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Planning Considerations Related to Contamination Control for the Return and Analysis of Martian Samples

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Abstract

The joint National Aeronautics and Space Administration and European Space Agency Mars Sample Return (MSR) Campaign is a proposed multi-mission effort to bring selected geological samples from Mars to Earth for the purpose of scientific investigation. Significant parts of these investigations could be affected by Earth-sourced contamination that is either misinterpreted as having a martian origin or that masks a martian signal. The Mars 2020 Perseverance rover implemented strict contamination control requirements to limit contamination of the samples during sample collection. Contamination control and contamination knowledge requirements have not yet been established for the samples after they arrive on Earth. The MSR Sample Receiving Facility (SRF) Contamination Panel (SCP) was tasked with defining the terrestrial biological, organic, and inorganic contamination limits for martian samples during their residence inside the SRF. To reach our recommendations, the SCP studied (i) the previously proposed limits and rationale of the Organic Contamination Panel, (ii) cleanliness levels achieved for sampling hardware by the M2020 mission, (iii) recent improvements in analytical technology and detection limits, (iv) updated information regarding the organic content of martian samples (*e.g.*, from the Sample Analysis at Mars instrument on the Curiosity rover and laboratory analyses of martian meteorites), and (v) information about the composition and geologic context of samples being collected by the Perseverance rover for return to Earth. Key Words: Mars Sample Return—Contamination control—Extraterrestrial materials. Astrobiology 00, 000–000.

Executive Summary

The Mars Sample Return (MSR) Campaign planned jointly by National Aeronautics and Space Adminsitration (NASA) and European Space Agency (ESA) is currently planned as a multi-mission effort to bring selected geological and atmospheric samples from Mars to Earth for the purpose of detailed scientific investigation (Kminek et al., 2022b). Significant components of these investigations have the potential to be affected by Earth-sourced contamination that

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could be misinterpreted as having a martian origin or mask a martian signal. Prior to the launch of NASA's Mars 2020 (M2020) Perseverance rover, an Organic Contamination Panel (OCP) provided recommendations (Summons et al., 2014) that formed the basis of the contamination control and contamination knowledge implementation plans for the M2020 mission. In 2023, the MSR Sample Receiving Facility (SRF) Contamination Panel (SCP) was tasked with recommending the terrestrial biological, organic, and inorganic contamination limits for martian samples during their residence inside the SRF. To reach our recommendations, the SCP studied (i) the previously proposed limits and rationale of the OCP, (ii) cleanliness levels achieved for sampling hardware by the M2020 mission, (iii) recent improvements in analytical technology and detection limits, (iv) updated information regarding the organic content of martian samples (*e.g.*, from the Sample Analysis at Mars instrument on the Curiosity rover and laboratory analyses of martian meteorites), and (v) information about the composition and geologic context of samples to be collected by the Perseverance rover for potential return to Earth. Major findings of our work include the following:

Background

- With the exception of Ni, Co, Nb, Ta, and W, M2020 successfully met its contamination limit requirements for inorganic and organic contamination. Total organic carbon (TOC) contamination of sampling hardware is known to be <40 ppb (in-sample equivalent), and Tier 1 individual compounds (which represent the compounds of highest interest) to be <1 ppb. Moreover, our review of M2020 test data suggests that most or all Tier 1 compounds were very likely <0.1 ppb.
- MSR sample tubes were sterilized and then sealed with a fluid mechanical particle barrier to yield a probability of <0.01 of a single living terrestrial organism inside the sample tube. This level of sterility was maintained until the opening of the adaptive caching assembly on the surface of Mars.
- Perseverance has collected both igneous (mafic to ultramafic) and sedimentary (sand to silt) rocks, as well as regolith. As a result, a diverse set of geologic materials, with widely varying organic and inorganic compositions, would need to be handled and assessed in the SRF.
- Our expectations for the organic contents of martian rocks remain similar to those of Summons et al. (2014), with individual compounds in the low-to-mid ppb range and TOC, the majority of which is in the form of kerogen, in the low-to-mid ppm range. Currently, there is no evidence from *in situ* measurements on the surface of Mars or the analysis of martian meteorites to suggest that these returned samples would contain extant martian life.

Findings

- Living and nonliving contamination should not be treated as equivalent in the SRF. The OCP treated living and dead organisms as equivalent with respect to contamination. Because of the potential for growth of terrestrial microbes in the SRF, a renewed focus on avoiding contamination by viable organisms is warranted for this step of the MSR campaign.
- We recommend further investigation of cold (<−20°C) sample storage and handling. Unless absolute sterility inside the SRF can be guaranteed, sample storage conditions should be designed and maintained to inhibit cellular growth and activity.
- Working conditions within the SRF should, at a minimum, reflect the state-of-the-art and best practices within fields with microbial cleanliness standards. Microbial cleanliness standards have been established for a variety of scientific and industrial applications, such as biologically sensitive applications in the pharmaceutical industry.
- We recommend using expected analyte concentrations as a basis for setting contamination limits, that is, with contamination limited to at most <1% of expected signals. It remains impractical to achieve sample cleanliness levels that are below detection limits for all (or even most) instrumentation.
- We adopt a similar approach as OCP in defining a limited list of organic contaminants of highest priority (Tier 1) and include everything else in a second list (Tier 2) with higher allowable levels. We differ from OCP in recommending separate treatment of volatile (boiling point, BP <200°C) versus nonvolatile contaminants to accommodate the extensive use of organic solvents within the SRF. TOC requirements have thus been modified to reflect volatility and solubility.
- We support the same approach as M2020 in using the Tissint meteorite to guide inorganic concentration limits; however, we note that several of the elements that were not explicitly limited for M2020 (Fe, Cr, Mo, Al, Si) are of significant geochemical interest, and thus best efforts should be made to limit contamination by these elements.
- We recommend several new, highly sensitive assays of biomolecules as specific means to test for terrestrial life. These assays are for lipopolysaccharide, β-glucan, and adenosine triphosphate.
- We strongly recommend the implementation of an active environmental DNA monitoring program that tracks the presence of nucleic acids, along with their sequence and identity. When changes to the SRF DNA baseline are observed, we recommend a cessation of activities followed by the identification and remediation of contamination sources. These recommendations correspond to a "no change in the SRF DNA baseline" contamination limit.
- The SCP did not explicitly consider requirements that may be needed to avoid noble gas contamination or other inorganic volatiles such as water. These considerations should be included in future discussions.

Proposed contamination requirements

- A summary of our proposed contamination requirements is provided in Table 1.

1. Introduction

1.1. Introduction to the sample receiving facility contamination panel

The Mars Sample Return (MSR) Campaign being planned jointly by the National Aeronautics Space Administration

Table 1. Proposed Contamination Limits for the SRF

	Description	*Elements or compounds*	*Proposed limit*
Inorganic	Elements of general geochemical interest	Li, B, Mg, P, S, Cl, Sc, Mn, Co, Ni, Zn, Br, Y, Zr, Nb, Cs, La, Ce, Eu, Gd, and Ta	1% of Tissint
	Elements used for geochronology	K, Rb, Sr, Nd, Sm, Lu, Hf, W, Re, Os, Pb, Th, U	0.1% of Tissint
	Necessary elements also of geochemical interest	Na, Al, Si, Ca, Ti, V, Cr, Fe, Cu, Ge, Mo	Best effort
Organic	Volatiles of highest concern (Tier 1a)	C_5–C_{11} *n*-alkanes, benzene, toluene, ethylbenzene, xylene, methanol, ethanol, acetone, acetonitrile, dichloromethane, dichloroethene, chlorobenzene, dichlorobenzene, dimethylsulfide, thiophene, methanethiol, ethanethiol, acetic acid, pyruvic acid, formaldehyde, acetaldehyde	1 ppb
	Volatiles of lesser concern (Tier 2a)	All other compounds with BP <200°C	10 ppb
	Total volatile organic carbon	All organics with BP <200°C	40 ppb
	Nonvolatiles of highest concern (Tier 1b)	C_{12}–C_{30} *n*-alkanes, pristane, naphthalene, phenanthrene, palmitic acid, squalene, cholesterol, benzothiophene, dibenzothiophene, benzoic acid, benzonitrile, oxalic acid, alanine, glycine, *N*-acetylglucosamine, dipicolinic acid, DNA, urea, glycerol	0.1 ppb
	Nonvolatiles of lesser concern (Tier 2b)	All other compounds with BP >200°C	1.0 ppb
	Extraction blank	Solvent-extractable, nonvolatile organic carbon	10 ppb
	Kerogen blank	Non-solvent-extractable, nonvolatile organic carbon	100 ppb
Biological	Culturable cells	Colony forming units	<1
	Special biological contaminants	Lipopolysaccharide, β-glucan, ATP	1 ppt
	DNA	No change in the SRF DNA baseline (see Section 5.4.2)	

List of targeted inorganic, organic, and biological contaminants and proposed sample contamination limits for the SRF.
ATP = adenosyl triphosphate; BP = boiling point; SRF = Sample Receiving Facility.

(NASA) and the European Space Agency (ESA) is intended to be a multi-mission effort to bring selected geological and atmospheric samples to Earth for the purpose of detailed scientific investigation. Significant parts of the scientific research and planetary protection strategies could be affected by Earth-sourced contamination that is either misinterpreted as being of martian origin or that masks a martian signal. Potential contamination is likely to be most significant during two primary phases of the Campaign:

1.1.1. During sample acquisition and storage by NASA's Mars 2020 (M2020) Perseverance rover. Several of the rover's components come into contact with the samples—most notably the drill bits and the Returnable Sample Tube Assemblies. These can be a vector for the transfer of contaminants that originated on Earth, either inorganic or organic (biogenic or abiogenic), to the samples. Once the sample tubes are sealed on Mars, further contamination during the transportation stages of the MSR Campaign is expected to be very limited given predicted leak rates for the sealed tubes of $<10^{-13}$ standard cubic centimeters per second (scc/s) (Osterhout et al., 2024).

1.1.2. During residence inside the Sample Receiving Facility. Upon arrival at Earth, the samples would be transported to a high-containment facility, currently referred to as the SRF. While the samples are in the custody of the SRF, they would need to be protected from unacceptable contamination. The Sample Receiving Project (SRP) that is planning the development of the SRF would have additional aspects for which contamination control/contamination knowledge (CC/CK) needs to be planned (*e.g.*, in making use of external laboratories), but these requirements and processes can be planned for later. An immediate problem is understanding how the CC/CK constraints contribute to the design and operating requirements of the SRF.

CC and CK requirements for the phases above would have important and lasting impacts on our ability to successfully carry out sample science investigations that are sensitive to terrestrial contamination. Potential contamination during sample acquisition was considered by the OCP, which was chartered and completed its work in 2014 (Summons et al., 2014). Their recommendations formed the basis of the CC/CK implementation plans for M2020. CC/CK requirements for the so-called ground segment (*i.e.*, SRF) have not yet been addressed; this is the focus of the current SRF Contamination Panel (SCP) (Fig. 1).

The SCP's stated task was to define proposed terrestrial biological, organic, and inorganic contamination limits needed to protect the scientific integrity of the MSR samples during residence inside the SRF. The SCP's starting point was an analysis of the total amount of terrestrial biological, organic, and inorganic contamination that would potentially already be present in the MSR samples as a result of M2020's operations, based on the prelaunch requirement verification analyses. The SCP then studied the amount of additional contamination from within the SRF that could be tolerated without negatively impacting science goals.

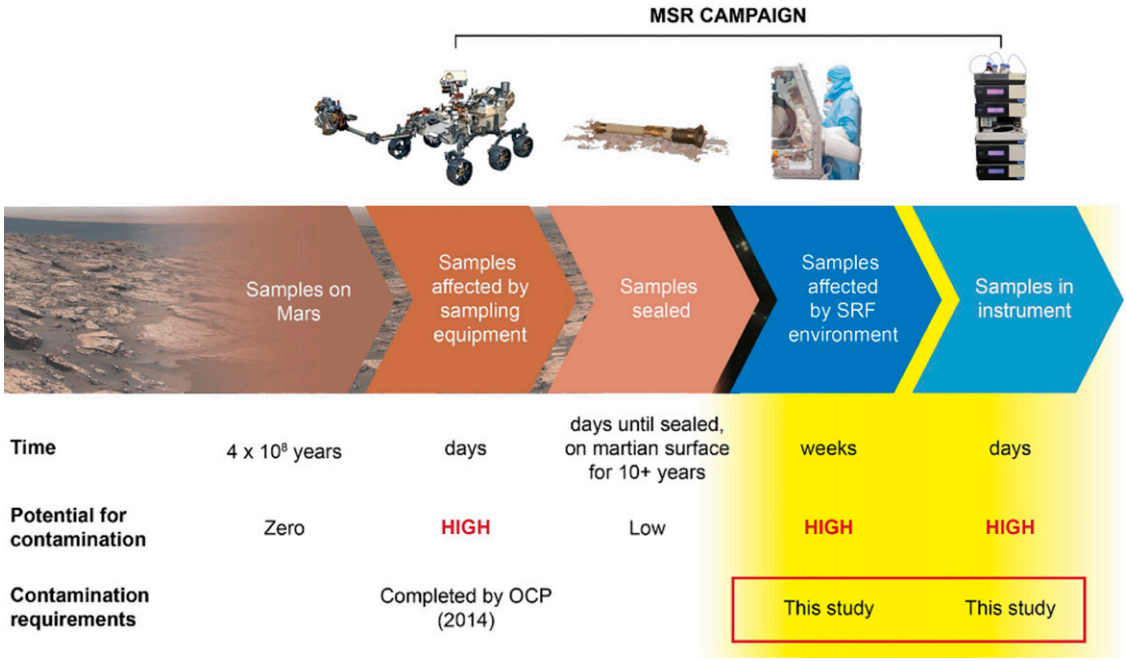

FIG. 1. History and lifetime of MSR samples (adapted from Summons et al., 2014). Our study recommends contamination requirements for the samples during their stay and through their initial characterization in the SRF (yellow box). MSR, Mars Sample Return; SRF, Sample Receiving Facility.

The SCP consisted of 15 scientists from the United States and Europe, who were selected based on their scientific backgrounds, covering expertise and knowledge in astrobiology, microbiology, organic chemistry and geochemistry, analytical chemistry, inorganic and isotope geochemistry, as well as theory and state-of-the-art lab practices, CC, and instrumentation. An additional five *ex officio* participants from NASA and ESA contributed to the panel's discussions and helped to administer the activities of the SCP.

1.2. Previous work on returned-sample curation and contamination

More than 250 years ago, the Vienna Imperial Natural History Cabinet started the first dedicated collection of meteorites. Since then, the curation of astromaterials has seen many innovations regarding sample collection, handling, analysis, and conservation, which in combination led to the multidisciplinary endeavor that is advanced curation today (McCubbin et al., 2019). Sample return missions from other planetary bodies have been of particular importance for the continuous development of curation practices and CC and will continue to be so in the future. The era of planetary sample return began with the design and construction of the Lunar Receiving Laboratory at NASA Johnson Space Center (JSC; then called the Manned Spacecraft Center) for the handling, processing, and storage of samples (and, briefly, astronauts) from NASA's Apollo missions (Compton, 1989; McLane et al., 1967). The Lunar Receiving Laboratory was designed and constructed from 1964 to 1967 and included the capability to contain and assay biological hazards and characterize prebiotic compounds (as understood in the mid-1960s).

In the Lunar Receiving Laboratory, samples were initially handled under vacuum, but the vacuum system was unreliable, tended to introduce contaminants, and made work very difficult, both ergonomically and by making lunar dust more difficult to remove (King, 1989). The vacuum requirement was dropped after Apollo 12 and replaced with dry nitrogen, which remains the standard approach today. The sterilization and quarantine protocols (including UV irradiation of nude astronauts before they could leave the facility) and biological testing by feeding lunar samples to animals were viewed as a waste of sample and unnecessary by many of the scientists, astronauts, and technicians involved. These views led to lapses in adherence and a communication breakdown between senior managers and those in the labs. The quarantine requirement was dropped before Apollo 14 (Allton, 1994).

