Science-Driven Contamination Control Issues Associated with the Receiving and Initial Processing of the MSR Samples

A Report from the Workshop “MSR Contamination Control,” May 1st-3rd, 2019 in Leicester, UK

The workshop was designed and implemented by the MSR Science Planning Group (MSPG), in response to Terms of Reference received from NASA and ESA.

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The decision to implement Mars Sample Return will not be finalized until NASA’s completion of the National Environmental Policy Act (NEPA) process. This document is being made available for information purposes only.

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EXECUTIVE SUMMARY

The Mars Sample Return Campaign in planning by NASA and ESA is composed of three flight mission concepts, the NASA-led Mars 2020 mission, the NASA-led Sample Return Lander (SRL) mission, and the ESA-led Earth Return Orbiter (ERO) mission. The fourth element encompasses the ground-based receiving and analysis facilities that would have to be in place to enable major science return from returned Mars samples.

Samples would notionally be received at a US-located Sample Receiving Facility (SRF) where initial activities would take place to permit their distribution to and analysis in external laboratories. Throughout the receiving, analysis and curatorial processes of returned Mars samples, it would be critical to preserve the information stored in them by minimizing their contamination by Earth-derived sources, and where that is unavoidable it is crucial to put in place procedures to ensure our understanding of the nature and magnitude of those sources so that science discoveries are not compromised.

Throughout 2019 the MSR Science Planning Group, appointed by ESA and NASA, dedicated work to science planning activities in advance of formalization of the inter-agency MSR Campaign agreement, and funding decisions by national stakeholders. Via regular in-person and remote meetings, dedicated workshops and community interaction via town hall meetings and panel discussions, MSPG worked on a number of areas critical to advance the state of science planning for the MSR Campaign.

This report constitutes output from their 2nd workshop, ‘Contamination Control in the SRF’, held at the University of Leicester, UK, 1-3 May 2019. The workshop involved 31 scientists, engineers and agency representatives (attendee list in Appendix A).

The objectives of the workshop were to determine high-level strategies related to the future preparation of contamination control requirements associated with sample receiving facilities and activities. This is seen as an essential input to functional requirements definition, and cost/schedule estimation of campaign facilities.

Assumptions made when undertaking this work are:

- Not all types of contamination are equal, or equally difficult to control--this workshop focused primarily on the solid samples (rock, regolith, dust), for which the range of scientific measurements is extensive.
- Managing the contamination of gas samples also needs attention, but was not specifically considered within this workshop.
- The contamination control requirements are expected to be a first-order driver on cost of the SRF

Conclusions

The most important messages of this report are presented as “Findings” and “Key Science Strategies”.

Pre-decisional information, for planning and discussion only
Findings

MAJOR FINDING #1: Even though the Mars 2020 Sample Contamination Control (CC) Requirements are very stringent, the workshop participants were collectively not aware of reasons why these requirements could not also be implemented in isolation cabinets on Earth. The Mars 2020 Sample CC requirements should therefore be the starting point for CC planning in the notional SRF and/or sample curation facilities.

FINDING #2: Both new and existing BSL-4 containment facilities should be considered for the SRF since, with modifications, it may be possible to meet CC requirements within re-purposed space. However, there are design and operational limitations of pre-planning an existing facility that may make (re)use or shared use of existing facilities hard, impossible, or sub-optimal.

FINDING #3: Initial CC and CK activities for each sample tube would need to accommodate the following proposed sequence of events: A) collecting the martian dust on the outside of the sample tubes, B) puncturing each tube, and extracting and measuring the headspace gas, C) extraction of the solid sample from the flight sample tubes, so as to enable Basic Characterization (BC), Preliminary Examination (PE), and objective-driven science, and D) the execution of time-sensitive science activities.

FINDING #4: Because the rock and soil sample tubes will be sent to Mars open, their interiors will be exposed to, and contaminated by, the Earth’s atmosphere during and, potentially, after launch. For contamination-sensitive experiments (e.g., noble gas, N and C compounds, etc.), we reaffirm a previous finding that 1-2 dedicated atmosphere sampling vessels on the proposed retrieval mission are extremely important (see iMOST, 2019).

FINDING #5: The quantity/type of data that would need to be collected during Preliminary Examination would need to be optimized for each sample depending on the primary scientific objectives, identification of priorities can occur only after samples have been collected by M-2020, such that their contents have been preliminarily characterized in-situ by rover instruments.

FINDING #6: The science and sample curation communities are unlikely to accept a one-size-fits-all solution for the materials that would be allowed to touch pristine martian samples within a sample processing cabinet. These materials would need to be tailored to each of the sample(s). This includes the composition of the interior portions of the cabinets, the processing tools, storage containers, and any atmosphere that would fill the cabinets.

FINDING #7: We expect that it would be possible to group the samples based on the primary scientific objectives for each sample into about 4-8 sets that would have a common set of CC-related environmental attributes (i.e., atmosphere and types of materials that can touch the samples). If so, the number of sample sets could be used to help define the number of isolators required during the initial set of activities. Each isolator would be cleaned between samples, even if the samples were from the same sample set.

