial fungi and other microbes could also be allergens

[Accidental similarity of amino acids forming neurotoxins such as BMAA](#_hzzu3tos299k)

**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,

[Martian microbes better adapted to terrestrial conditions than terrestrial life, example of more efficient photosynthesis](#h_Martian_microbes_better_adapted)

New: Discussion of impact on our biosphere of cyanobacteria more efficient at photosynthesis than terrestrial life

[Example of a mirror life analogue of chroococcidiopsis, a photosynthetic nitrogen fixing polyextremophile](#h_example_of_mirror_life_analogue)

(...)

[[Example of mirror life nanobacteria](#h_example_of_mirror_life)](#_kj1pr29b8qxs)

(...)

[Possibility of extraterrestrial Martian life setting up a “Diminished Gaia” on Earth](#_kuefq6e917l6)

(...)

[Worst case scenario where terrestrial life has no defences to an alien biology - humans survive by ‘paraterraforming’ a severely diminished Gaia](#_p8mguenm8mn)

**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

[Worst case where alien life unrecognized by terrestrial immune systems spreads to pervade all terrestrial ecosystems](#_9t3at4mgnlzi)

**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

[Could Martian microbes be harmless to terrestrial organisms?](#h_Could_Martian_microbes)

(...)

[Enhanced Gaia - could Martian life be beneficial to Earth’s biosphere?](#_qg4dre3vy8vr)

**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.

[A simple titanium sphere could contain an unsterilized sample for safe return to Earth’s surface - but how do you open this “Pandora's box”?](#_imt6dv36x0tk)

(...)

[**Variations on the precautionary principle - which is appropriate for a Mars sample return?**](#_fp1s7mhe7n2d)

(...)

[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](#_e0vzd0xwbzsw)

**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.

[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](#h_any_requirement_similar_synthetic_life)

(...)

[Adaptive approach - return an unsterilized sample to Earth’s biosphere only when you know what is in it](#_qtpt6qowfe1f)

(...)

[Vulnerability of early life on Mars in forwards direction - legal protection is weak, but strengthened by the laws for backwards protection of Earth](#h_vulnerability_early_life)

(...)

[Why Mars sample returns are no longer enough to prove astronauts are "Safe on Mars" or safe in Jezero crater - with the modern more complex understanding of Mars](#_nrm1zt3alb7)

(...)

[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 a single sample of a biohazard such as mirror life COULD be enough to prove Mars unsafe](#_10rh8sqhumhb)

(...)

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](#_l06mtkyk3pfy)

(...)

[Design specifications for 100% sterile rovers for fast safe astrobiological surveys throughout the solar system](#_4f9mmbuo6v1t)

(...)

[Mars less habitable than a plateau higher than Mount Everest, so high our lungs need a pressure suit to function](#_nywdspjuqul)

(...)

[Dust as one of the greatest inhibitors to nominal operation on the Moon - and likely on Mars too](#h_dust_as_one)

(...)

[Planetary protection as an essential part of an ambitious, vigorous approach to human exploration](#_nywdspjuqul)

(...)

[**Conclusion - legal process is both understandable and necessary**](#_1zy6z1urmm8)

(...)