The Soviet Union handled the lunar samples returned by Luna 16, 20, and 24 in ultra-high vacuum followed by helium or nitrogen in a ground-based receiving complex at the Vernadsky Institute in Moscow. The sample chambers contained sterilization devices to exclude contact of terrestrial microorganisms with lunar soil and a temperature sterilizer in the pumping line to destroy organisms that could be present in lunar materials (Slyuta, 2021). Since Apollo, there have been few sample return missions with an interest in organic compound preservation, although the Apollo curation techniques have done an admirable—albeit imperfect—job of maintaining both inorganic and organic cleanliness over the last five decades and counting (Day et al., 2018; Elsila et al., 2016; Lunar and Planetary Science Institute, 2023; Thomas-Keprta et al., 2014; Wright et al., 1992).

As different samples have been collected and archived, JSC now curates various types of returned astromaterials in dedicated facilities (Table 2), as well as Antarctic meteorites and airborne interplanetary dust particles. Most recently, the collection now includes samples returned from asteroid

Table 2. Previous Sample Return Missions

Mission	*Country*	*Destination*	*Sample mass*	*Returned to Earth*
Apollo 11	USA	Moon: Mare Tranquillitatis	22 kg	1969
Apollo 12	USA	Moon: Oceanus Procellarum	34 kg	1969
Luna 16	USSR	Moon: Mare Fecund	101 g	1970
Apollo 14	USA	Moon: Fra Mauro Highland	43 kg	1971
Apollo 15	USA	Moon: Hadley–Apennine	77 kg	1971
Luna 20	USSR	Moon: Apollonius Highlands	30 g	1972
Apollo 16	USA	Moon: Descartes Highlands	95 g	1972
Apollo 17	USA	Moon: Taurus Littrow	110 g	1972
Luna 24	USSR	Moon: Mare Crisium	170 g	1976
Long Duration Exposure Facility	USA	Cosmic dust in low Earth orbit	μg	1990
Mir Orbital Dust Collector	USSR	Cosmic dust in low Earth orbit	μg	1997
Genesis	USA	Solar wind	ng	2004
Hayabusa	Japan	Asteroid 25143 Itokawa	<1 mg	2005
Stardust	USA	Comet Wild2/Interstellar dust	μg	2006
Tanpopo	Japan	Cosmic dust in low Earth orbit	μg	2018
Hayabusa2	Japan	Asteroid 162173 Ryugu	5.4 g	2020
Chang'e-5	PRC	Moon: Oceanus Procellarum	1.7 kg	2020
OSIRIS-REx	USA	Asteroid 101955 Bennu	∼250 g	2023

List of previous and in-progress missions returning samples. All but Tanpopo and Chang'e-5 have material curated at JSC.

101955 Bennu by NASA's OSIRIS-REx mission. Of the sample facilities, only those for Hayabusa2 (Righter et al., 2023; Sakamoto et al., 2022; Sugahara et al., 2018), OSIRIS-REx (Righter et al., 2023), and to some extent Stardust (Sandford et al., 2010) and the meteorites (McCubbin et al., 2019) were designed and operate explicitly to limit organic contamination. None of them are designed to control biological contamination (McCubbin et al., 2019). Like the Hayabusa samples curated at the Japan Aerospace Exploration Agency (ISAS/JAXA), the Hayabusa2 samples are also curated under vacuum but with additional organic requirements (Sakamoto et al., 2022; Sugahara et al., 2018). The improved success of JAXA's vacuum curation relative to Apollo 11 and 12 may be related to both the improved technology and the significantly smaller sample that needed to be manipulated.

1.3. Key points of consideration of the SCP

During the course of our deliberations, a number of key questions and concerns arose repeatedly and in ways that were central to our decision-making. We highlight and briefly discuss those issues here.

1.3.1. There is an inherent tension between biosafety and contamination control. The principal concern of biosafety containment is to prevent the transfer of any infectious cells, viruses, or molecules from samples (and other infected materials) to other living creatures, including the humans working on them. At the highest levels of containment (termed biosafety level 4 in the United States and Europe), this is normally achieved either by having samples sequestered in cabinets with glove access or by having humans in positive-pressure suits. In either case, the concept is that air flows exclusively from the lab in toward the samples and then on to be decontaminated. The lab itself must have negative pressure relative to the outside, such that any air leaks flow inward, to prevent escape of infectious agents to the outside world.

In contrast, CC is normally achieved in exactly the opposite way, by having air flow from the sample out toward the scientist, for example, in a laminar flow hood. Clean labs are commonly over-pressured relative to ambient, such that any leaks flow outward. These measures effectively prevent contamination (volatiles, particles, cells) from the lab environment from reaching ultra-clean samples.

Both sets of requirements can, in theory, be satisfied by the concept of a double-walled isolator, with sample and scientist separated by a lower-pressure "airlock." Such isolators are already used in various industries such as radiopharmaceutical production (International Atomic Energy Agency, 2005). Nevertheless, there are many challenging and consequential decisions that lie at the intersection of these two requirements. For example, in the event an isolation cabinet fails, would we prefer to (potentially) contaminate samples or technicians? Given that large labs cannot be perfectly airtight, should the SRF be at positive or negative pressure relative to the outside environment? We did not attempt to answer such questions, but they will require significant attention by another group. For our deliberations, we focused only on the contamination requirement aspect.

1.3.2. The case for remotely operated sample handling. Human scientists with their arms reaching inside biosafety cabinets is the traditional model for interacting with samples, although technology already exists to use remotely operated mechanical manipulators or autonomous robotics. The question of how people should best interact with the Mars samples generated lively debate among the panel. On one hand, humans in the lab likely offer greater operational flexibility and lower development costs; however, they are impossible to sterilize and, thus, present a very significant risk of biological contamination of the samples (not only by human cells but also by all the bacteria and fungi that live on us). In-person sample handling also puts the humans at risk, and their presence would drive many expensive containment requirements within the lab, as well as testing and quarantine requirements outside the lab. Also, gloves or biosafety suits

that separate human workers from samples are generally constructed of organic polymers to provide needed flexibility but may be incompatible with organic cleanliness requirements.

On the other hand, remotely operated manipulators could be completely sterilized, would not need to leave the high-containment facility or be quarantined, and could be constructed of materials less likely to contaminate samples (albeit with concerns about how they would be lubricated). They would—almost certainly—cost more and take longer to develop and deploy, though we note that significant technology currently exists in robotic surgery, within the nuclear energy sector, high-tech manufacturing (*e.g.*, semiconductor chips), and deep-sea exploration. There was debate about whether remotely operated hands would be any more or less capable of "doing science" than humans wearing cumbersome gloves. The issue requires further study, particularly the capabilities and limitations of remote operation with respect to lab work on small samples. If remote operation appears feasible, it likely offers the simplest way to resolve the biosafety versus CC dilemma discussed above.

1.3.3. Solvents and volatile contamination. Organic molecules with a high enough vapor pressure to partition significantly into the vapor phase (*i.e.*, "volatiles" or VOCs) are ubiquitous on Earth, particularly in laboratories. We use them as solvents, for cleaning and disinfecting, in paints, coatings, markers, electronics, etc. They are by far the hardest component of organic contamination to control, because they move in the gas phase by diffusion and/or advection and are impervious to physical filtering. They are also key compounds of interest in the returned Mars samples, with *in situ* analyses by SAM on Curiosity finding detectable volatiles in the martian regolith (Eigenbrode et al., 2018). Keeping samples (or sample aliquots) free from terrestrial VOC contamination would be an important and extremely challenging job and likely require severe limitations on the use of solvents in the vicinity of those samples.

At the same time, extraction with organic solvents would be needed for many of the other organic analyses that would be of high priority for astrobiology and the sample safety assessment, for example, gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS). Samples would literally be submerged, stirred, or sonicated, and perhaps even boiled in organic solvents such as dichloromethane (boiling point 40°C) that would then be evaporated to concentrate analytes. It is virtually inconceivable that the airborne VOC blank in such a lab could also be maintained at sub-part-per-billion levels. We, therefore, strongly recommend that planning for the SRF incorporate ways to physically isolate lab spaces that are storing samples and/or measuring volatile organics from those that are using solvents (to extract samples, clean thin sections, sterilize surfaces or samples, etc.). We have structured our recommendations for contamination limits to facilitate this separation, that is, by distinguishing between volatile contaminants and nonvolatile ones.

1.3.4. Is "TOC" a useful contamination metric for the SRF?. Total organic carbon (TOC) is generally intended to be a catch-all category that includes all possible organic compounds and polymers, from gases like methane to high-molecular weight solids like kerogen and everything in between. TOC is typically derived by removing inorganic carbon and then burning samples and measuring released CO_2, or by using methods that respond generically to C-C or C–H bonds. In the context of MSR, the utility of TOC lies in the ability to specify a contamination limit for most organic compounds without having to explicitly list—much less measure—all of them. TOC is not, in and of itself, a critical scientific measurement, although it does hold interest regarding the total amount of accumulated organics.

There are several methods available for reducing TOC contamination to very low levels, including solvent cleaning, plasma ashing, and high-temperature combustion. Experience shows, however, that once surfaces are clean, recontamination of surfaces by "adventitious carbon" (*i.e.*, volatile organic species) in the air is rapid (*e.g.*, Calaway and Fries, 2015; Greczynski and Hultman, 2022). This is true even in clean-air labs, because the contaminants move in the vapor phase rather than as particles. Success at maintaining low TOC limits, therefore, requires limiting access of the samples to ambient air. For example, for the M2020 mission, recontamination of sample tubes was limited by keeping them capped until time of use.

In a laboratory environment, the entire concept of TOC becomes problematic due to the extensive use of solvents (Section 1.3.3). How exactly should we think about the "TOC contamination" of a sample that has just been submerged in organic solvent? And since solvent-like molecules are not measured in such analyses, should this be a metric that is measured?

In recognition of these realities, our panel has retained the concept of "TOC contamination," including volatile adventitious carbon, only for those sample aliquots that would be subjected to VOC analysis and for which solvent extraction is not used. For everything else, including those samples requiring solvent extraction, we have modified the concepts to be "solvent-extractable, nonvolatile organic carbon" and "nonextractable, nonvolatile organic carbon." The former category is applicable to techniques looking at extractable components (LC-MS, GC-MS), while the latter applies to techniques that analyze the residual organic matter, such as nuclear magnetic resonance (NMR) or pyrolysis-GC-MS.

1.3.5. The case for cold storage. The panel debated at length the pros and cons of maintaining Martian samples at subzero temperatures (likely −20°C or lower), either just during storage ("cold storage") or also during laboratory handling (a "cold" SRF). The primary perceived benefit of keeping samples cold is to prevent growth of any viable terrestrial cells that might end up on the samples. Unless the SRF can guarantee that there is no significant chance of live contamination, this would seem to be an important safeguard. Additional benefits include the retention of volatile compounds, slowing of unwanted reactions, controlling humidity, and reducing the amount of VOCs in lab air.

On the other side of the balance sheet, the primary drawback of a cold SRF is the difficulty for humans working under these conditions. Many commercial analytical instruments would have to be modified to work under such conditions, and some established measurement protocols would have to be altered. For example, any that called for use of liquid water. Keeping the SRF labs at room temperature while

storing samples cold could alleviate many of these concerns but would introduce the possibility that repeated freeze/thaw cycles could alter the physical structure of the sample (cracking mineral grains, etc.), condense volatile contaminants onto the samples, and result in other deleterious effects.

The consensus of the panel was that cold storage, at a minimum, should be implemented, at least for those samples that are to be archived for longer timescales (years), if not for all samples. For example, the OSIRIS-REx mission, at the recommendation of ExMAG, will be archiving 2.5 wt % of the Bennu sample in hermetically sealed containers at −80°C for long-term storage (Nittler, 2023). There was no consensus as to whether to recommend cold SRF laboratories. Nonetheless, the potential benefits are compelling enough that we feel this is worthy of further detailed study. In particular, the use of remotely operated manipulators might substantially alleviate the primary concern of a cold SRF, namely, the difficulty for humans working in such conditions.

1.3.6. Living and nonliving contamination are not equivalent. Life is self-replicating and able to transform its environment. In the context of Mars samples, this means that contamination by a single living cell has the potential to multiply and cause disproportionate harm to sample science, not only the search for (past or present) life but also the study of oxidation states, molecular and elemental distributions, gases, isotopic compositions, etc. This growth potential is difficult to capture in requirements that specify maximum amounts of materials deposited onto the samples. Requirements for biological contamination, therefore, need to go hand-in-hand with requirements for preventing growth. Modest levels of biological contamination might be tolerated if samples are kept at −20°C or below, while storage in conditions permissive of growth might lead us to require zero biological contamination.

1.3.7. Artificial isotope tracers. Valuable insights would be gained into the origins, processing, and age of virtually all returned materials from their isotopic compositions. In particular, identifying organics as unambiguously of martian origin would lean heavily on the stable isotopes of H, C, N, and O, while efforts to establish a martian geochronology would be based on radiogenic isotope variations in Sr, Nd, Hf, W, Os, and Pb. Making useful measurements of isotopic composition requires the ability to discern martian signals from terrestrial contamination. Fortunately, the isotopic compositions of most terrestrial materials vary within a fairly narrow range, and one that is often distinct from the analogous martian range. To preserve the utility of such isotopic measurements, it is therefore imperative that no artificially enriched or depleted isotopic tracers are allowed to contaminate the samples. This is more likely for the light stable isotopes, though not inconceivable for the heavier elements. We considered recommending a formal contamination limit for all isotopes of potential interest but feel that this would be quite onerous to implement given the diversity of possible isotopes and measurement techniques. Instead, we recommend simply that no materials be used within the SRF that have a non-terrestrial isotopic composition. If such a tracer needs to be used, then a formal program to measure and eliminate contamination of the samples by that specific tracer would need to be implemented.

1.3.8. The thorny issue of units. A question that arose often during our discussions is that of the appropriate unit(s) of measurement for the specified contamination limits (per area, per mass, number of cells, etc.). This has been previously discussed at length by the OCP (Summons et al., 2014). For analyses that require bulk sample extraction, powdering, or digesting aliquots, a per-sample-mass basis is appropriate. However, for surface-based measurements, a per-surface-area basis would be more apt. On the other hand, microbial bioburden levels are frequently defined by the number and types of organisms observed in a given area over a specific period of time (Peacos, 2018). Rather than provide contamination limits with differing units, the limits described below are all given on a weight/weight basis for the bulk sample, even if solid samples might be broken into several pieces within the tube, in which case the surface/volume ratio would be significantly different. For CCs that are performed on surfaces, including witness plates, the contamination level in the bulk sample we propose must be converted to a surface area requirement.

1.3.9. Contamination measurements within the SRF. Our report lists many elements, compounds, and biological materials that would need to be measured with regard to meeting contamination requirements. There will undoubtedly be many others of interest to the CC/CK program. A current uncertainty relates to whether such measurements can (or should) be made within the SRF. From a biosafety perspective, once martian samples are brought into the SRF, any CC/CK samples (swabs, witness plates, air filters, etc.) must be considered as potential biohazards. Some sample types (*i.e.*, trace metals) could presumably be sterilized and brought outside the SRF for analysis, while other sample types (*i.e.*, organic molecules, DNA) clearly cannot. A preliminary comparison of the analytes recommended for monitoring herein with a list of proposed instruments included in a draft report of the SRP Measurement Definition Team (MDT) suggests that most, or all, of the proposed contaminants in our report could be targeted by instrumentation within the SRF. Whether such instruments could reach the stringent concentration limits set herein remains to be seen, though it seems probable that most selected instruments would have state-of-the-art sensitivity. Regardless, the CC program for the SRF would present a heavy and demanding analytical workload and potentially compete with the analysis of samples for instrument and technician time. It may be preferable to use dedicated instrumentation for contamination monitoring that is separate from the analytical instruments used for analysis of martian samples. Our specific recommendation, therefore, is that the group(s) that make final recommendations about SRF analytical instrumentation need to consider their capabilities and throughput with respect to the CC/CK program needs, not just martian samples.