FINDING #8: The Double Walled Isolator (DWI) Breadboard (prototype) shows that it would be feasible to minimize cross-contamination between Earth and Mars materials in a cabinet sized isolator without requiring the entire laboratory room being managed to the same levels of contamination control and containment.
MAJOR FINDING #9: Effective strategies for contamination knowledge for Mars returned samples are judged to be extremely important due to the inevitable contribution of some level of contamination. Primary strategies include collecting witness plates and procedural blanks from before, during, and after flight, preparing a genetic inventory, modeling, tests using analog materials, the application of Bayesian statistics, and evaluation of internal surfaces of samples which will not have been exposed prior to sample subdivision.

Key Science Strategies

1. The dust on the outside of the tubes, though more contaminated than the samples inside the tubes, would be an important scientific sample in its own right, and it must be collected (see also Finding #1 of MSPG et al., 2019).

2. For reasons of gaseous contamination, it would be beneficial/required to have the capability to select different gases (or under vacuum) for the environment in which to open, evaluate, and store the MSR geological samples.

3. The prevention of contamination by direct contact of the Mars samples with curation tools, trays, etc., needs to be carefully planned. There may be rationale for some specific CC requirements for these materials to exceed those of the sample intimate hardware of M-2020.

4. Certain time-sensitive measurements would need to be made as soon as possible after the sample seals are opened (see also Finding #5 of MSPG et al., 2019).

5. We recommend using metagenomics in order to construct a complete genetic inventory of spacecraft contamination. This methodology has the benefit of extracting and sequencing all DNA collected from spacecraft surfaces, including that of dead cells and microorganisms which cannot be cultured.

6. Contamination knowledge MUST continue after BC and PE. Scientists who may handle MSR samples in their home laboratories should demonstrate sufficient contamination knowledge prior to handling the samples. It is considered advantageous to enforce rules that allow contamination tracking of samples after they have left MSRF, so that full records of possible contamination may accompany the samples to the next science PI.
1. INTRODUCTION

1.1. What Question are we Trying to Answer?

In order to design the lowest-cost Sample Receiving Facility (SRF) that is able to meet the requirements necessary to support Mars Sample Return (MSR) science, it is important to answer the following question: **What are the contamination control (CC) standards necessary to ensure that the science objectives of MSR can be achieved?**

The contamination-related aspects of Mars sample return are both challenging and critical for the scientific outcomes. The sample-collecting rover has been built on planet Earth where both life and trace molecules associated with life are ubiquitous. The Mars 2020 engineers will clean the sample-intimate hardware before launch to minimize Earth-sourced contamination, but traces of Earth-sourced contamination, both biologically-derived and otherwise, are expected to remain nevertheless on the sample-contact surfaces. The martian samples in their native state are quantitatively free of Earth-sourced contamination, but the act of collecting and storing the martian samples into the Mars 2020 sample tubes would alter their native state. Those samples would fly back to Earth in sealed sample tubes, where they would be opened in some sort of an isolation cabinet. Like the outbound sample tubes, the isolation cabinet and the associated sample tools would be cleaned to minimize Earth-sourced contamination, but traces of residual Earth-sourced contamination would inevitably remain. Then, finally, the samples would be analyzed using methods and instrumentation that also contribute Earth-sourced contamination (Fig. 1).

Within this backdrop, science would be attempting to achieve detection limits of Mars-sourced signal as low as is reasonably achievable (ALARA). However, we recognize that the way this overall experiment would be carried out, there are certain possible results that may be impossible to distinguish. For example, if there is trace life (or even a life-related molecule) on Mars that is identical to some life form on Earth, it may be impossible to distinguish from the minimized Earth-sourced background contamination. The reality is that we need to find something on Mars that is different from the life on Earth to achieve the most credible detections. There are good reasons to believe that life on Mars, if it exists, would have evolved away from life on Earth, so that this ideal case would be possible.

Given challenges and realities stated above, the essential question for this workshop was how clean does the receiving environment (including the instrumentation) need to be in order to carry out the experiment that is MSR? We recognize that for every order of magnitude of increased cleanliness, the cost may increase significantly. In addition, the cleanliness of the outbound spacecraft can no longer be changed—its requirements have been finalized. What does the established front-end contamination values imply for the contamination requirements for the back end?

**Key Acronyms & Concepts**

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Definition</th>
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<tbody>
<tr>
<td>BC</td>
<td>Basic Characterization. The first step in sample characterization including imaging and weighing the samples.</td>
</tr>
<tr>
<td>CC</td>
<td>Contamination Control. The process of reducing contamination</td>
</tr>
<tr>
<td>CK</td>
<td>Contamination knowledge. The process of identifying and characterizing the properties of contamination</td>
</tr>
<tr>
<td>MSR</td>
<td>Mars Sample Return. A campaign of flight elements that would deliver scientifically selected samples to Earth.</td>
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Preliminary Examination. The process of characterizing the samples in enough detail to produce a sample catalog from which scientists can request sample allocations.

**Figure 1.** Making scientific measurements on MSR samples would need to account for Earth-sourced contamination, including the flight hardware responsible for collecting the samples and the systems that interact with the samples after they get to Earth (e.g. isolators, instruments). It is important to define strategies for limiting this contamination and mitigating its effects on the science results. Note that as of this writing, the expected contamination state of the Mars 2020 sample-collecting rover can no longer be changed because although not yet launched, the design of the mission has been finalized. The essential questions for this study therefore relate to how much money/effort should go into CC and CK strategies on the sample receiving and analysis.