2. What Do We Expect with Regard to the Returned Samples?

2.1. Introduction to Jezero Crater

Jezero Crater is a Noachian-aged, 45-km diameter impact basin located at 18.4°N, 77.7°E near Nili Fossae on the northwestern rim of the Isidis basin (*e.g.*, Goudge et al.,

2015). Jezero was selected as the landing site for the Perseverance rover due to the geologic diversity of the rocks present within the crater, the variety of minerals observed from orbit, and recognition that the late Noachian/early Hesperian age of the crater lithologies dates to a time period on Mars that was warmer and wetter than Mars today, and hence could have been habitable to ancient microbial life (*e.g.*, Grotzinger et al., 2014). Igneous units with orbital spectral signatures of olivine and hydrated Mg carbonate are present on the crater floor (Goudge et al., 2015). The crater has multiple sedimentary fans, at least one of which is consistent with subaqueous deposition in a lake that occupied the crater floor ~3.8 billion years ago (Goudge et al., 2017). The westernmost fan is a primary target for observation and sample collection by Perseverance. The western fan shows variable mineralogy from orbital visible/shortwave-infrared spectroscopy, including Fe/Mg smectite and hydrated Mg carbonate (Ehlmann et al., 2008; Goudge et al., 2015). Some of these carbonates are concentrated along the inner margin of the crater and may have precipitated in an ancient lake margin environment (Horgan et al., 2020). Since landing on Mars in February 2021, the Mars 2020 Perseverance mission has tested many of the pre-landing hypotheses, corroborated many, and provided details about the Jezero Crater fluviolacustrine system.

2.2. Expected rock and sediment types

Returned samples collected by the Perseverance rover are expected to include igneous and sedimentary rocks as well as regolith (*i.e.*, unconsolidated surface sediment) and martian atmospheric samples (empty sealed tube or headspace gas in the sample tubes) that were collected within at least five campaigns (Crater Floor, Fan Front, Upper Fan, Margin, and Crater Rim). A summary of samples from the first four campaigns is provided in the work of Herd et al. (2025). The samples include a suite of igneous rocks from the crater floor, which have been interpreted as olivine cumulate intrusive rocks and ferroan basaltic/trachy-basaltic to trachy-andesitic lava flows with variable degrees of aqueous alteration recorded, fluvial to deltaic sediments transported and deposited from the Jezero watershed, diverse locally derived clasts and potentially global dust in regolith samples, and lacustrine, beach, or possibly igneous rocks from the Margin unit (Herd et al., 2025 and references therein). Most recently, the sample Sapphire Canyon appears to be a sedimentary rock from the layered unit within Neretva Vallis that preserves a signature of organic matter and reduction spots; both are potential biosignatures of significant astrobiological interest (Hurowitz et al., 2025; Murphy et al., 2025). The Crater Rim campaign has provided access to pre-Jezero Crater rocks that potentially record habitable environments in Noachian crust (Mayhew et al., 2025).

2.3. Expected organic materials

We speculate on the organic materials that may be present in the returned Mars samples based on a broad organic survey of igneous and sedimentary rocks at Jezero Crater by the Perseverance rover and a more in-depth characterization of organic matter in sedimentary rocks and regolith by NASA's Curiosity rover in a different area, Gale Crater. Additional information is collected from martian meteorite samples and terrestrial analog environments.

The fluorescence signatures observed by the SHERLOC instrument on Perseverance for igneous rocks in Jezero Crater are consistent with emission either from aromatic organic compounds containing one or two fused aromatic rings and/or aromatic heterocycles, or from Ce^{3+} in apatite or anhydrite. Currently, the inorganic hypothesis could explain two out of the four fluorescence responses outlined by Scheller et al. (2022) and Sharma et al. (2023). These fluorescence phenomena and the nuances demonstrated by the spectra are the subject of current research testing several hypotheses. Thus far, there has been a single above-background detection in Raman of a signal consistent with macromolecular organic carbon in the Montpezat sample (Sharma et al., 2023).

Additional information comes from Gale Crater, where the Sample Analysis at Mars (SAM) instrument on the Curiosity rover has worked to characterize organic molecules for the past decade (Mahaffy et al., 2012). Despite their differing paleodepositional conditions, Gale and Jezero share many similarities, and it seems reasonable to presume that organics at both sites—at least those due to meteoritic inputs—could be similar. Heating rocks from Gale Crater released hundreds of ppb of chlorinated organic compounds, mostly aromatic, at relatively low temperature (<500°C) (Freissinet et al., 2015; Szopa et al., 2020), sulfur-containing compounds at high temperature (>500°C) (Eigenbrode et al., 2018), aromatic carboxylic acids (Millan et al., 2021), and linear hydrocarbons up to C_{12} (Freissinet et al., 2025). Many of these compounds could also be present in the samples from Jezero. Indeed, the chlorinated compounds detected at Gale Crater are thought to be reaction products of martian perchlorates and aromatic functionalized precursors, possibly benzoic acid or macromolecular carbon, present at up to ppm level (Freissinet et al., 2020). The concurrent presence of both Na-perchlorate and supposedly aromatic compounds at Jezero, under surface radiation, should lead to similar chlorinated compounds. In a similar chemical process, the presence of sulfate (inorganic sulfur) and organic compounds could lead, over time, to the formation of sulfur-containing organic matter, especially within the "sulfate-bearing unit," the younger layer of rock above the clay-bearing unit that is currently being explored by Curiosity on Mt. Sharp.

The SAM instrument onboard Curiosity has also detected inexplicably high relative abundances of CO_2 being released from heated samples (Sutter et al., 2017). If this "excess" CO_2 results from the oxidation of organic matter, it would imply ppm levels or greater of TOC in the martian samples, despite the strongly oxidizing conditions at the surface. Indeed, the result of combustion in oxygen of the Cumberland samples has recently yielded measurements of 950 μg C/g of carbon at low temperature, the majority of which is carbonate. A high-temperature step produced 273 μg C/g, with a C isotope ratio that is consistent with organic material (Stern et al., 2022). This uncharacterized organic matter could represent the oxidized remains of martian sources or abiotically synthesized organic material (*e.g.*, igneous, hydrothermal, atmospheric, or biological; (Benner et al., 2000) or exogenous sources such as meteorites, comets, or interplanetary dust particles) and be present in the form of organic salts

(Lewis et al., 2021). It is also conceivable that the original organic matter has been processed into kerogen-type macromolecular organic matter, akin to the insoluble organic matter in meteorites. That type of macromolecular material would be largely invisible to our rover-based instruments but more easily characterized on Earth by using techniques such as NMR.

The variety of minerals detected at Jezero, including sulfates, carbonates, and silicates, are favorable for long-term preservation of organic matter. The reduced organic matter may represent a large variety of chemical families: chlorine- and sulfur-containing compounds as detected by Curiosity, carboxylic acids they may originate from, organic salts generated via primary synthesis or by oxidation pathways, amino acids as detected on meteoritic sources, or more mature kerogen-like material. If preserved over geological times at Jezero Crater's formation, those compounds could be detected in the returned samples.

Analyses of martian meteorites have also revealed a slew of organic compounds present, many of which have been confirmed as having a martian origin by analysis of the D/H ratio of carbon-containing materials (McKay et al., 1996; Schmitt-Kopplin et al., 2023; Sephton, 2004; Steele et al., 2022, 2018, 2016, 2012; Thomas-Keprta et al., 2022). Analysis of 12 martian meteorites, including the Tissint meteorite fall, has revealed a macromolecular organic material spatially resolved to iron-rich minerals and aqueous alteration processes (Steele et al., 2012). Raman and Fourier transform-infrared spectroscopy (FTIR) spectroscopy of martian meteorite NW-11220 also reveals an abundance of aliphatic compounds (Goodwin et al., 2024).

2.4. Expected martian biology investigations

An important science objective identified by the international MSR Objectives and Samples Team (iMOST) is to "assess and interpret the potential biological history of Mars, including assaying returned samples for the evidence of life" (Beaty et al., 2019). To achieve this, extensive planning related to the scientific and sample safety assessment strategies of martian samples has occurred. The most recent planning documents related to these strategies are those developed by the Sample Safety Assessment Protocol Tiger Team (SSAP-TT) (Teece et al., 2025—this issue) and the SRP MDT (Carrier et al., 2025—this issue).

The SCP was tasked with defining the CC/CK requirements necessary for the aforementioned science and safety programs.

A central assumption in MSR is that carbon-based martian biology is far more likely than other forms of life due to carbon's utility in terrestrial biology and prevalence throughout the solar system (*e.g.*, Kminek et al., 2022a; Sephton and Botta, 2008; Teece et al., 2025). Within the samples, martian biology may exist in the forms of extant life, extinct life, and/or prebiotic precursors, and there are plans for all forms of martian biology to be investigated within the SRF. As terrestrial life is carbon-based and there is growing evidence that similar prebiotic chemical reaction networks could have occurred on Earth and Mars (for a review, see Sasselov et al., 2020), minimizing and cataloging terrestrial contamination should be an important priority within the SRF. Within the MDT, a three-part life investigation strategy has been proposed: "1) structures of biological and martian origin, specifically geologically short-lived biomolecules (or molecular structures with biomolecular characteristics) and cell-like structures; 2) thermodynamically improbable distributions, including elemental, molecular, and spatial, indicative of a living system; and 3) changes over time that result only from biological processes" (Carrier, et al., 2025). Complementing the scientific strategy of MDT is the sample safety assessment protocol (SSAP) proposed by the SSAP-TT to determine whether samples are "safe" for release from the SRF. Within the SSAP-TT proposed protocol, biological activity would only be assessed on samples that exceed the "abiotic baseline" established in earlier characterization steps of the protocol (for details, see Teece et al., 2025). These scientific and safety strategies necessitate a rigorous biological CC/CK program to ensure that positive life detections within martian samples are not of terrestrial origin (*e.g.*, cellular material and microbial activity).

3. What Levels of Cleanliness Did the M2020 Project Achieve for Sample-Contacting Hardware, and How?

The level of cleanliness achieved by the M2020 mission with respect to collected samples is relevant to our task in two regards. First, this represents a baseline of contamination that is likely already present within the samples that would be returned to the SRF. In some cases, it may be pointless to recommend SRF contamination limits that are far below what is already known to be present. Second, it demonstrates the technical capability to clean and verify hardware at certain levels. Here, we briefly summarize relevant information from the M2020 project regarding their cleaning and verification of sampling hardware.

Contamination modeling of the rover environment considered two possible sources of contamination to samples: preexisting contamination on sampling hardware (also known as "initial state" contamination) and the transfer of volatiles from other parts of the rover. Measurements of outgassing rates from rover hardware indicated that the latter pathway would be negligible, so the focus was primarily on initial state contamination of sample-intimate hardware (SIH; includes sample tubes, seals, and drill bits).

Sampling hardware was cleaned via multi-solvent sonication, then a TiN coating was applied using a 670°C plasma nitriding process that should have further oxidized any organic residues. Sample tubes were assembled, then a final rinse of water isopropyl alcohol (IPA), and hexane was conducted, followed by a final 24 h vacuum dry heat microbial reduction (DHMR) heating at 150°C (Maltais et al., 2023). A variety of analytical techniques were used to establish that the sampling hardware surfaces met the cleanliness requirements with residual molecular contamination levels of <1 ng/cm^2 TOC and <0.3 ng/cm^2 for restricted Tier 1 and Tier 2 compounds and met the inorganic cleanliness requirements (Maltais et al., 2023), as described in more detail in Section 3.2.

3.1. Inorganic contaminants

While all the naturally occurring elements of the periodic table can provide useful information on Mars' geochemical evolution, the inorganic requirements for CC/CK as applied to M2020 focus on elements that are particularly important to

high-priority science objectives (McLennan et al., 2012). Because the levels of inorganic contamination that are relevant to martian samples vary as a function of each element's abundance, they can be conveniently expressed as a fraction of the natural concentration of each element in martian meteorites. Specifically, the choice was made to report contamination limits relative to the composition of a single meteorite, Tissint, which is a depleted shergottite (Chennaoui Aoudjehane et al., 2012). M2020 further decided to define two different categories of elements with different sensitivity toward contamination. For elements of general geochemical interest (Fig. 2; Li, B, Mg, P, S, Cl, Sc, Mn, Co, Ni, Zn, Br, Y, Zr, Nb, Cs, La, Ce, Eu, Gd, and Ta), they recommended a limit of less than 1% of Tissint concentration as Earth-sourced inorganic contamination. In contrast, elements used in geochronology (K, Rb, Sr, Nd, Sm, Lu, Hf, W, Re, Os, Pb, Th, and U) require strict CC due to the very high precision of measurements needed to infer an age and were assigned a limit of less than 0.1% of Tissint content (Fig. 2).

To quantify the levels of cleanliness achieved by the M2020 project, contamination of the various sample-contacting components was measured by using ICP-MS for 34 separate elements. The contributions of the different mechanical parts (drill bits, teeth, seal particulate) and the transfer of elements to the samples were then estimated via a contamination model (Maltais et al., 2023). For most elements, the contributions of the materials to the core are at least 10 times lower than the contamination requirements. B, Re, U, Nd, Pb (as well as Rb and Cs, in the case of a new drill bit and the uniform Saddleback basalt) are closer to the contamination limit but still compliant. However, five elements (Ni, Co, Nb, Ta, and W) exceeded the contamination requirements by more than an order of magnitude for engineering reasons. For example, the estimated contribution of W from materials—depending on the drill bit (new or old) and the nature of the sample (sandstone or basalt)—varies between 1.9×10^{-2} and 3.1 ppm compared with the 4.1×10^{-5} ppm allowed in the core according to contamination requirements. Elemental exceedances in the core samples are primarily due to tungsten carbide teeth (for W, Ta, Co, and Nb), the custom drill bit (Nb and Ni), and seal particulate (Ni).

In the case of the regolith samples, the drill bit is composed of TiN and Ta. Since no tests were performed to evaluate contact transfer wear of TiN from the regolith bit to the sample, two theoretical transfer amounts were considered to determine what chemical elements might be noncompliant. Considering 0.1 mg of TiN wear in the samples, Ta is the only exceedance observed from other sources, namely, seal cup gold shedding. It should be noted that characterization of Ta transfer is still pending; for now, the calculation relies on an estimate. With 1.0 mg of TiN wear in the sample, which is considered a conservative upper limit for TiN wear, U, Rb, Cs, and Sr show exceedances for a 10 g regolith sample, as does Ta.

Finally, no exceedances are observed for Pb from sampling-related contributions, although the Gaseous Dust Removal Tool results indicate that sampling within 0.5 m of an abraded patch could potentially lead to an exceedance of the Pb limit.