1.2. Why Does this Matter?—Some relevant lessons learned from analogous scientific studies

A primary hypothesis being tested by MSR is that martian life existed or exists, and trace evidence of past or present martian life may exist either in or on the surface rocks and regolith. An alternate hypothesis, that life is abundant in Mars surface rocks and soils, is now widely viewed as unlikely. Thus, we may be trying to distinguish the above hypothesis from the null alternative (i.e., that indigenous martian life does not presently exist at the martian surface, and never did). It is likely that the ability to differentiate signal from noise in “low-yield” samples, in which the signal we are trying to detect is comparable to (or lower than) background contamination levels, would be essential for testing these hypotheses. In such experiments, CC and CK are especially important (see Fig. 2). There are valuable lessons learned in similar recent high-profile case histories where insufficient attention was paid to contamination issues, in which scientific results were later discredited. For MSR it is imperative that we do everything possible to avoid a similar fate.
Three examples follow.

- In 2000, Vreeland et al. reported the isolation and growth of a bacterium from a 250-Myr-old salt crystal—a spectacular announcement. They claimed it was trapped inside the crystal at the time of formation and persisted as an endospore until the present day. However, the scientific community raised serious doubts about the ability of an endospore to survive for that length of time. Graur & Pupko (2001) compared the DNA sequence data to modern bacteria, revealing close relatives separated by less than 100,000 years of evolution rather than the purported 250 Myr.

- In a more recent example, Santiago-Rodriguez et al. (2016) sequenced the gut microbiome of pre-Columbian Andean mummies, finding multiple high-prevalent pathogens and identifying microbial process involved in natural mummification. These data were widely questioned because the authors neglected to use appropriate ancient DNA authentication measures (Eisenhofer et al., 2017). Notably, this included failure to properly catalog potential contaminants.

- A third high-profile case of contamination involving organic molecules relates to the reported find of organic biomarkers such as steranes and hopanes in 2.7-2.5 Ga rocks in Australia (Brocks et al., 1999, Brocks et al., 2003a) which were used prove the presence of of eukaryotes and cyanobacteria during the late Archean (Brocks et al., 2003b). These findings were later discredited as younger contaminants possibly introduced from drilling fluids (Rasmussen et al., 2008). Currently all finds of steranes in Archean rocks (French et al., 2015) and even rocks older than 600 Ma are considered by most as contamination(Brocks 2009). This is shows the importance of both CC and CK in working on organic material in low.yield samples.

Even questionable or discredited studies commonly used rigorous sterilization and cleanliness protocols. Indeed, the anti-contamination measures of Vreeland et al. (2000) were described as “heroic” even by their vocal critics (Graur & Pupko 2001). A common theme to the three examples presented here (and there are many others) is that modern contamination was not thoroughly and systematically excluded as a possible source for the observed results. This could have been remedied by a combination of DNA sequence analyses and inventorying possible contaminants. The lesson to be learned is that rigorous contamination-prevention is not sufficient in the light of extraordinary claims. For MSR to have the potential to make “civilization-scale” discoveries, it would be necessary to 1) Have a rigorous contamination control program, and 2) Document and quantify the contaminant inventories, both organic/biologic and inorganic, of the spacecraft and sample processing environments as part of contamination knowledge (see Section 5 of this report for further details).
Figure 2: Illustration of some of the contamination-related complexities involved in analyzing DNA from low-yield samples (figure credit: Rachel Mackelprang). Shown in this figure is an analysis of a mock microbial community used to determine the lower limits of DNA sequencing protocols and illustrate how sequence data becomes more susceptible to contamination when DNA inputs are low. The number of cell equivalents assumes a 5 megabase genome. DNA weight is in picograms (pg).

1.3. This study
1.3.1. Process
The Mars Sample Return Science Planning Group (MSPG), established by ESA and NASA in November 2018, is an international team of scientists with a charge to ensure that planning activities undertaken by the two space agencies in support of Mars Sample Return (MSR) are coordinated and consistent. The main objective of MSPG is to produce reports from a series of workshops to establish and document positions amongst a diverse set of sample scientists related to planning assumptions and/or potential requirements involving the handling and analyses of returned samples. The first workshop “Science in Containment”, the subject of a prior report, was focused on investigations that need to be performed while under biological quarantine, defined there as “in containment”. The second workshop “Contamination Considerations”, the subject of this report, was focused on the logic associated with setting contamination control specifications at different levels.

The workshop participants discussed a set of prepared questions during three breakout sessions. Participants were assigned to one of the three groups such that each group was approximately equal in composition. Each group considered all the questions and the resulting outputs have been integrated to
form this report. In each section below, the original discussion prompt from the workshop is repeated, followed by a synthesis of the workshop participants’ responses. It was not our intent to establish consensus positions using the workshop discussions—that would require follow-up work. However, for many of the questions discussed, it was possible to identify preponderance of opinion, and the most significant of those were flagged as findings. It is anticipated that the report could be used to support future planning, including international partnership formation and SRF costing exercises. Other inputs into this planning, such as what science needs to be done within containment and planetary protection recommendations, as mentioned above, are topics addressed in subsequent workshops. At the time of writing MSPG intends to finalize an overall summary of its conclusions after the workshop series is complete.

1.3.2. This report

This is a report from the second of the series of three planned workshops. It was held at the College Court facility in Leicester, UK between May 1-3, 2019 with 30 participants (see Appendix A). The overall host of the meeting was the University of Leicester (John Bridges, primary point of contact).

1.3.3. Assumptions

For the purpose of MSPG planning activities, MSPG was asked by its sponsors to work from the following assumptions. If these assumptions change in the future, the conclusions from this and other workshops may need to be reconsidered.

1. The proposed scientific objectives of MSR are those described by iMOST (2019).
2. The sample-related facility scenario would be as follows:
   a. Additional uncontained (i.e. not BSL-4) curation facility(s) in the US and/or Europe would exist. A European facility could be able to receive a subset of samples after initial receipt by the US-based SRF. The European facility may or may not have equivalent containment to the SRF. If it does, then investigations regarding life that are dependent on bio-containment could be performed in Europe. If it does not, receipt of samples by a European facility would occur after transfer criteria are met to permit transfer out of containment.
   b. Principal Investigators, located around the world in academic institutions, research institutes, government laboratories, and elsewhere, would desire access to the SRF and curation (primary) facilities, and eventually if safe, access to samples distributed outside the curation facilities.
   c. The decision on where to locate the U.S. SRF or a potential European bio-contained facility would need to be made in the context of the local and national laws and optimizing for capabilities; thus, this is not known (or knowable) at this time.

2. MARS 2020 PRECURSORS
Summary Conclusion: The workshop participants could not identify reasons why it would be impossible to apply the Mars 2020 Sample Contamination Control (CC) Requirements (in the areas of organic, biological, particulate, and inorganic contamination) to the environment of the isolators (with or without gloves) in the SRF on Earth. The group would endorse starting the design process with these contamination requirements and challenging engineering to find solutions (Fig. 3).

Discussion/Analysis:
Mars 2020 includes the Sampling and Caching Subsystem (SCS) which is capable of acquiring core and regolith samples for potential Mars sample return. Because of the requirement to support return sample science investigations (RSS), more stringent contamination requirements have been placed on Mars 2020 than any previous NASA mission. Specifically, the three key and driving Mars 2020 contamination requirements for organic, inorganic and biological contamination are as follows:

1. **Organic Contamination.** “The Mars 2020 landed system must be capable of encapsulating samples for return such that the returned sample meets the organic cleanliness standards.” This includes keeping “Tier 1” compounds below 1 ppb in the sample. The Tier 1 compounds are specific organic compounds of astrobiological significance (e.g., DNA), and keeping total organic carbon (TOC) below 10 ppb (see Appendix F).

2. **Inorganic Contamination.** Inorganic requirements on the acquired samples includes 34 specific elements and mandates that, depending on the element present, their concentration be no greater than 0.1% or 1% of their concentration in Mars meteorites (see Liu et al., 2014). The Tissint meteorite (a depleted shergottite) was chosen as the reference in order to be able to establish quantitative values, as it has had a brief and well documented terrestrial residence time since its fall.

3. **Biological Contamination.** “The Mars 2020 landed system must be capable of encapsulating samples for return such that each sample in the returned sample set has less than one viable Earth-sourced organism.”

In order to achieve the requirements described above, new protocols, cleaning procedures and materials restrictions as well as facility requirements were levied on the flight hardware and assembly facilities to achieve and maintain these high levels of cleanliness. The flight hardware and assembly facilities on Mars 2020 must adhere to strict requirements in 6 key areas (Fig. 3) in order to achieve the overall requirements above. The areas include viable organisms (VOs), outgassing, particulate cleanliness level (PCL), Total Organic Carbon (TOC), Inorganic contamination and non-volatile residue. A full genetic inventory as well as multiple curation points is also required throughout the hardware build and assembly.
Pre-decisional information, for planning and discussion only

Figure 3. Mars 2020 sample intimate hardware contamination requirements, which constrain the contamination state of the samples at the point of their collection. These are proposed here as a starting point for the SRF planning.

MAJOR FINDING #1: Even though the Mars 2020 Sample CC Requirements are very stringent, the workshop participants were collectively not aware of reasons why these requirements could not also be implemented in isolation cabinets on Earth. The Mars 2020 Sample CC requirements should therefore be the starting point for CC planning in the notional SRF and/or sample curation facilities.

3. CONTAMINATION CONTROL FOR RETURNED SAMPLES

3.1. Contamination Considerations at the SRF Level
3.1.1. Contamination associated with repurposing existing space vs. new construction

Summary Conclusion: The workshop concluded (to within the limits of its collective knowledge) that contamination control is not a valid reason to exclude the option of re-purposing pre-existing “dirty” facility space for SRF functions (i.e., contamination control is not a rationale to require that the SRF be restricted to new construction). However, while using an existing facility is theoretically possible, it may not be an optimal solution given an array of potential issues related to sample safety, short- and long-term facility flexibility, facility access, and governance which all may affect contamination control. Although a full analysis of these additional considerations is outside the scope of this workshop some of these are discussed in Appendix D.
Discussion/Analysis:
Restricted sample return missions are defined as collecting samples from planetary bodies considered by scientific opinion to be “of significant interest to the process of chemical evolution and/or the origin of life” by the Office of Planetary Protection. These types of samples return missions require the implementation of an array of safety precautions. One of these major precautions is the requirement that the samples be handled under BSL-4 containment until deemed safe for release.