3.2. Organic contaminants

For Mars 2020, organic contamination of surfaces was assessed by using GC-MS analysis of final hexane rinse for Tier 1 compounds and FTIR, diffuse reflectance infrared Fourier transform, and direct analysis in real time-mass spectroscopy to estimate TOC on surfaces. Because these techniques are measuring hardware surfaces, the native units of measurement are ng/cm^2. A sophisticated contamination

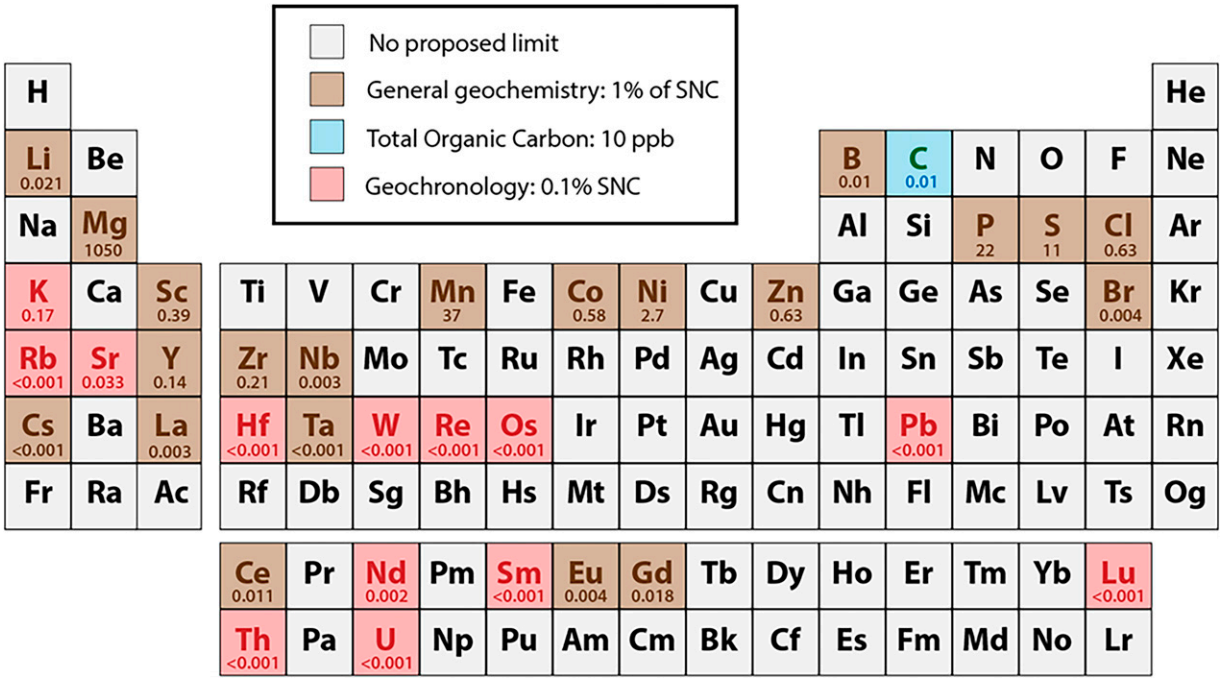

FIG. 2. Summary of inorganic contamination limits imposed by the M2020 mission. Values correspond to contamination limits (w/w) expressed in parts per million (ppm). Figure adapted from Maltais et al., 2023.

model was developed to consider sample-accessible surface areas, transfer coefficients, and exposure times (Katz et al., 2018; Maltais et al., 2023). Based on that model, a limit of <1.0 ppb (w/w) in the sample for Tier 1 compounds (the OCP for M2020 requirement) is equivalent to <0.3 ng/cm^2 on the SIH surfaces using conservative values for model parameters (Boeder and Soares, 2020). Using this conversion, all of the SIH that was tested met the required limit of <0.3 ng/cm^2—in other words, the Tier 1 requirements were all met. Although many of the project reports list the measurements as "not detectable" at a limit of <0.3 ng/cm^2, in reviewing their data it appears that real instrument detection limits for these analyses were 0.03 ng/cm^2 or lower for most of the Tier 1 analytes. Thus, *contamination within the cores by most or all of the Tier 1 compounds is likely at least an order of magnitude below the OCP limit of 1 ppb*. The two exceptions to this statement are acetic acid and glycine, with detection limits of <0.1 and <0.2 ng/cm^2, respectively, for which we can only say that contamination was below the 1 ppb limit (Table 3).

TOC contamination was assessed via a combination of techniques that measure organic molecules directly on surfaces and respond generically to features shared by all organic compounds (*e.g.*, C–H bond vibrations). Converting such measurements into concentrations is not straightforward, but based on the M2020 team's calibration they achieved <1.0 ng/cm^2 TOC for all SIH pieces and parts. Using "best estimate" parameter values in the contamination model, this translates to 8.1 ppb TOC. Using "best conservative estimate" parameter values, this translates to 13.5 ppb, slightly above the OCP requirement of 10 ppb. Using even more conservative parameters, the predicted contamination is up to 38 ppb, which is still below the 40 ppb threshold requirement. For our purposes, it appears that 10 ppb is a suitable order-of-magnitude estimate for TOC contamination already in the samples. M2020 data suggest that most of the TOC detected was likely adventitious carbon, for example, volatile compounds that were transported in the gas phase and then adsorbed onto surfaces after they were cleaned.

Tier 2 compounds were—by design—not explicitly monitored. The M2020 team's approach was to use broad-based screening techniques, such as full-scan GC-MS, to show that none of the detected background components (individual molecular species) were approaching 25% of the total, and since TOC was <40 ppb they could confidently predict that no single organic compound would reach 10 ppb. Given the observation that most of the TOC was likely volatile adventitious carbon, it seems unlikely that any of the nonvolatile Tier 2 compounds (*i.e.*, other *n*-alkanes and polyaromatic hydrocarbons [PAHs]) were above even 1 ppb. However, that statement was not explicitly tested and so must be taken with appropriate caution.

3.3. Biological contaminants

The Mars 2020 mission adhered to a bioburden reduction protocol for which monitoring was conducted according to the

Table 3. Detection Limits for M2020 Project Contamination Screening of Organics

Tier 1 compounds	*Tube, sheaths ng/cm^2*	*Witness plate ng/cm^2*	*Swab (PE) ng/cm^2*	*Seals ng/cm^2*	*Comments*
Hexadecanoic acid	<0.02	<0.08	[b]	<0.14	
Naphthalene	<0.004	<0.02	—	<0.03	Semi-volatile, sublimes at room temperature
Alanine	(<0.02)[c]	<0.002	<0.007[a]	—	
Glycerol	<0.004	<0.012	—	<0.03	
Urea	<0.004	<0.003	[b]	<0.03	
Pyridine dicarboxylic acid	<0.004	<0.003	[b]	<0.03	
Glycine	(<0.2)[c]	<0.05	<0.15[a]	—	
Pyruvic acid	<0.02	<0.12	[b]	<0.14	Semi-volatile and reactive, evaporates at room temperature
Acetic acid	<0.1	<0.1	[b]	<0.15	Semi-volatile and reactive, evaporates at room temperature
DNA (pyrolysis)	(<0.01)[c]	<0.007	—	(<0.1)[c]	Associated with particulate contamination
N-acetyl glucosamine	<0.003	<0.014	—	<0.02	
Dichloromethane	(<0.05)	—	—	—	Volatile, rapid evaporation at room temperature
Squalene	<0.006	<0.02	—	<0.04	Semi-volatile evaporates and degrades at room temperature
Chlorobenzene	<0.02	<1.2	—	<0.14	Volatile evaporates at room temperature
Heptacosane	<0.02	<0.06	—	<0.14	
Pristane	<0.02	<0.03	—	<0.14	
Total organic carbon	<0.1	<0.05	—	<0.2	

Source: "Returned Sample Contamination Control and Knowledge: Pre-Launch Review" slide deck (Personal Communication from Paul Boeder, JP.). [a]Analyzed using the direct, dry polyethylene (PE) swab; [b]May also be detected by the direct PE swab; [c]Sampling dependent on solvent removal of particles containing analyte.

NASA standard assay outlined in the handbook for the microbial examination of space hardware, NASA-HDBK-6022 (NASA, 2010). In total, assays were conducted to assess bioburden on ~10% of the spacecraft-exposed surfaces throughout the building of the spacecraft at JPL and during Assembly, Test, and Launch Operations (ATLO), constituting 16,881 swabs and wipes that were analyzed (Cooper et al., 2023). Estimates of bioload across the spacecraft, including entry, descent, and landing hardware but outside of SIH, were initially set at $<50 \times 10^4$ spores at launch. Cooper et al. (2023) reported the final prelaunch swab and wipe tests to indicate the number of spores at 37.3×10^4 after 5745 swabs and 674 wipes.

The sample-contacting and sampling hardware were treated to a higher standard by the mission requirement of having to provide a less than 1 in 1000 probability of a single microbe contaminating a sample tube. To meet this requirement, special aseptic protocols were developed and monitored. Chen et al. (2023) outlined these protocols in detail and estimated the probability of a single terrestrial organism existing within a sample tube as between 1 and 2 orders of magnitude beneath the level one requirements.

4. Recommendations for Construction, Cleaning, and Monitoring the SRF

4.1. General considerations

It is near axiomatic that research facilities are constructed for relatively narrow scientific goals, and different facilities can use quite different materials both in their construction and sample manipulation methods to achieve those goals. For example, organic labs rely heavily on glass, ceramics, and metals; cleaning is achieved via solvent washing or combustion in O_2. Inorganic labs on the other hand, particularly trace-metal and geochronology labs, make wide use of Teflon, polyethylene, epoxy, and other organic polymers, while cleaning is generally through leaching with mineral acids. Microbiology labs offer yet a third perspective, with an emphasis on presterilized—and largely disposable—plasticware. If needed, cleaning is typically accomplished with detergents, followed by a sterilization step via irradiation (UV), heat (flame, autoclave), solvents (ethanol), or other chemical agents (*e.g.*, bleach and hydrogen peroxide).

The unique challenge of the SRF is that it seeks to combine all three of these scientific goals into a single facility, with additional biosafety containment requirements. The returned martian samples would be sufficiently unique and valuable that practically any element, compound, isotope, mineral, or morphological characteristic is of interest. The entire periodic table could presumably be eliminated from consideration on the basis of "potential interest," yet we obviously must use something to construct the SRF. Inorganic materials (stainless steel, glass, etc.) would likely only interfere with specific elements but probably would not be easily distinguished from their martian counterparts—for example, terrestrial silica from martian silica. In contrast, organic polymers like Teflon would be easily recognized as "not martian," but because of their behavior, for example, in pyrolysis, they have the potential to interfere with a broader swath of organic analytes. Ultimately, while our panel discussed the pros and cons of various building materials, we did not reach a consensus about what materials should be used. There was definitive agreement about certain materials that should *not* be used, as detailed below.

Surface properties of the SRF materials are just as important as their chemical compositions. Material surfaces, including welds and joints, must be highly polished to reduce surface area (important for adsorption of volatiles), limit trapped particles that include biological material, and make them easier to clean and sterilize. Electrostatic charging of surfaces must also be well controlled, as this would determine the attraction of charged volatile species and aerosols to the surface and the ease of removing them during cleaning. Any polymers used should preferably be hydrophobic to limit interaction with water and aerosols. An electrostatic management program should be part of the clean room design and operation.

A second area of discussion involved the isolation of different analytical "tracks" into separate physical spaces or compartments. The main point of such a scheme is to reduce the number of contamination requirements that a given space would be subject to, as well as provide greater flexibility in materials and construction. For example, the "organic wing" could be all stainless steel, while the "inorganic wing" could be all Teflon. The primary downside to such a recommendation is that it would significantly reduce operational flexibility—for example, the ability to do inorganic analyses on sample residues that have just been solvent extracted. Ultimately, we feel there are some significant potential benefits to the compartmentalization concept, but doing so intelligently would require first defining the analytical strategy (and sample allocation) that would be followed in the SRF.

4.2. Geochemical perspective

NASA has long-term experience with handling returned samples (Apollo, Stardust, Hayabusa, and Hayabusa2, OSIRIS-REx) and collected non-terrestrial material in general, including interplanetary dust particles and meteorites. The questions related to inorganic contamination limits in the SRF are quite similar to those of M2020 and have already been addressed in these previous contexts. Thus, established and well-known procedures and materials (*e.g.*, McCubbin et al., 2019) can be considered as the starting point. Following the experience of NASA JSC, limited materials should be used for the SRF, characterized by low particle-shedding and outgassing properties. These include specific types of glass (*e.g.*, Schott glass, fused silica, and borosilicate BK7), aluminum (*e.g.*, 6061, 6063, and 2024), stainless steel (*e.g.*, 304, 316, 316L), and fluorinated polyethylene (Teflon™, PTFE, etc.) and with specific finishes (clear anodized, electropolished, passivated, etc.). The list above provides some of the most common materials. The reader is referred to McCubbin et al (2019) for a more in-depth discussion. The SCP did not anticipate a need to develop or invent new materials for curation. In all cases, the SRF needs to be fitted with a capability to clean and sterilize tools and equipment that would come in contact with the samples. The number of organic solvents used for cleaning and sterilizing should be limited to as few as possible. IPA was viewed as acceptable for this purpose and unlikely to be a major analyte of interest in martian samples; however, alternatives including both fluorinated and inorganic solvents should be considered.

Samples and subsamples should be stored and processed in controlled environments, including controlled temperature, pressure, humidity, and composition of the atmospheric gas in storage containers and laboratories. This is a key point to safeguard the thermodynamic equilibrium of the samples and hence their redox state and mineralogy. This aspect is particularly important for deciphering the alteration processes and pathways that occurred on Mars to produce the secondary minerals observed today. Understanding the martian geological history and water–rock–gas interactions on the planet are among the key objectives of MSR. In this regard, it is also necessary to keep samples under gaseous nitrogen, argon, or a synthetic martian atmosphere rather than vacuum. The selected high-purity gas must be consistent throughout the isolators, and the pressure must be controlled.

It seems reasonable to assume that, aside from water vapor and possible VOCs, the majority of contaminants would occur as particles. Therefore, particle contamination limits have to be determined as well. Generally speaking, the sample handling room should be expected to be class 100–1000 (ISO 5–6), while the sample preparation room should be class 1000 (ISO 6) with closed containers and isolators when the sample is not being processed. These particle limits are less stringent than those required to control biological contamination (see Section 4.3). Potential transfer of particles from various operations, such as tube cutting, subsample sawing, and thin section polishing, also has to be taken into account.

4.3. Biological perspective

In nature, organisms can either be alive and metabolically active, in a dormant state (*e.g.*, as an endospore), viable but non-culturable (VBNC), or simply dead (Barer and Harwood, 1999; Oliver, 2010, 2005; Xu et al., 1982). Here, we define biological contamination as including compounds or organisms that confound instrument readings and/or transform, consume, or produce compounds of interest. A variety of pathways for biological contamination, which include personnel, surfaces, personal protective equipment and instruments, reagents, and air, need to be controlled and monitored within the SRF to understand the potential sources and nature of introduced biological contamination. Personnel are the primary source of biological contamination in clean room environments, directly affecting air, surfaces, and samples (Favero et al., 1966). The contamination risk posed by personnel in clean rooms is further discussed in Section 1.3.2. *The case for remotely operated sample handling* and sources of contamination in clean rooms are further described in Fig. 3.

4.3.1. Biological contamination control. To minimize biological contamination, the ideal conditions for sample handling should be completely aseptic.[1] Such conditions are commonly applied during aseptic packaging for food, beverage, pharmaceutical, and other industries to ensure final products are protected from contamination and safe for patients or consumers. To be "as aseptic as currently feasible," the facility and operations should *at least* be designed and operated to fulfill the regulations currently existing for current *Good Manufacturing Practice* (cGMP) aseptic pharmaceutical processing rooms and follow the biological contamination limits and monitoring programs set for such facilities, equipment, and operations (WHO, 2011). Preferentially, air is sterilized through filtration and UV-C light or hydrogen peroxide, and surfaces are cleaned and sterilized with nonorganic biocides (disinfectants and antiseptics) to prevent the introduction of organic compounds that could potentially promote microbial subsistence or growth. Activated carbon molecular filtering systems in combination with high energy particulate air filter particulate filtering systems with low molecular contaminant release were used within the ExoMars clean rooms (Comecer, 2021). The use of hydrogen peroxide vapor for sterilizing air and surfaces that come into contact with samples is preferred over organic detergents and disinfectants. Surfaces within the sample processing cabinets should be actively sterilized with UV-C to kill any living cells and denature some longer-chain polymers such as DNA and RNA; however, the detritus from the death of these organisms will not be removed during the UV-C sterilization process and would accumulate over time.

The ESA ExoMars program provides a baseline for biological CC. Key components of the ExoMars Rosalind Franklin rover were assembled in an isolator classified as ISO 3 and Airborne Molecular Contamination (AMC)-9 (Comecer, 2021; Giuliani et al., 2009). ISO 3 has a limit of

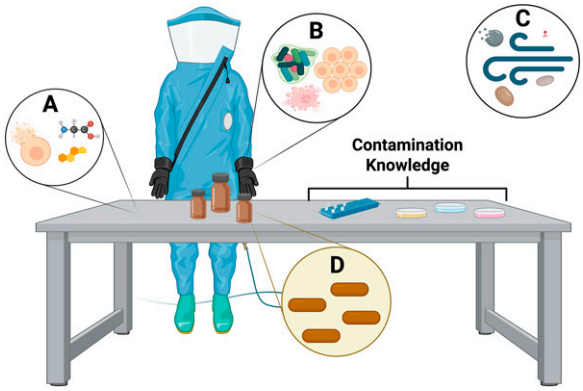

FIG. 3. Biological contamination pathways. Biological contamination in the form of living cells and cellular components could enter the SRF through a variety of pathways, and a detailed contamination knowledge program should follow samples during their stay at the SRF. Surfaces **(A)** can accumulate cells and biomolecules and should be cleaned and sterilized with nonorganic biocides. The personnel and personal protective equipment (PPE) used by workers within the SRF are another vector of biological contamination **(B)**. PPE should be cleaned and sterilized in the same manner as working surfaces. Biological contamination may also be delivered by way of the air **(C)** or reagents used for analysis within the facility **(D)**. We, therefore, recommend handling samples under ISO Class 3 conditions and the use of reagents that are Molecular Biology Grade or higher for analysis. We also recommend that a physical barrier be placed between workers and samples to minimize biological contamination, for example, by placing the samples in an isolator to facilitate aseptic handling. Figure created in BioRender. Magnabosco, C. (2025) https://BioRender.com/yywzmi0.