Traditionally there is one prime functionality for a high containment facility: to protect the community from exposure to the known hazard(s). However, samples returning from Mars add two extra complexities: 1) the samples may contain “unknown unknown” hazards, which complicates the hazard assessment and 2) the samples need to be protected from terrestrial contamination so Planetary Protection and Science investigations are not impeded. This combined effort requires the integration of both negative and positive pressure environments to meet the needs of planetary protection (PP) and contamination control (CC), respectively (Fig. 4). Furthermore, in order to help meet the PP and CC requirements, double walled isolators (DWIs) would likely need to be integrated into the chosen BSL-4 facility. While there are a number of proposed solutions, the two main facility considerations are whether to contain the samples within a modified existing BSL-4 facility or a new fully integrated facility.

Figure 4. Example of possible 3-Wall configuration integrating positive pressure and negative pressure paradigms (adapted from Rummel et al., 2002).

Whether utilizing an existing facility or constructing a new one, there would be certain requirements levied in order to meet contamination control and planetary protection objectives. These recommendations are based on the anticipated contamination control requirements that are as yet undefined. For more details not provided below, see Appendix D.

Infrastructure Clearance and Capacity
In order to achieve proper airflow requirements, both the space above the cleanroom (or plenum) and the interior of the cleanroom itself need to be of an adequate size for suitable airflow circulation (Fig. 5). The internal size of the cleanroom would likely be driven by the size and number of DWIs/gloveboxes.
required for sample handling and processing. Given the necessity to avoid cross-contamination, accommodation of different micro-environments (e.g., nitrogen, argon, helium), and an array of functions, a suitable footprint should comfortably fit multiple DWIs/gloveboxes, in addition to analytical equipment or desiccators for storage. In addition to space, the size of the points of entrance/egress and pass-throughs is also important. Any containment facility would have to be able to accommodate new large pieces of equipment, like the DWIs, to be brought in and moved around. Given the number of unknowns surrounding Mars sample return (e.g., duration of quarantine, range of analytical equipment within containment) facility adaptability and modularity is important, particularly if samples are not deemed safe for release.

![Figure 5. ISO Class 5 Raised Floor System from Xu, 2014.](image)

**Figure 5. ISO Class 5 Raised Floor System from Xu, 2014.**

**Challenges Unique to the Utilization of an Existing Facility**

To our knowledge, there is no facility in existence with both the required bio-safety containment and clean room standards. It is uncertain whether any existing facility is able to meet both bio-safety containment and cleanroom standards, and whether such standards can be sustained long-term. BSL-4 facilities typically have negative pressure, meaning they are made increasingly dirty with time; even if one can be made clean with an internal clean volume, the starting conditions work against cleanliness. Therefore, it may not be possible to make an existing BSL-4 facility clean enough, especially when shared with other users whose work may demand less stringent cleanliness levels.

Conversion of existing “dirty” space to required levels for high cleanliness industrial processes (light-emitting diode, LED, manufacturing) has been demonstrated for an ISO-8 facility (Fig. 5). In such cases, implementation and maintenance of cleanliness levels may not be the principle engineering challenge, but rather the accommodation of infrastructure to do so in the available space, or perhaps the need to
have the operational solution to, in parallel to its own, also meet requirements imposed by users sharing the facility building, or existing building codes/infrastructure needs.

**FINDING #2:** Both new and existing BSL-4 containment facilities should be considered for the SRF since, with modifications, it may be possible to meet CC requirements within re-purposed space. However, there are design and operational limitations of pre-purposing an existing facility that may make (re)use or shared use of existing facilities hard, impossible, or sub-optimal.

### 3.1.2. Cleanroom Concepts Presented at Workshop

**Cleanliness Technology Concept to control Contamination**
The control of contamination in clean handling environments for a curation and analysis facility of Mars samples requires several different measures. Not only the environment alone can guarantee a contamination risk-reduced handling of mars samples, a number of other measures need to be taken to avoid cross-contamination.

All measures to control contamination are grouped together under the term “cleanliness technology” and include influencing factors including, but not limited to,

- cleanliness suitable environment,
- personnel,
- logistics,
- processes,
- manufacturing equipment.

Taken together, these contribute significantly towards the contamination of a clean handling and manipulation environment (Fig. 6).
To avoid cross-contamination during the handling of Mars samples, particulate, chemical and microbiological contamination is especially critical and has to be considered and controlled. It has to be taken into account that cleanroom technology can only control airborne particulate contamination.

Consequently, it is beneficial to consider an overall rating of cleanliness that incorporates not only the cleanliness rating of specific facilities, but also the associated influences on cleanliness of the sample handling environment (Fig. 6). If necessary, the controlled cleanroom in regard of particles\(^1\) can be combined with a filtration of airborne chemical contamination (see also ISO 14644-8\(^2\)). Also, approaches of GMP controlled environments (e.g. design aspects such as rounded edges, non-metabolizable materials) can be used for a comprehensive cleanroom concept to control the most relevant contaminants.