[1]Sterile or sterilized, free from contamination caused by bacteria, viruses, or other microorganisms.

1000 particles >0.1 μm/m^3 and, therefore, is more stringent than ISO 5, ISO 6, and cGMP Grade A designation. The level of air molecular contamination is classified as AMC and has been codified within ISO standard 14644-8. AMC-9 corresponds to an AMC concentration of $<10^{-9}$ g/m^3. Microbial contamination in this isolator was monitored through an online optical detection system (Laser-Induced Fluorescence Emission), with molecular contamination monitored using GC-MS (Comecer, 2021). Technology to achieve these levels of cleanliness is also used outside the space industry for sterile processes, large-scale pharmaceutical production, and production within the semiconductor industry.

Based on these industry and mission cleanliness standards, the SRF must fulfill an absolute level of cleanliness that corresponds to (at minimum) an isolator of ISO Class 3 particle class and AMC-9. The samples should additionally be handled according to procedures that prevent biological contamination from contact with non-sterile air and non-sterile surfaces. Surfaces that would be in contact with the martian sample material are preferentially made out of bacteriostatic materials, which are highly polished to limit crevices larger than 1 μm, prevent dirt and cells from accumulating and facilitate cleaning and sterilization. Avoiding electrostatic charges should be part of the design and operation management of the clean room, as this would strongly impact contamination levels and cleaning efficacy. Furthermore, a comprehensive CK program on biological contaminants, further described in Section 4.3.2, should be developed and used throughout the sample processing. Lastly, in the biological contamination monitoring program it is also important to consider: (i) the detection limit of the sampling procedures (*e.g.*, filters, plates, swabs, and wipes; dry or wet sampling) used to monitor the cleanliness of the facility, equipment, and operations in addition to the detection limit of the analysis procedure used (see Section 3), and (ii) the frequency (incidence rates) over a certain time span at which biological contaminants are detected (Peacos, 2018).

4.3.2. Biological contamination knowledge. Air and surface microbial contamination levels should also be continually assessed in the sample handling area during both work and rest. Microbial cultivation plates to quantify colony-forming units (CFU) during the isolator's work and rest periods should be used to monitor microbial contamination. As cultivation plates can be a source of organic contamination, we recommend swabbing isolators but inoculating cultivation plates with these swabs in an area separated from the samples. A zero-tolerance (no growth) requirement should be maintained in the isolator and atmosphere supplied to the isolator during work and rest. If microbial growth is observed, all work in the isolators should be stopped and the contamination sources should be assessed and removed. Additionally, CFUs from facility controls or contaminated samples should be identified to the species level when practical, to assist in the determination of the likely source of the contaminant. As many viable microorganisms are not cultivable and, therefore, will not be detected by CFU monitoring programs, biological CC strategies should include biomolecule-based analysis tools for contamination detection, such as those methods used in pharmaceutical facilities (see Section 6.3).

Nucleic acids in the form of DNA and RNA are essential components of terrestrial cells. As DNA is more stable than RNA, it can serve as a proxy for presumed contamination by any nucleic acids and can also be sequenced as part of a CK program (Chomczynski, 1992; Eigner et al., 1961; Jo et al., 2023). There are numerous technologies that can be employed to identify and sequence environmental DNA, thereby enabling the identification of the biological source of these fragments through various bioinformatics algorithms and databases (Athanasopoulou et al., 2021). While quantitative PCR has traditionally been used to determine the presence of environmental DNA, new technologies and approaches such as emulsion PCR followed by metagenomic sequencing (Lan et al., 2017) have been able to reduce the level of detection into the femtogram range (Abellan-Schneyder et al., 2021; Hatzenpichler et al., 2020; Pavšič et al., 2016). Such techniques are well developed and can be adapted to target both intracellular and extracellular DNA pools (Guo and Zhang, 2013; Lever et al., 2015; Sanders et al., 2011). Nonetheless, amplification of DNA from low-biomass samples is particularly susceptible to contamination from the trace amounts of nucleic acids found in the kits and buffers used to analyze them, and it is, therefore, important that blank controls are used to distinguish the "kitome" from the contaminating environmental DNA being examined in the SRF (Salter et al., 2014; Stinson et al., 2019). With these considerations in mind, we highly recommend a CK program that includes the sequencing of air and surface samples within the facility, paying special attention to changes in both the quantity and composition of DNA over time (this is further discussed in Section 5.4.2).

5. Sample-Based Contaminants of Concern

5.1. General considerations

As with the original OCP, we employed a three-part strategy for developing possible contamination limits: (1) *What are the detection limits of instrumentation that are likely to be used in the SRF?* Contamination levels below those detection limits are certainly safe but would be very difficult to verify and of questionable necessity. As has been noted previously (Summons et al, 2014), contamination does not have to be non-detectable for reliable measurements to be achieved. In many applications, subtraction of a well-characterized "background" is a longstanding analytical strategy. (2) *What levels of cleanliness can plausibly be achieved?* We considered this question both for each prospective contaminant in isolation and for the entire ensemble of proposed contaminants. We have attempted to recommend limits that we believe are feasible to implement in their entirety. (3) *What analyte concentrations do we expect in the returned samples?* In combination with desired signal-to-noise (S/N) levels for analytical measurements, this provides a rational basis for setting contamination limits that should not interfere significantly with science objectives while simultaneously avoiding being unnecessarily stringent.

One of the biggest dilemmas we wrestled with was the breadth of possible sample contamination. It would also be impossibly expensive and time-consuming for the SRP to quantify and track every single element and compound. On the organic side, we have adopted the approach of the OCP of

asking for explicit monitoring of a relatively short list of compounds deemed to be representative of compounds that are particularly interesting for returned-sample science (Tier 1) and sweeping all other compounds into the catch-all Tier 2 list of "everything else." For inorganic contamination, we recommend adding several elements to the list of monitored contaminants but have left off several other elements of potential interest because of their high utility for the SRF, for example, iron that will be used in stainless steel.

Our panel also debated whether to recommend total in-sample contamination limits, that is, limits that would subsume contamination already present in the samples, or to recommend additive limits just for the SRF, regardless of what might already be present. Although the former is perhaps more intuitive, given that analyses would sample all of the past contamination, the latter is much simpler to understand and implement. For example, the detection limits for many organics in the clean M2020 sample tubes were near the limits we are proposing; thus, we would not know how much of this contamination "budget" was already used up by M2020. Given that our understanding of the maximum allowable contamination levels is at best only order of magnitude, we did not feel that the former approach was necessary and so opted for the latter (SRF-specific) limits. These values could be converted to total in-sample limits if so desired.

5.2. Inorganic contaminants

The SCP inorganic sub-team reviewed the inorganic contamination limits previously proposed by the M2020 mission and decided to recommend a similar strategy. Note that we focused on solid samples and did not consider specific additional requirements that may be needed to avoid noble gas contamination or other inorganic volatiles such as water. This aspect should be included in future discussions.

The original inorganic contamination requirements for the M2020 mission (*i.e.*, sample collection and caching) were based on a limit of 1% of the average concentration in Tissint (depleted shergottite) for elements of general geochemical interest (Li, B, Mg, P, S, Cl, Sc, Mn, Co, Ni, Zn, Br, Y, Zr, Nb, Cs, La, Ce, Eu, Gd, and Ta), and of 0.1% of the average concentration in Tissint for elements used for geochronology (K, Rb, Sr, Nd, Sm, Lu, Hf, W, Re, Os, Pb, Th, and U). As mentioned earlier (Section 3.1), the M2020 mission met the requirements for most elements and, in many cases, achieved contamination levels several orders of magnitude lower than the requirements. However, for elements B, Cs, Rb, Re, Nd, U, and Pb, the contamination was expected to be relatively close to the contamination limits, and for Nb, Ta, Ni, Co, and W, it was expected to slightly exceed the requirements. Similarly, M2020 does not meet contamination requirements for Sr in regolith samples. It is important to note that several of these elements (Rb, Nd, U, Re, W, and Pb) are involved in geochronology and thus of high scientific priority. Although the presence of above-limit contamination will certainly complicate those analyses, we believe they will nevertheless still be attempted and thus are worth further protecting.

Following a similar approach as M2020, we recommend a contamination limit within the SRF of 1% of the average concentration in Tissint (depleted shergottite) for Li, B, Mg, P, S, Cl, Sc, Mn, Co, Ni, Zn, Br, Y, Zr, Nb, Cs, La, Ce, Eu, Gd, and Ta, and a contamination limit of 0.1% of the average concentration in Tissint for K, Rb, Sr, Nd, Sm, Lu, Hf, W, Re, Os, Pb, Th, and U. Regarding rare-Earth elements (REEs), they are not all included in the list of elements as they follow a predictable pattern. As long as selected elements in LREE (light REE), MREE (medium REE), and HREE (heavy REE) are known, other REEs are deducible. These contamination limits preferably apply to the entire returned sample. However, in cases where this may be deemed unfeasible, a viable alternative would be to ensure that interior portions of the sample can meet the contamination limits. For example, it is common in trace element geochemistry to abrade sample surfaces to reach relatively cleaner inner material. This strategy would also be viable for Mars samples but is relatively wasteful if the abraded/cleaned material is discarded. The proposed limits are in addition to those for the M2020 mission. Thus, for an element that accumulated close to the 1% contamination limit during M2020, and again in the SRF, a total contamination load of close to 2% (of the average concentration in Tissint) is theoretically possible. As discussed above, these are order-of-magnitude estimates of allowable contamination, and we do not believe that 1% is significantly different from 2%.

No contamination limit was previously proposed for some other elements (Na, Al, Si, Ca, Ti, V, Cr, Fe, Cu, Ge, Mo), even though they will be relevant to scientific questions stated among the scientific goals of MSR. For instance, Fe and Mo, as redox-sensitive elements, are useful tools to assess the redox state of the martian environment as emphasized in the iMOST proposed measurements list (Beaty et al., 2019). Aluminum, Fe, Cr, and Ti have been previously considered as "uncontrolled" for engineering reasons, yet it is not obvious to us that abundant use of an element in the rover or SRF *necessarily* means its contamination must be totally uncontrolled. As a concrete example, a contamination limit exists for W even though the drill bits are made of W. Recognizing the inherent difficulties of proposing hard limits on contamination by elements that will be used abundantly, the SCP chose not to recommend formal contamination limits for these elements. Nevertheless, we do strongly recommend limiting contamination by these elements on a "best effort" basis, and that they be explicitly targeted as part of the CK program.

One area of potential concern is whether Tissint (an igneous rock) is a suitable benchmark for contamination in sedimentary rocks and/or regolith, because of their differing compositions. For example, alpha-particle X-ray spectrometer measurements of sedimentary rocks from Gale Crater show MgO concentrations that are 2–5× lower than those in Tissint glass (*e.g.*, Berger et al., 2020; Chennaoui Aoudjehane et al., 2012). Using Tissint-derived contamination limits for such samples might allow for contamination levels that would interfere with measurements for certain elements. Collected solid samples to date include both igneous and sedimentary rocks as well as regolith (*i.e.*, unconsolidated surface sediment), so this is not just a hypothetical possibility. If, in the future, instruments on M2020 indicate that relative concentrations of certain elements are far below those of

Tissint, then our proposed contamination limits may need to be revisited.

5.3. Organic contaminants

Many of the MSR scientific objectives relate to detecting and characterizing potential organic matter hosted within the samples. Because we anticipate that the returned Mars samples will have relatively low organic contents, and that discriminating between martian and terrestrial organic matter will be challenging, limiting organic contamination is essential to confidently identify authentic martian organic molecules. To form quantitative limits for organic contamination, SCP considered the previous recommendations for M2020 (Summons et al., 2014), the concentrations of organic contamination that M2020 achieved for MSR-relevant components (*e.g.*, sample tubes and seals; Boeder and Soares, 2020), and updated our understanding of the inventory of organic molecules in martian materials (Eigenbrode et al., 2018; Freissinet et al., 2015; Millan et al., 2021). Our resulting recommendations for organic contamination limits have two components, namely, *which* organics to monitor and limit, and *how much* of each compound to permit. We address these two components in order.

5.3.1. Which contaminants to limit?

A principal challenge in organic CC is that there are millions of distinct compounds, and we potentially care about all of them at some level in martian rocks, yet it is physically impossible to monitor all of them simultaneously. The SCP considered several alternative solutions to this dilemma but ultimately decided to adopt the same strategy as the OCP (Summons et al., 2014) of using two "tiers" of priority. Accordingly, Tier 1 compounds represent a relatively small list of representative high-priority analytes that should be explicitly monitored. Following Summons et al. (2014), these include biomolecules used by terrestrial life, molecular remnants of ancient terrestrial life (molecular fossils or organic biomarkers), and molecules that have been detected in meteorites, in martian materials, or may be relevant to prebiotic chemistry. This approach of using selected compounds as representative of entire classes of concern is analogous to the use of "indicator species" in ecological studies or "indicator compounds" in environmental pollution monitoring (Siddig et al., 2016; Zhang et al., 2021). Tier 2 then encompasses all other organic compounds not specified within Tier 1, with the expectation that they would *not* be explicitly monitored. Instead, the SRP should use other indirect approaches to establish the likelihood of meeting Tier 2 limits, such as the absence of significant unknown peaks in multiple analytical methods. TOC is operationally defined; we take as our definition the carbon that is liberated by high-temperature combustion in O_2, exclusive of carbonate minerals. This definition effectively includes all compounds in both Tier 1 and 2.

In addition to this previously established structure, we recommend dividing the contaminants into two categories based on vapor pressure: volatile and nonvolatile (or "semi-volatile"). We suggest a boiling point of 200°C at 1 atm pressure as a convenient demarcation between the two; this would include compounds lighter than C_{12} *n*-alkane in the volatile category. Alternatively, the EU definition of vapor pressure >0.01 kPa at 293.15 K could be used and results in a similar division of compounds. Our recommendation arises from the knowledge that nonvolatile analytes must be extracted with organic solvents, which are themselves volatile organic compounds (see Section 1.3.3). Although it might be possible to do all of the extractions with inorganic solvents (*e.g.*, supercritical CO_2), the use of organic solvents for extraction is so deeply ingrained that this potentially risks the credibility of the analyses, so we think it both simpler and safer to just separate the two. In our proposed scheme, samples would have to meet *both* categories of requirements during sample storage and the upstream stages of handling and workup, until the point where subsamples are separated and dedicated to specific (volatile or nonvolatile) measurements. After that branch in the workflow, subsamples would only have to meet the requirements for their analytical category. We believe this proposed categorization of organic contamination will provide important flexibility for their implementation within the SRF compared with a single umbrella of organic contamination limits. And, if it later proves to be unnecessary, it would be trivial to revert back into a single category.

Table 4 presents our list of recommended Tier 1a (volatile) compounds. The list includes low molecular weight alkanes (C_5–C_{11} *n*-alkanes) and aromatic compounds (BTEX [benzene, toluene, ethylbenzene, xylene]) that are major constituents of petroleum-derived products and thus common contaminants; a number of common laboratory and industrial solvents (methanol, ethanol, acetone, acetonitrile, dichloromethane, dichloroethane); volatile chlorinated (chlorobenzene and

Table 4. Proposed Tier 1a, Volatile Compounds of Concern[a]

Compound(s)	*Rationale*
C_5–C_{11} *n*-alkanes	Common terrestrial contaminants, petroleum constituents, and solvents
Benzene, toluene, ethylbenzene, xylene*	Common petroleum constituents and solvents
Methanol, ethanol, acetone, acetonitrile	Common solvents
Dichloromethane, dichloroethene[b]	Chlorinated solvents, possible analytes of interest in samples
Chlorobenzene, dichlorobenzene[b]	Chlorinated organics previously detected on Mars
Dimethylsulfide, thiophene, methanethiol, ethanethiol	Organosulfur compounds, possible analytes of interest
Acetic acid, pyruvic acid	Important biomolecules
Formaldehyde, acetaldehyde	Common in building materials, possible analytes of interest

[a]These are the "indicator" compounds we have chosen to be explicitly monitored, but we emphasize that they are representatives of entire compound classes of potential concern. Any evidence suggesting that other related compounds are present as contaminants should lead to them being directly addressed.