Even in very inhospitable environments (basement vault of a burned-out factory building with long-standing water damage—See Appendix D, Fig. D1), a functioning cleanroom can be implemented if the right measures are taken, i.e. dehumidification systems, selection of suitable materials like wall/floor coatings, implementation of air filtration, permanent conditioning (temperature and humidity) of air, etc.

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\(^1\) In this context, microbiological contamination can be also considered as airborne particle as microorganisms are either physical bodies in the same size range of airborne particles (> 0.1 µm) or attached to airborne particles; microorganisms are therefore also removed with standard HEPA/ULPA particle filtration systems.

\(^2\) Depending on the defined critical chemical contaminations, special filters to remove acids, bases, condensables, dopands, etc.
Conclusion:
For a curation and analysis facility of Mars samples, a suitable cleanliness technology concept consisting of
- a suitable environment (e.g. a biosafety lab combined with standard cleanroom, clean zone, DWI, etc.),
- cleanliness suitable logistics (material and personnel flow),
- a set of guidelines to manufacture the instruments and RM systems in contact with the Mars samples
to avoid false positive or false negative analysis results due to (cross-) contamination of critical contaminants, as well as
- cleanliness guidelines to integrate equipment, such as robotic arms, grippers, instruments, cameras, tools, ..., inside the DWIs
has to be derived. If this is done, the risk of uncontrolled contamination in all directions (out of the facility as well as into the facility) can be effectively reduced.

3.2. Description of Returned Sample-Related Operations up to and Including Preliminary Examination

3.2.1. Definition and Scope of some activities required in advance of PE

Once the Sample Return spacecraft has been recovered from its Earth landing site, it would be transported to the SRF, where it would be received. The spacecraft would then be progressively opened, to get down to the samples. The outer parts of the spacecraft would have been part of ERO—the Earth Return Orbiter. Therefore, these should be dust-free and not of obvious scientific interest, having never been to the martian surface. However, the Orbiting Sample container (OS) would have been loaded with samples at the martian surface (as part of SRL—the Sample Retrieval Lander), so its interior and exterior surfaces are likely to have dust on them. The sample tubes almost certainly would have a coating of dust, since they would have been laying on the martian surface for a period of up to seven years (if deposited on the surface in 2022 and picked up in 2029). Therefore, beginning with the opening of the OS, initial CC and CK activities for each sample tube would need to take place in the context of the following sequence of events (see Fig. 7):
- collecting the martian dust on the outside of the sample tubes,
- puncturing each tube (for example, in a closed vacuum cabinet) and extracting and measuring the headspace gas (the likelihood of seals remaining intact on the flight tubes are defined by probabilities, but after receipt, we would need to know their actual state),
C. extraction of the solid sample from inside the flight sample tubes, and when necessary to protect the sample, repackaging in curation-grade sample vessels (once each sample is out of its tube, and in an appropriate environment, it can be further characterized) and

D. the initiation of time-sensitive science activities such as that involving Mars-sourced water vapor in the tube.

For reasons of gaseous contamination, it would be beneficial/required to have the capability to select different gases (or under vacuum) for the environment in which to open, evaluate, and store the various MSR samples. It is likely that it would not be possible to make the decision on which environmental conditions to use for different samples until all samples have been collected by Mars 2020, and the reasons for collecting them are known.

**Figure 7.** Block diagram showing four key science-related activities that would have to be planned for inside of the notional SRF before PE can take place.

3.2.2. Contamination considerations associated with four initial activities in the isolators

**Summary Conclusion:** Initial CC and CK activities for each sample tube would need to take place in the context of the following sequence of events: a) collecting the martian dust on the outside of the sample tubes, b) puncturing each tube (for example, in a closed vacuum cabinet) and extracting and measuring the headspace gas (the seals on the flight tubes are defined by probabilities, but after receipt, we would need to know their actual state), c) extraction of the solid sample from inside the flight sample tubes (and repackaging in curation-grade sample vessels as needed to protect the sample) (once each sample is out of its tube, and in an appropriate environment, it can be characterized) and d) the execution of time-sensitive science activities such as that involving Mars-sourced water vapor in the tube (Fig. 7).
**FINDING #3:** Initial CC and CK activities for each sample tube would need to accommodate the following proposed sequence of events: A) collecting the martian dust on the outside of the sample tubes, B) puncturing each tube, and extracting and measuring the headspace gas, C) extraction of the solid sample from the flight sample tubes, so as to enable BC, PE, and objective-driven science, and D) the execution of time-sensitive science activities.

**Discussion/Analysis:**

**Dust on the exterior of the sample tubes**

The sample tubes collected by Mars 2020 are designed to be deposited on the ground, and their exteriors will be exposed to the martian environment, potentially for many years. Thus, they are expected to be coated with air-fall dust at the point when they are recovered for transport to Earth. Although the insides of the sample tubes will be cleaned or baked out to very high tolerances, and methods of preventing their recontamination prior to the deliberate collection of a martian sample have been planned for, the same is not true of the exterior of the sample tubes. They will have a higher level of contamination than the tube interiors, but would still be useful for many types of analyses.