[b]Multiple isomers exist; treat limit as the sum of isomers.

dichlorobenzene), and sulfur-containing (dimethyl sulfide, thiophene, methanethiol, ethanethiol) compounds that have been previously detected on Mars or are of particular interest given the detection of related compounds; two ubiquitous biomolecules, acetic acid and pyruvic acid; and low molecular weight aldehydes (formaldehyde and acetaldehyde) that are common in building materials but will be of interest from an origins-of-life perspective in returned samples. All, or nearly all, of these compounds should be detectable in a single GC-MS analysis. Additional volatile compounds of concern not listed in Tier 1a could (and should) be explicitly monitored if they are discovered to be present during construction of the SRF. The SRP should also continue to monitor recent results from the SAM instrument on Curiosity, and if new organic analytes are detected in the future, should consider whether to add them as new Tier 1 compounds. We note that not *every* organic compound detected on Mars need necessarily become a Tier 1 contaminant. However, justification for doing so could include: (i) different functionality or chemical properties than are currently captured by our Tier 1 list, (ii) compounds of particular interest with respect to possible biotic origins, or (iii) compounds at particular risk of contamination, for example, because of possible use in building or cleaning materials, etc. For compounds with multiple isomers (*e.g.*, xylene, dichlorobenzene, dichloroethene), we recommend treating the limit as applying to the sum of all isomers rather than tracking individual isomers separately.

Virtually any organic solvent used to clean the SRF would be a potential contaminant of concern. Even those that are unquestionably of human origin (*e.g.*, fluorocarbons) pose a risk of interfering with other analytes of interest. The SCP carefully considered whether to propose a much longer list of solvents for Tier 1a but decided against this to preserve flexibility for the SRP. Some solvent(s) will inevitably be needed for cleaning, and any detection of it in samples thereafter will be assumed to be contamination. The choice of which solvent(s) to use, therefore, requires further study. We recommend that the solvent(s) *not* contain any chlorine, given the interest in chlorinated organic molecules on Mars, and that as few different solvents as possible be used. IPA has commonly been used in this role; though we are not opposed to its use in the SRF, there was concern that it has not been shown to be an effective solvent for large, nonpolar (*e.g.*, sterane or PAHs) molecules, so a second, apolar solvent may be needed.

Table 5 presents the list of proposed Tier 1b (semi-volatile) compounds. The list is similar to that proposed by Summons et al. (2014), with several alterations motivated by recently available data. The previous list included only a single *n*-alkane; we recommend including all of the C_{12}–C_{30} alkanes. The representation of hydrocarbons was expanded in both Tier 1a and Tier 1b categories because of their importance as organic biosignatures in ancient rocks on Earth and their vulnerability to contamination through pervasive use of petroleum-based products (French et al., 2015). They can all be detected in the same GC-MS analysis, so this should not entail much extra analytical effort. Pristane and naphthalene (two-ring PAH), common petroleum hydrocarbons, are retained from the prior list, and we recommend adding phenanthrene, a three-ring PAH, to monitor a wider range of PAHs. This compound class has been detected in meteorites and martian materials and is common in petroleum- and combustion-products (Clemett et al., 1998; Lima et al., 2005; Steele et al., 2012). The common lipids palmitic acid and squalene are retained, with the addition of cholesterol (an important human sterol) in recognition of the increased human contact potential within the SRF. We suggest adding two organosulfur compounds (benzothiophene and dibenzothiophene) as well as benzoic acid, benzonitrile, and oxalic acid, all of which have been recently detected in martian samples and are susceptible to contamination from sources such as petroleum-based products or solvents (Eigenbrode et al., 2018; Freissinet et al., 2015; Millan et al., 2021). We retain several important biological compounds: amino acids (glycine, alanine), components of cell walls or spores (*N*-acetylglucosamine and dipicolinic acid, respectively), nucleic acid (DNA), a nitrogenous compound and prebiotic precursor (urea), and a polyhydroxy compound (glycerol).

The list of Tier 2 compounds includes everything that is not included in Tier 1a or 1b. It can be divided into volatile (Tier 2a) and semi-volatile (Tier 2b) categories as for Tier 1, with the caveat that not all possible compounds' boiling points are known. Retention time on an apolar GC column (*i.e.*, before or after *n*-C_{12}) would serve as a suitable proxy for boiling point. A more consequential challenge regards how to prove that there are no organics of any type above a certain quantitative threshold. The M2020 mission was able to use TOC measurements for this purpose (Section 3.2). By showing that TOC was near the Tier 2 limit, they were able to argue that no individual compound could be above the

Table 5. Proposed Tier 1b, Semi-Volatile Compounds of Concern[a]

Compound(s)	*Rationale*
C_{12}–C_{30} *n*-alkanes	Common terrestrial contaminants, petroleum constituents
Pristane	Important hydrocarbon biomarker
Naphthalene, phenanthrene	PAHs, common combustion products and meteorite constituents
Palmitic acid, squalene, cholesterol	Common bio-lipids
Benzothiophene, dibenzothiophene	Petroleum constituents, analytes of interest
Benzoic acid, benzonitrile, oxalic acid	Previously detected on Mars
Alanine, glycine, *N*-acetylglucosamine, dipicolinic acid, DNA, urea, glycerol	Common biomolecules

[a]These are the "indicator" compounds that we have chosen to be explicitly monitored, but we emphasize that they are representatives of entire compound classes of potential concern. Any evidence suggesting that other related compounds are present as contaminants should lead to them being directly addressed.

Table 6. Biomolecules Included by OCP as Tier 1 Contaminants and Which Are Common in Most Forms of Terrestrial Life

Contaminant class	*Chemistry*	*Cellular component*
Nucleic acid	DNA	Information storage polymer for all terrestrial life[a]
Spores	Dipicolinic acid	Primary polymer for bacterial spore coat formation
Cell walls	*N*-acetylglucosamine	Primary monomer in bacterial peptidoglycan and fungal cell walls
Amino acids	Glycine	While there are many amino acids used by terrestrial life, glycine is the simplest stable amino acid found in proteins
	Leucine	Leucine is typically the most abundant amino acid found in meteorites and in terrestrial proteins (Fountoulakis and Lahm, 1998)
Lipids	Palmitic acid	Common fatty acid in bacteria and eukaryotic cells
	Squalene	Important in formation of lipids, found in human skin
Short-chain carboxylic acids	Acetic acid	Important molecule in central metabolism, lipid, and amino acid anabolism, and a fermentation product
Polyhydroxy compound	Glycerol	Primary structural component of lipids
Hydroxy carboxylic acid	Pyruvic acid	Primary metabolite of central metabolism

[a]There are also DNA-based and RNA-based viruses; however, viruses are cellular parasites that require a living cell to provide the energetic processes and synthesis pathways to allow replication, and while a virus's dependence on a DNA-based host for replication places these entities as nonliving matter, they are still a potential source of biological contamination.

limit. However, without the ability to "bake" the entire SRF to get rid of cleaning solvents, it may not be possible to achieve very low TOC measurements (see below), and thus the same type of argument may not be viable. We do not have any specific recommendation for how to achieve proof of Tier 2 limits and acknowledge that it could be challenging to meet. A possible (less conservative) alternative could be to formulate the requirement as "no analytes detectable by a combination of specified analytical techniques," such as GC-MS, LC-MS, and FTIR. This would require further evaluation to pick the right combination of techniques and still leaves some ambiguity about how such analyses should be conducted to prove that there is nothing detectable.

We recommend retaining the concept of TOC contamination for archived samples and for subsamples subjected to VOC analysis. Without solvents in use, it can be implemented in the same way as for the M2020 sample handling hardware. For subsamples subjected to solvent extraction, placing limits on TOC contamination no longer makes sense; they have literally been immersed in an organic liquid. We, therefore, recommend modifying the requirements to target "solvent-extractable, nonvolatile organic carbon" and "non-extractable, nonvolatile organic carbon." The former is effectively an extraction blank and can be measured using standard solvent extraction techniques, with the solvent (and any volatile analytes) removed by evaporation and the residue burned or measured by FTIR, etc. The latter type of TOC limit is intended for pyrolysis-based analyses, NMR, and other methods that characterize kerogen-associated organic matter. In effect, this limit would represent everything remaining in a blank (or sample) after the solvent-extractable fraction is removed. This residual organic carbon is analogous to a kerogen blank, and it could contribute contamination-derived products to subsequent pyrolysis experiments. We use the term "kerogen" here to denote any non-extractable, non-hydrolyzable organic material regardless of origin or composition.

5.3.2. How much of these contaminants to allow?. The SCP took as a starting point for our discussion the original quantitative limits proposed by the OCP (Summons et al., 2014) and the achieved Mars 2020 cleanliness of the SIH (Boeder and Soares, 2020). Those limits were well above state-of-the-art instrument detection limits at the time, because it was deemed technically infeasible to reduce contamination to below detection limits. Instrument detection limits have only improved in the ensuing nine years, and thus we conclude that this would still be an infeasible basis for establishing contamination limits. The OCP limits were instead obtained by estimating the analyte concentrations that might be plausibly observed in the samples given the best available data at the time and setting contamination levels roughly 100-fold lower. Here we have followed that same approach, updating expectations of analyte concentrations based on more recent Mars-based data. That resulted in us recommending identical limits for volatile components but 10-fold lower limits for semi-volatile compounds.

OCP adopted a quantitative limit of 1 ng/g (1 ppb) of each Tier 1 compound based on measured concentrations in meteorites and martian materials and the detection limits for survey-type analyses that have a broad analytical window rather than more targeted, sensitive analytical methods with lower detection limits (Summons et al., 2014). The Tier 2 limit was set at a 10-fold higher level of 10 ng/g (10 ppb) relative to the Tier 1 compound limits. OCP set a TOC limit of 40 ng/g (40 ppb) based on reported TOC values in martian materials, detection limits for measuring TOC, and to ensure an additional protective cap on molecular contamination without directly monitoring all compounds (Summons et al., 2014).

Mars 2020 met these requirements recommended by OCP, so a conservative assumption would be that the returned samples would already contain organic contamination at the level of the M2020 contamination requirements by the time they arrive at the SRF. However, Mars 2020 achieved contamination cleanliness levels that were significantly lower than the required limits recommended by OCP. Specifically, the Tier 1 compounds were below detection on the interior surfaces of the sample tubes and seals (Boeder and Soares, 2020). Thus, the returned samples could contain even lower levels of organic contamination than the conservative assumption. This success presumably reflects the greater ease of limiting

recontamination by nonvolatile organics that do not travel in the gas phase. Based on the reported analytical detection limits of the methods (Maltais et al., 2023, Supp table E-1) used to monitor and quantify organic contamination for the Mars 2020 SIH, we estimate that the returned samples likely contain Tier 1 compound concentrations that are at least an order of magnitude lower than the Mars 2020 requirements at the time of their arrival at the SRF.

Based on these considerations, the SCP recommends the same quantitative limits that OCP proposed for the volatile organics category (Tier 1a: <1 ng/g [1 ppb], Tier 2a: <10 ng/g [10 ppb], and TOC: <40 ng/g [40 ppb]). For the semi-volatile components, recent martian data suggest that analyte concentrations may be lower than was previously expected; and as discussed above, the data indicates that the rover SIH was successfully cleaned to 0.1 ppb or better. Accordingly, the SCP recommends a semi-volatile limit for Tier 1b compounds of <0.1 ng/g [0.1 ppb], an order of magnitude lower than previous. The recommended Tier 2b limit is still set at 10× higher than Tier 1b compounds (Tier 2b: <1 ng/g [1 ppb]), consistent with the previous quantitative relationship between Tier 1 and Tier 2 recommended by OCP. We further recommend a limit on solvent-extractable, nonvolatile organic carbon (aka "bitumen") of <10 ng/g [10 ppb] for the semi-volatile category. This is 10-fold higher than the Tier 2b limit. We considered proposing the same fourfold ratio as between TOC and Tier 2a (volatiles) but decided that was unnecessarily strict given that the extractable, semi-volatile blank is quite likely to be divided among dozens, if not hundreds, of trace compounds, none of which would exceed the Tier 2b limit.

We recommend a limit of <100 ng/g [100 ppb] for nonextractable, nonvolatile organic carbon (aka "kerogen") based on recent estimates of kerogen content in martian samples. These estimates range from ~1 to 500 mg organic carbon/g (Stern et al., 2022 and included references), such that our proposal of 100 ng/g [100 ppb] is on the order of 1% of the expected kerogen concentrations in returned samples containing low to moderate organic concentrations (*e.g.*, ~10 mg organic carbon/g), assuming that most of the organic carbon is in the form of kerogen.

5.4. Biological contaminants

The original OCP was tasked with recommending separate contamination limits for organics and living cells but chose not to separate them. In large part, this was because it was "*. . .not currently possible to reliably and easily distinguish organics in living and non-living matter using molecular methods*" (Summons et al., 2014) and the fact that the most sensitive assays for cells of any type involve the detection of organic biomolecules. Implicit in this argument was the assumption that any viable cells present on the sampling hardware would be very unlikely to multiply, because the rover and sampling hardware would be in very cold environments. The M2020 project further implemented a sterilization procedure that involved DHMR to yield a predicted <1 viable cell per tube, satisfying Planetary Protection as well as contamination concerns.

Compared with M2020, the situation for the SRF is markedly different. Preventing contamination within the SRF requires the handling, separation, and analysis of returned samples within an environment that must be protected against an enormous background of terrestrial organic carbon (Tait et al., 2022) and cannot be completely sterile with humans working in it (Sections 1.3.5 and 4.3). In returned martian samples, our ability to distinguish terrestrial-based biological contamination from indigenous material conclusively is also of the highest priority, given that such findings are critical to our ability to determine the likelihood of past or present life on Mars, as well as directly tied to PP objectives (Clark et al., 2021; Kminek et al., 2022a). Growth of terrestrial microbes on the samples within the SRF poses a potentially dire risk to both objectives through the presence of cells, production of biomarkers, and transformation of the chemical nature or structure of returned samples within a relatively short time. Thus, the recommendation of this committee is that contamination by viable organisms must be explicitly and seriously considered, and we recommend a multilayered approach. First, we recommend retaining all the previous Tier 1 biomolecules, most of which now have lower (<0.1 ppb) limits. Second, we recommend implementing new, highly sensitive assays for three biomolecules (lipopolysaccharide [LPS], adenosine triphosphate [ATP], and β-glucan) that can be detected at sub-parts-per-trillion (<1 ppt) levels. Third, we recommend working under at least ISO-3/AMC-9 conditions with a contamination monitoring program. Such a program should include standard culturability assays that, despite their many limitations, provide an important baseline for viable cellular contamination given the presence of humans (and their microbiomes) in the SRF, as detailed in Section 4.3. Fourth, we recommend maintaining sample storage and laboratory conditions that are inhospitable to growth, as detailed in Section 1.3.5. Notably, we have not proposed specific DNA- or RNA-sequencing-based limits, though they will be invaluable for CK; the rationale for this decision is presented in Section 5.4.2. Biological contamination recommendations are detailed below.

5.4.1. Biomolecules covered under Tier 1a and 1b limits. All the biomolecules originally proposed by the OCP (Table 6) remain on our list of recommended Tier 1 contaminants. Most of them fall within the "nonvolatile" category and thus have new, lower limits of <0.1 ng/g in the sample. Our expectation is that transfer of these components will primarily be in the particulate phase as cells or cellular debris, and thus they remain an effective means of monitoring contamination by materials that could confound our search for martian life. Although there are other biomolecules that can now be monitored with higher sensitivity, we recommend retaining all the compounds listed by the OCP as part of the CK program. As these compounds have been explicitly monitored throughout the entire MSR campaign, they would offer us the best opportunity at understanding the origins of any potential future contamination.

Two of the compounds—acetic acid and pyruvic acid—appear on the Tier 1a volatiles list and are thus subject to higher <1.0 ppb limits. There is some ambiguity here because they could also be described in their anionic form (acetate ion, pyruvate ion) and would then be nonvolatile and subject to lower (<0.1 ppb) limits. The acidic form is more relevant at low pH (< ~4.5), whereas the anionic form is more relevant at circum-neutral pH, including within cells. We recommend retaining the acidic form on the Tier 1a list

for three reasons: (i) consistency with the prior OCP approach, (ii) because of relatively high detection limits for these compounds, we do not know whether the M2020 sampling hardware was cleaned to <0.1 ppb levels, and (iii) because of concern about the possible transport within the SRF of either acetic acid or pyruvic acid *as a volatile*.