The dust on the exteriors of the tubes, though “dirty” compared to the samples in the tube, would be an important scientific sample in its own right. Therefore, we would certainly want to collect it as quantitatively as possible for curation. Since this air-fall dust is likely to be little more than a surface coating, its total mass is likely to be low. While numerous mass-intensive scientific studies would be impossible, there are several reasons why this dust may be interest:

- It may be our only sample of martian dust that has not undergone the thermal cycling within a fixed volume container.
- The grain size distribution may reflect dust-lifting and dust-depositing processes that are not well-represented in the rest of the collection.
- This material may represent our best chance to study the adhesion of martian dust to metal surfaces—an important issue for astronaut planning (iMOST, 2019).

Establishing the contamination state of this dust sample is likely to be difficult, and it is unlikely that contamination control requirements associated with this sample would meaningfully affect the overall requirements structure of the SRF.

**KEY SCIENCE STRATEGY:** The dust on the outside of the tubes, though dirty compared to the samples inside the tubes, will be an important scientific sample in its own right.

**Contamination of the sample tubes by the Earth’s atmosphere en-route to Mars**

As for other potential contaminants, the requirements for post-return contamination should be as low as or lower than those of Mars 2020 sample intimate hardware for volatile elements (e.g., nitrogen, noble gases, water/hydrogen). However, the tubes to be used by Mars 2020 will not be evacuated and sealed, so they will be in contact with the terrestrial atmosphere before launch. Thus, the level of terrestrial contamination could be significant, particularly for noble gases and N, and possibly also for H and H$_2$O. Terrestrial atmospheric gases will be adsorbed on the walls of the containers and would desorb under vacuum during the transit to Mars, but desorption would likely not reduce terrestrial contamination to a negligible level. On Mars, the tube walls will be in contact with partial pressures of volatiles two orders of magnitude lower than on Earth and these will exchange with adsorbed terrestrial volatiles. It would be difficult to evaluate and quantify the extent of contamination, and further tests...
should be made in conditions analogous to exposure to space at the relevant temperatures and to a Mars-like atmosphere. Dedicated tubes for atmospheric sampling may minimize the ratio between sampled martian gases and adsorbed terrestrial gases, so a dedicated atmospheric sample to be collected by the retrieval mission is very important. In further sampling of martian atmosphere after Mars 2020, specific handling of dedicated tubes should be envisioned, e.g., baking and degassing under vacuum.

Contamination of the samples by the Earth’s atmosphere after they are opened
Further contamination by atmospheric gases in the SRF should be avoided as much as possible. The tubes from which the martian headspace gases would be extracted and analyzed should be opened in a box having a window and put under vacuum. After puncturing of the tubes, martian gases should be retrieved (e.g., cryogenic trapping in a removable trap). The tubes should be transferred to another volume filled with a specific gas composition, where they would be opened and the cores would be extracted. The nature of the ambient gas should be chosen depending on the samples and on the targeted analyses. Nitrogen is a key element of the martian environment; the martian atmosphere and the martian mantle are 60% richer (Wong et al., 2013), and 3% poorer (Mathew et al., 2001), respectively, in $^{15}$N compared to nitrogen in the terrestrial atmosphere. Excess $^{15}$N recorded in nitrogen-containing organic compounds has the potential to trace a martian origin. A terrestrial N$_2$ atmosphere may alter the pristine martian signal. Alternative gases should be evaluated for isolators, like argon or helium. However, argon is also a key tracer of the martian environment. Advantages with helium are that it does not adsorb easily, the martian atmosphere is poor in helium, and this noble gas does not appear to be a key tracer of the martian environment. Helium can also be easily purified and recycled, since every gas except He and Ne is trapped in activated charcoal at the temperature of liquid nitrogen.

Once a gas composition for the boxes has been chosen, the same gas should be used during processing of the tubes and cores and during characterization and curation. Alternatively, some of the samples could be processed and stored under vacuum as the Hayabusa2 samples will be. This would require significant technological development to handle the tubes and the cores. As for hydrogen compounds, any type of storage environment would affect the samples, which would dehydrate and/or exchange isotopically with the environment. Storing the samples under controlled hygrometry would not help since isotopic exchanges of D and H would inevitably occur. Considering this problem, isotope measurements coupled with precise mass balance may permit back-calculating the original D/H ratios. Regardless, H-compounds should be rapidly extracted and analyzed.

Sample processing should permit the separation of subsamples and their removal from the confinement in order to execute experiments requiring the solvents and hoods, which cannot be done in environment-controlled containers. These operations should not alter the samples environment (gas, pressure, temperature).

KEY SCIENCE STRATEGY: For reasons of gaseous contamination, it would be beneficial/required to have the capability to select different gases (or under vacuum) for the environment in which to open, evaluate, and store the MSR geological samples.