5.4.2. Special considerations for DNA. In addition to the Tier 1 status of DNA, the panel strongly recommends the implementation of an active environmental DNA monitoring program that tracks the *presence* of nucleic acids, along with their sequence and identity, to help identify the source of the contamination of these DNA sequences (*e.g.*, human microbiome, fungi, and skin cells). When changes to the SRF DNA baseline are observed, we recommend a cessation of activities followed by the identification and remediation of contamination sources. These recommendations correspond to a "no change in the SRF DNA baseline" contamination limit.

5.4.3. Special category biological contaminants. In considering whether certain materials should be subjected to lower detection thresholds for the Special category contaminants, the committee considered that such analyses must be rapid, reasonably affordable, and viable within the limitations of SRF containment (Tait et al., 2022). To address this, we looked to solutions used within the pharmaceutical industry, where biological contamination of cleanroom facilities can lead to harmful and dangerous consequences. Here, a number of rapid, signal-enhancing methods that rely on the bio-active properties of certain organic compounds for detection have been developed. We, therefore, propose the addition of a third category of (organic) contamination limits for these bio-active compounds: LPS, β-glucan, and ATP, all at <1 pg/g [<1 ppt] in sample.

5.4.3.1. Lipopolysaccharide. LPS is a polymer that plays an important role in stabilizing the outer membrane of Gram-negative bacteria (GNB). Given that life-threatening infections are caused by GNB, it is no surprise that the human immune system has evolved to recognize LPS as a marker for infection, even at single-molecule concentrations to activate a nonspecific inflammatory response (Guha and Mackman, 2001). The response to LPS is conserved across many animal species, including amoebocytes from the horseshoe crab (*Limulus polyphemus*). Clotting upon exposure to LPS in these amoebocytes is the basis for the amoebocyte lysate (LAL) assay, which can detect LPS at sub-pg/mL levels (Levin et al., 1970).[2] Notably, gram-positive bacteria (GPB) lack LPS and are, therefore, not detected through the LAL assay. Teichoic acids (TA), on the other hand, are found in the cell wall of GPB and can account for up to 50% of a GPB cell's dry weight (Dos Santos Morais et al., 2022), which makes it an attractive compound for GPB contamination monitoring. Quantification of cell wall TA is typically performed by using polyacrylamide gel electrophoresis (Pollack and Neuhaus, 1994); however, the biological contamination subgroup was unable to identify a rapid and commercially available TA assay and, therefore, decided not to recommend a specific TA contamination limit. Based on these findings, we recommend that GNB LPS contamination is actively monitored using the LAL assay and that GPB contamination is monitored using the results of DNA-based CK programs and the ATP assays described below. If a commercial TA assay becomes available in the near future, this would likely be a preferable alternative.

5.4.3. 2. β-glucan. Fungi have been identified as a major biological contaminant in JSC clean rooms (Regberg et al., 2018). The presence of fungal cells can confound organic analyses and has the potential to alter martian samples. Therefore, a stringent fungal contamination monitoring program is strongly recommended. β-glucan is a polysaccharide that is found in the cell walls of fungi, cereals, and bacteria and can be detected at pg/mL concentrations using a modified LAL assay (Obayashi et al., 1995).

5.4.3.3. Adenosine triphosphate. ATP is the energy currency of all terrestrial cells and can be used as an indicator of biological cleanliness and *active* cells. Preventing microbial activity should be an essential priority of the SRF CC program, as active organisms can dramatically transform the chemical nature or structure of returned samples within a relatively short time. Bioluminescent ATP assays utilize the enzyme luciferase to catalyze an ATP-dependent reaction and produce light. These assays can detect ATP at sub-pg/mL concentrations and are beginning to be used to monitor hospital cleanliness due to their sensitivity, ease of use, and speed (van Arkel et al., 2021). Additionally, the cultivation-free nature of ATP assays enables users to monitor a much wider range of active and viable cells than standard cultivation assays.

5.4.4. Culturability. While the methods described above are highly sensitive to the presence of detectable cellular materials, cultivation remains one of the most established ways to identify the presence of certain microorganisms in the environment and is capable of detecting a single viable cell under appropriate conditions (Austin, 2017; Houpikian and Raoult, 2002). This committee, therefore, recommends that the protocols employed include microbial assays for culturable cells on surfaces and air within the SRF (as described in Sections 4.3 and 6.3.2), with a specific limit of <1 culturable cell per sample. Nonetheless, as most environmental microorganisms cannot be cultivated, cultivation assays can only assess the presence of a subset of contaminating microorganisms within the SRF environment (Fisher et al., 2017; Kehe et al., 2021; Oliver, 2005; Pace, 1997; Stewart, 2012). The committee, therefore, recommends using both cultivation-dependent and cultivation-independent methods to monitor biological contamination and activity, further described in Sections 4.3.2 and 6.3.

6. Strategies for Detecting and Characterizing Contamination (Under Containment)

6.1. Inorganics

The returned samples will be of geological nature: they consist of drill cores of rocks and minerals that formed in igneous or sedimentary settings on Mars, and loose regolith samples (NASA, 2023). With respect to detecting and

[2]Gram-positive bacteria (GPB) release components of peptidoglycan, which can similarly activate a cellular response (IL-12 response); however, this has not been developed into a common detection method for cleanliness (Hessle et al., 2005).

characterizing inorganic contamination, it is thus straightforward to turn to the best practices of sample curation and CC/CK of previous geologic sample return missions. These are, in particular, the procedures developed for the curation of the ~380 kg of lunar material brought back during the Apollo era (McCubbin et al., 2019), as well as the procedures established for the curation and sampling of the ~5 g sample returned from asteroid Ryugu in the Hayabusa2 mission (Abe, 2021), and the 120 g sample of asteroid Bennu brought back by OSIRIS-REx in 2023 (Lauretta et al., 2024).

All these samples are (or will be) handled and stored in specialized curation facilities with ISO-certified controlled environment (temperature, pressure, humidity) and cleanliness requirements (level of airborne particulates, specific building materials, sterilization protocols). To assess and characterize potential inorganic contamination, CC/CK requires that all materials in contact with the samples are monitored, analyzed, documented, and archived for future reference. This includes the sample tubes, parts of the sampling device, and the witness plates and tubes employed during spacecraft construction and sampling on Mars, as well as any lab-based sample manipulation tools, lab construction materials, air filters, glove boxes, desiccators, and airborne molecular and particulate contaminants regularly collected within them. Sampling and analysis of airborne molecular and particulate contaminants follow protocols developed for the semiconductor industry (ISO 14644-8 and ISO 14644-10). Other materials may be analyzed (after sterilization, if needed) by laser ablation inductively coupled plasma mass spectrometry, electron microprobe analysis, and scanning electron microscope/energy dispersive X-ray for their inorganic elemental (and isotopic) composition.

6.2. Organic compounds

The detection of organic compounds indigenous to returned martian samples will be of critical importance in the search for evidence of ancient life and will require highly sensitive and selective analytical techniques and relevant controls to constrain residual contamination in the samples and possible artifacts introduced during the mission (Summons et al., 2014). The most effective means to ensure that Earth-sourced contamination can be convincingly distinguished from martian material in the MSR samples are: (i) limit terrestrial contamination through a stringent program of CC, especially of materials and molecular species of astrobiological interest, as detailed in Section 5.3; and (ii) implement a comprehensive CK program that makes it possible to characterize terrestrial contamination sources throughout the various mission phases of the MSR campaign (Fig. 4).

The interior surfaces of the M2020 sample tubes and seals are the most contamination-sensitive elements of the mission and were cleaned using a multistep, high-precision solvent (sonication in isopropanol and acetone) rinsing process and 150°C for 24 h vacuum bakeout (Boeder and Soares, 2020). After cleaning, the sample tubes were protected from particulate contamination (<0.15 mm dia.) by the fluid mechanical particle barrier until their use on Mars. These SIH CC measures were necessary to meet the key biological (each tube has a >99.9% probability of being free of any viable Earth-source organism) and organic cleanliness (each sample has <10 ppb TOC and <1 ppb of any Tier 1 compound) requirements (Boeder and Soares, 2020). Analyses of the final solvent rinses of the M2020 SIH using FTIR spectroscopy and GC-MS techniques did not identify any Tier 1 compounds above the analytical detection limits of the instruments. Based on experimental test results and molecular transport modeling, the final organic cleanliness of a returned 15 g rock core was also estimated to be 8.1 ppb TOC and 0.7 ppb of Tier 1 compounds (Boeder and Soares, 2020).

To track potential contamination exposure of the sample tubes post-launch, the M2020 rover carries five flight witness tube assemblies (WTAs) with a TiN mesh particle trap containing an assembly of 12 polished gold witnesses and 2 aluminum getter foils, designed to passively capture vapor-deposited and particulate contaminants (Moeller et al., 2021). One of these flight witnesses, called the bit carousel WTA (hereafter WB1), was activated by puncturing a hermetically sealed foil on Earth during ATLO prior to placing the WTA in the bit carousel and then subsequently sealed after landing on Mars on Sol 120 to provide CK during the early phases of the mission. The remaining four ordinary WTAs (WB2, WB3, WB4, and WB5) are all identical and will be exposed and sealed periodically during the surface mission to capture any evolution of the contamination environment (Moeller et al., 2021). WB2 was activated and sealed on Sol 499, and WB3 was activated and sealed on Sol 586. Based on a review of the CK strategy by the MCSG and M2020 project science, and community input received during the Mars 2020/ MSR Sample Depot Science Community Workshop held in September 2022, a recommendation was made to place WB3 in the first depot, "Three Forks," and retain all other WTAs in the Rover Cache (Czaja et al., 2023). The Perseverance rover also has a second type of control sample, which is externally accessible to the drilling mechanism on the Rover in the form of the Drillable Blank Assembly (DBA). The main benefit of the DBA is to witness any sample contamination associated with the coring process. The one-time-use DBA consists of a baked SiO_2 ceramic brick (FS-120) that is hermetically sealed inside a canister with a titanium foil top. The corer can puncture through the foil and collect a sample of the FS-120 material inside using the same methods as acquiring a martian sample. In addition to the M2020 flight spare sample tubes and other hardware, several flight spare DBAs were also archived and will be available for future CK testing.

To ensure that the scientific integrity of the returned samples is maintained throughout curation and sample allocation, planning for the SRF must include a rigorous CC/CK program. In addition to the flight witness materials that would be available for investigation (including the round-trip OS flight witnesses and an M2020 WTA and/or DBA), additional controls would be needed to document potential sample contamination inside the SRF during sample processing. These include not only witness plates but also trapping of VOCs from facility air using adsorbents, swabs of surfaces, and used air filters. Sampling within the SRF should be deployed at regular time intervals to document any changes in the contamination environment, and with a variety of analytical techniques to detect potential contaminants beyond those listed as explicit Tier 1 compounds (*e.g.*, Dworkin et al., 2018; Maltais et al., 2023). Multiple techniques are

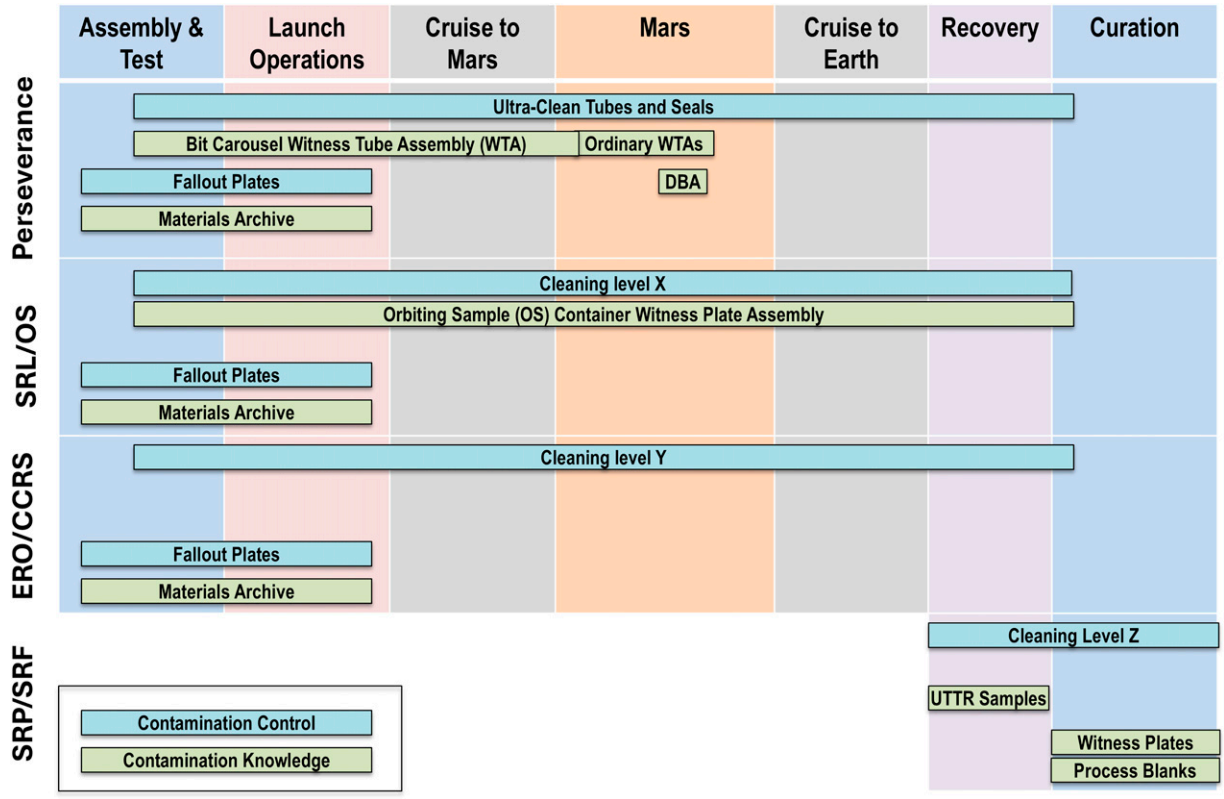

FIG. 4. Contamination control and contamination knowledge are required for all phases of the Mars Sample Return campaign to preserve the scientific integrity of the samples and reconstruct the contamination and alteration history of the samples throughout the course of the mission.

required to identify a range of organic compound classes with different molecular weights and volatilities, and with appropriate detection limits depending on the amount of surface area available for analysis (Summons et al., 2014). Special attention is also required for the preservation of instrumental data from this CK monitoring plan; simple tables of abundance are not sufficient. Future scientists will very likely want access to raw chromatograms, spectra, tuning and calibration files, etc.

A further challenge regards using the measured concentration data from air filters or witness plates to determine a contamination level in a solid sample. This requires knowledge of volatile or particulate adsorption efficiency differences between the witness plate substrate and the solid sample, and other variables. Given these uncertainties, the organic abundance results from witness plates are often used qualitatively rather than quantitatively; nevertheless, they are critical for documenting which compounds *could* represent contamination. Although perfect witness materials for the returned samples do not exist, additional procedural controls will be needed in the SRF to accurately track and measure organic contamination exposure of the returned samples during processing. Ideally, an organically clean and simple synthetic material (*e.g.*, the DBA flight spare FS-120) could be collected and sealed using an M2020 flight-like corer and adaptive caching assembly (ACA). The sealed FS-120 core could then be processed using the same steps as a martian core sample, from headspace gas extraction, tube opening, core extraction, and preliminary examination. Portions of the FS-120 core could then be analyzed directly or solvent extracted, and the extracted residues analyzed for organic contamination using a variety of instruments available in the SRF. The organic concentrations in the processed and exposed FS-120 core could then be determined from the mass of the extracted sample (*e.g.*, ppm or ppb). In addition to providing valuable information about potential contamination during the martian sample core extraction and processing, the results from this process blank would also provide a valuable procedural control for the analyses that will be included in the Sample Safety Assessment (Kminek et al., 2022a). Potential cross-contamination between a martian sample and the process blank could be mitigated by processing the samples in different lines and/or cleaning the isolators between samples and controls.

6.3. Terrestrial cells and molecules of biological origin

Detection methods for biological contamination generally fall within three classes: (1) visualization of intact cells and/or cellular components (2) growth of organisms and cellular activity assays, (3) detection of biomolecules.

6.3.1. Visualization of intact cells and/or cellular components. The field of microbiology began after the invention of the microscope, and microscopy continues to be an essential tool in the study of microorganisms today. Currently, there are several different kinds of microscopic techniques to study intact cells or even cellular components. First and foremost, light and fluorescence microscopy,

which enable differentiation of living and dead microbial cells in an environmental sample (Boulos et al., 1999), have generally acquired much popularity in environmental microbiology (Emerson et al., 2017). At the same time, fluorescence *in situ* hybridization methods can link cellular activity with taxonomic information (Amann and Fuchs, 2008; Pernthaler et al., 2001) and are used to determine many different aspects of microbial communities, from tagging rRNA (Amann et al., 1990), mRNA (Pernthaler and Amann, 2004), and even specific genomic regions of microorganisms or viruses (Barrero-Canosa et al., 2017). On the other hand, (ultra)structural information of microorganisms is usually achieved from different types of electron microscopy (Rachel et al., 2010); however, light microscopy and electron microscopy both necessitate a certain concentration of biological entities in the sample or extended periods of scanning the sample area. In many laboratory and environmental settings, microscopy-suitable concentrations of cells are easily achieved; however, some natural ecosystems are naturally low in biomass, for example, drinking water or desert soil. If the sample matrix is water, microorganisms can be concentrated by using a variety of different filtering techniques to facilitate light and electron microscopy. Water samples are additionally amenable to particle counting techniques such as flow cytometry and biofluorescent particle counters (*e.g.*, Ichijo et al., 2022), although autofluorescent minerals and nonspecific staining can result in overestimates of biomass (Veal et al., 2000). Other matrices like soil, sediment, or sludge need specific preparation protocols even if the biomass is high, to avoid interference of components of the matrix with the imaging technique or flow cytometry for cell enumeration (Kallmeyer et al., 2008; Morono et al., 2013). Hence, light microscopy techniques have so far only been applied as qualitative measures for bioburden monitoring in cleanrooms (Moissl et al., 2008) and have not achieved a level at which quantitative measurements of such environments are possible. On the other hand, particle counters have been used to monitor particle loads in cleanrooms and estimate the particle load within potable water on the ISS (*e.g.*, Behrens et al., 2022; Comecer, 2021; Ichijo et al., 2022); however, SRF-specific sample preparation and monitoring protocols using this approach would require development and validation.

Additional technical hurdles will need to be addressed if biological contamination monitoring requires the identification of contaminant terrestrial cells within martian soil/sediment samples due to the (presumably) low number of contaminant cells introduced in the SRF and the interference of the martian sample mineralogical matrix. Rather than traditional microscopy, advanced techniques that supplement microscopy methods with the detection of chemical signatures and biomolecules can improve the biological signal-to-noise within complex samples. Two enabling and minimally destructive technologies are FTIR spectromicroscopy (Wenning and Scherer, 2013) and Raman spectromicroscopy/microspectroscopy (Jarvis and Goodacre, 2004). While Raman spectromicroscopy can be applied to samples in aqueous solutions, the high infrared activity of water molecules usually requires desiccation of samples for FTIR spectromicroscopy. In contrast, the high laser frequency of Raman spectromicroscopy can result in the destruction of biological material, while FTIR does not damage the material in this way (Holman et al., 2009). Based on the large screening area, its high sensitivity, and enabling downstream analyses via its nondestructive nature, FTIR microspectroscopy bears a great potential for detection of cells, cellular material, and biomolecules in returned martian samples and, with the aforementioned microscopy caveats in mind, can additionally be used in SRF biological contamination monitoring.

6.3.2. Growth of organisms and cellular activity assays. Cultivation of microorganisms for bioburden monitoring has been the gold standard of Planetary Protection since the advent of Mars exploration during the Viking mission (Moissl-Eichinger et al., 2012; Venkateswaran et al., 2014). A significant focus of this work is toward spore-forming microorganisms that are resistant to high temperatures and can be identified via a heat-shock selection process followed by cultivation on plates, commonly referred to as the "spore assay." This screening has been performed for all NASA's and ESA's missions that are destined to Mars, enabling thorough comparability of the cleanliness of spacecraft and the creation of a rich database. The application of selective media to enrich for extremophiles or anaerobes (La Duc et al., 2007; Probst et al., 2010; Stieglmeier et al., 2009) has additionally led to the cultivation of microorganisms usually not captured via the standard spore assay and further broadens the diversity of cultivable microorganisms from highly contamination-controlled cleanroom environments where spacecraft are built.

Transferring the standard spore assay, defined media, and the existing knowledge of known cleanroom strains to CC of samples retrieved from Mars would enable an easily executable bioburden measure. Such a measure would be highly sensitive for living microorganisms that can grow under the defined conditions, since a single viable cell can be sufficient for successful cultivation. At the same time, taxonomically identified isolates can be compared against other isolates from previous space missions to identify bioburden patterns as a function of the activity of engineers and scientists. Nonetheless, as previously discussed (Section 4.3.2), the majority of microorganisms cannot be cultivated under defined laboratory conditions, missing over >99% of microbial species in cultivation assays (Amann et al., 1990; Tyson and Banfield, 2005). Moreover, the bioburden determined via the standard spore assay does not show any correlation with biodiversity assessed through 16S rRNA gene sequencing or bioburden measured via 16S rRNA quantitative PCR (Cooper et al., 2011), necessitating molecular methods in addition to cultivation and imaging for CC.

6.3.3. Detection of biomolecules. A general microbial survey using cultivation-dependent methods must be accompanied by molecular analysis to determine the bioburden and microbial diversity in the cleanroom facility and on sample devices. The constantly improving molecular methods provide valuable insights into the structure and function of VBNC microorganisms (Quince et al., 2017). A DNA-based microbial survey of the cleanroom facilities requires the samples to be collected and handled by using appropriate aseptic techniques to avoid further contamination from

external sources. It is anticipated that cleanroom samples would contain very few microorganisms, which makes it necessary to concentrate any material to a level at which cells, cell debris, and biomolecules can be detected. Air sampling on a membrane and surface swabs are the standard collection techniques used in the NASA and ESA planetary protection programs (Cooper et al., 2011; La Duc et al., 2012; Probst et al., 2011, 2010). Sample collection can also be augmented with additional concentration techniques such as filtration or centrifugation and additional amplification techniques such as cultivation, depending on the type of sample and the target microorganisms (Luhung et al., 2021). Once the material is concentrated or amplified, DNA extraction can be performed. A number of available commercial DNA extraction kits and customized extraction protocols have been optimized for low-biomass samples to maximize DNA yield and purity (Jiang et al., 2015; Maixner et al., 2022). Since any subsequent DNA-based method is biased toward the type of DNA extraction method applied, it is recommended to use a combination of methods to achieve the best quantification and capture of the greatest diversity of organisms in a sample (*e.g.*, Cooper et al., 2011; Wang et al., 2021).

PCR followed by DNA sequencing is still the most commonly used molecular technique to identify and classify microbial species present in a sample. PCR can target and amplify specific genomic regions, such as the 16S rRNA gene for bacteria/archaea or the 18S rRNA gene and ITS region for fungi (Loos et al., 2021; Yeh et al., 2021). Amplified PCR products can be quantified by using a variety of techniques, for example electrophoresis, UV spectrometry, or fluorometric methods (Nielsen et al., 2008; Robin et al., 2016). Quantitative PCR (qPCR) and digital PCR (dPCR) combine DNA amplification with quantification and can provide rapid estimates of specific microbial group abundances (Abellan-Schneyder et al., 2021; Zhu et al., 2020). Despite their sensitivity, PCR analyses are subject to various biases, including the overestimation of microbial abundance due to multiple 16S rRNA gene copies in one bacterial genome (Louca et al., 2018), or underestimation of microbial abundance due to the inability of conventional 16S rRNA gene primers to capture entire clades of organisms (Brown et al., 2015; Eloe-Fadrosh et al., 2016). Untargeted metagenomic analysis using shotgun next-generation sequencing can overcome these biases (Quince et al., 2017; Thomas and Segata, 2019) and produce a more representative view of microbial contaminants in cleanrooms; however, the low biomass present in cleanroom facilities challenges the metagenomic analysis workflow and may require DNA amplification steps such as random whole-genome amplification (MDA, multiple displacement amplification) of, for example, single cells before library preparation and sequencing (Hatzenpichler et al., 2020; Yilmaz et al., 2010). Another promising amplification-based technique that can produce single-cell or metagenomic libraries for DNA sequencing from only a few femtograms of biomass is emulsion PCR (Lan et al., 2017). The implementation of these approaches will help cleanroom facilities to obtain a broad overview of the structure and function of the microorganisms entering the facility and greatly aid in CC/CK programs.

Specific sample preparation and DNA sequence analysis protocols can additionally help identify whether contaminating biomolecules are derived from living or nonliving matter, information that is critical given the disproportionately high impact that living cells could have on sample integrity for analysis (Mahnert et al., 2015; Weinmaier et al., 2015). One of the most commonly used fluorescent dyes to determine cell viability is propidium iodide (PI). PI is a fluorescent DNA intercalating agent that can only penetrate compromised membranes of dead cells (Boulos et al., 1999). Propidium monoazide (PMA), a derivative of PI, is a photoreactive DNA intercalating dye that prevents enzyme-based amplification of DNA (*e.g.*, PCR) within membrane-compromised (dead) cells and, therefore, is selective for amplification and sequencing of viable cells, since the DNA of PMA-tagged cells can no longer be amplified due to a steric hindrance of the DNA polymerase in binding (Fittipaldi et al., 2012; Nocker et al., 2007). On the other hand, extracellular DNA can be selectively assessed through the application of a sample wash step and centrifugation (Lever et al., 2015). (Meta)genomic data can additionally enable *in silico* detection of modern and so-called "ancient DNA," since highly fragmented cell-free DNA accumulates damage patterns over time (Orlando et al., 2021). This ancient DNA will include the exogenous DNA remnants of dead cells in the cleanroom facility. Although these biomolecular contamination methods are highly sensitive and specific, it is important to note that the analysis of low-biomass samples can be challenging due to the potential for contamination during processing, low DNA yield, and the need for sensitive detection methods (Section 4.3.2). Careful experimental design, controls, and appropriate replication are crucial to ensure the reliability and validity of results obtained from such surveys.

7. Summary and Conclusions

Through the analysis of (i) the previously proposed limits and rationale of the OCP, (ii) cleanliness levels achieved for sampling hardware by the M2020 mission, (iii) recent improvements in analytical technology and detection limits, (iv) updated information regarding the organic content of martian samples (*e.g.*, from the SAM instrument on the Curiosity rover and laboratory analyses of martian meteorites), and (v) information about the composition and geologic context of samples being collected by the Perseverance rover for return to Earth, the SCP developed a set of terrestrial biological, organic, and inorganic contamination limit recommendations for martian samples during their residence inside the SRF (Table 1). These limits reflect an amount of contamination that could be added to the sample during its residency in the SRF rather than a total in-sample contamination limit, and they were established following the SCP's major findings:

- Living and nonliving contamination should not be treated as equivalent in the SRF. The OCP treated living and dead organisms as equivalent with respect to contamination. Because of the potential for growth of terrestrial microbes in the SRF, a renewed focus on avoiding contamination by viable organisms is warranted for this step of the MSR campaign.

- We recommend further investigation of cold (<–20°C) sample storage and handling. Unless absolute sterility inside the SRF can be guaranteed, sample storage conditions should be designed and maintained to inhibit cellular growth and activity.
- Working conditions within the SRF should, at a minimum, reflect the state of the art and best practices within fields with microbial cleanliness standards. Microbial cleanliness standards have been established for a variety of scientific and industrial applications, such as biologically sensitive applications in the pharmaceutical industry.
- We recommend using expected analyte concentrations as a basis for setting contamination limits, that is, with contamination limited to, at most, <1% of expected signals. It remains impractical to achieve sample cleanliness levels that are below detection limits for all (or even most) instrumentation.
- We adopt a similar approach as OCP in defining a limited list of organic contaminants of highest priority (Tier 1) and including everything else in a second list (Tier 2) with higher allowable levels. We differ from OCP in recommending separate treatment of volatile (boiling point, BP <200°C) versus nonvolatile contaminants, to accommodate the extensive use of organic solvents within the SRF. TOC requirements have thus been modified to reflect volatility and solubility.
- We support the same approach as M2020 in using the Tissint meteorite to guide inorganic concentration limits; however, we note that several of the elements that were not explicitly limited for M2020 (Fe, Cr, Mo, Al, Si) are of significant geochemical interest, and so best efforts should be made to limit contamination by these elements.
- We recommend several new, highly sensitive assays of biomolecules as specific means to test for terrestrial life. These assays are for LPS, β-glucan, and ATP.
- We strongly recommend the implementation of an active environmental DNA monitoring program that tracks the presence of nucleic acids, along with their sequence and identity. When changes to the SRF DNA baseline are observed, we recommend a cessation of activities followed by the identification and remediation of contamination sources. These recommendations correspond to a "no change in the SRF DNA baseline" contamination limit.
- The SCP did not explicitly consider requirements that may be needed to avoid noble gas contamination or other inorganic volatiles such as water. These considerations should be included in future discussions.

The SCP also identified several areas that warrant further investigation:

1. Contingency plans for equipment failure (Section 1.3.1).
2. Remotely operated sample handling (Section 1.3.2).
3. Sample storage (Section 1.3.5).
4. Integration of the CC/CK program with martian sample analysis (Section 1.3.6).
5. Construction, cleaning, and monitoring programs within the SRF (Section 4.2).
6. Noble gas or other inorganic volatile contamination limits (Section 5.2).

Finally, it is essential that the SRF's CC/CK program continues to develop in coordination with the latest mission findings and analytical methods on Earth. A robust and comprehensive CC/CK program would have significant and lasting impacts on our ability to conduct sample science investigations throughout the lifetime of the sample.

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Abbreviations Used

ACA = adaptive caching assembly
AMC = airborne molecular contamination
ATLO = Assembly, Test, and Launch Operations
ATP = adenosyl triphosphate
BTEX = benzene, toluene, ethylbenzene, xylene
CC = contamination control

Abbreviations Used (Cont.)

cGMP = Good Manufacturing Practice
CFU = colony forming units
CK = contamination knowledge
DBA = Drillable Blank Assembly
DHMR = dry heat microbial reduction
dPCR = digital polymerase chain reaction
ESA = European Space Agency
ExoMars = a Mars rover mission scheduled for launch in 2020
EU = European Union
FTIR = Fourier transform-infrared spectroscopy
GC/MS = gas chromatography-mass spectrometry
GNB = gram-negative bacteria
GPB = gram-positive bacteria
ISAS = Institute of Space and Astronautical Science
IPA = isopropyl alcohol
JAXA = Japan Aerospace Exploration Agency
JSC = NASA Johnson Space Center
LAL = amoebocyte lysate
LC/MS = liquid chromatography-mass spectrometry
LPS = lipopolysaccharide
MSR = Mars Sample Return
OCP = Organic Contamination Panel
MDA = multi-displacement amplification
NASA = National Aeronautical and Space Administration
NMR = nuclear magnetic resonance
PAHs = polyaromatic hydrocarbons
PCR = polymerase chain reaction
PI = propidium iodide
PIXL = planetary instrument for X-ray lithochemistry, an instrument on the M-2020 mission
PMA = propidium monoazide
PP = planetary protection
PPE = personal protective equipment
qPCR = quantitate polymerase chain reaction
REE = rare Earth elements
SAM = Sample Analysis at Mars, an instrument on the Curiosity rover
SCP = Sample Receiving Facility (SRF) Contamination Panel; Sample Receiving Facility Contamination Panel
SHERLOC = Scanning Habitable Environments for Raman and Luminescence, an instrument on the M-2020 mission
SIH = sample-intimate hardware
SRF = Sample Receiving Facility
SRP = Sample Receiving Project
TA = teichoic acids
TOC = total organic carbon
VBNC = viable but non-culturable
VOC = volatile organic carbon
WTAs = witness tube assemblies