Contamination during sample extraction and Basic Characterization
After the head gas is extracted, the tubes would be opened within their designated sample cabinet, and the core sample would be extracted. To make sure this is done as cleanly as possible, this procedure should be practiced beforehand on analogue samples and accounted for in procedural blanks. It is
important to preserve the orientation of the sample or at least document it during extraction from the sample tube. After extraction the sample would be described, weighed, and imaged. Additionally, other desired basic characterization analyses would occur on the samples, without compromising their pristine nature. Once basic characterization is completed, the sample would be repackaged within a curation-grade container for storage until the sample is selected for preliminary examination. After each sample is repackaged, the entire sample cabinet must be cleaned before the next sample can be introduced for gas extraction and basic characterization. The contamination control requirements during this stage should take into consideration the stringent Mars 2020 requirements as a starting point for required cleanliness. For example, Ti and N, which is part of inner TiN coating of the sample tube, and gold, which is part of the knife edge, had no requirements as a part of the Mars 2020 project, but may be required to be limited in the SRF for future science benefit. Also, for some elements, such as highly siderophile elements and isotopes, more stringent requirements may be required for specific scientific purposes.

**KEY SCIENCE STRATEGY:** The prevention of contamination by direct contact of the Mars samples with curation tools, trays, etc., needs to be carefully planned. There may be rationale for some specific CC requirements for these materials to exceed those of the sample intimate hardware of M-2020.

**Contamination of the head space gas**

Each sealed sample tube would consist of a fixed volume, within which a fraction of the volume would be filled with solid rocks/minerals/soils. The remaining volume fraction would contain headspace gas that would initially be at the pressure of the martian atmosphere at the time the seal was closed. As noted by iMOST (2019), the primary value of the headspace gas is twofold: 1) To determine if the sample tube seal leaked, and 2) To determine the extent to which the solid sample and the headspace gas reacted with each other during the time they were sealed within the sample tube. Issue 1 clearly has the potential to introduce Earth-sourced contaminants and/or to result in sample fractionation. Avoiding these two effects would help to facilitate high-end sample-related scientific investigations of the martian atmosphere, so it is therefore very important to collect and return 1-2 independent samples of the martian atmosphere, preferably sealed with a high-quality valve (iMOST, 2019).

**FINDING #4:** Because the rock and soil sample tubes will be sent to Mars open, their interiors will be exposed to, and contaminated by, the Earth’s atmosphere during and, potentially, after launch. For contamination-sensitive experiments (e.g., noble gas, N and C compounds, etc.), we reaffirm a previous finding that 1-2 dedicated atmosphere sampling vessels on the proposed retrieval mission are extremely important.

**Contamination planning related to time-sensitive measurements**

Time-critical scientific analyses need to be conducted prior to preliminary examination (or possibly even before basic characterization in the case of analysis of the headspace gases within each tube). These time-critical scientific analyses need to be identified, and a process must be developed to conduct the time-critical science while maintaining the pristine nature of the remaining sample that is not part of the time-critical analysis. These time-critical measurements could include characterization of hydrous mineral phases that are sensitive to changing relative humidity or analysis of samples for organic compounds for science and/or as part of a sample safety assessment.
3.2.3. Definition and Scope of PE

**Summary Conclusion:** The overall objective of the preliminary examination (PE) phase can be defined as the production of a catalog that the scientific research community can use to apply for sample allocation(s). The BC and PE processes should be maintained as separate activities from the initial science investigations that attempt to answer primary mission science objectives. Specifying which data need to be collected during PE should be optimized for each sample type based on the primary scientific objectives and can be better defined after the samples have been collected by Mars 2020. The fundamental purpose of PE is to enable objective-driven science, while not interfering with (or “scooping”) the competitive scientific discovery process. PE must collect enough data so that members of the scientific community are able to recognize and apply for samples that are appropriate for a proposed analysis, while at the same time minimizing sample manipulation that could contribute additional contamination that may be detrimental to primary scientific objectives. In addition, we assume that certain common sample preparations (e.g., thin sections, extractions; the details of which are expected to be discussed in successive committees or working groups) would be part of the PE process, and such prepared products can, and should, be made available for allocation after PE.

**Discussion/Analysis:**
The Basic Characterization (BC) phase of the returned samples would be ongoing, but once the first set of BC information is completed for a given sample, the next phase of activities for that sample, Preliminary Examination (PE), can begin. Details of the definition of BC and PE for returned Mars samples are presented by MSPG (2019) in the report from their first workshop: ‘The Relationship of Mars Sample Return Science and Containment’. The Preliminary Examination phase and the Sample Safety Assessment Protocol (SSAP) are expected to be somewhat simultaneous and synergistic, with results from PE measurements assisting with SSAP, and vice versa, in the SRF. Completion of both activities would enable distribution of samples for analysis outside the SRF.

There would be several streams of PE depending on the sample type and the key science objectives that need to be achieved using that sample. Specifying which data need to be collected during PE should be optimized for each sample type and can be better defined after the samples have been collected by Mars 2020. There would be comprehensive field data supplied on the material in the sample tubes from Mars 2020 to help the PE team before the samples have arrived at the SRF. From the range of Mars 2020 instruments, we would expect to know information such as the locality of material sampled, the rock type, general mineral distribution, macro-scale spectral information and visible images of the material.

The PE phase would build a catalog of relevant data such as lithology (e.g., thickness of sedimentary layers, distribution of mineralogy and organic components), mineralogy (e.g., constituents and trace and major elemental abundance and spectral properties), and organic measurements (spectral properties) for the samples, so that the most suitable sample can be selected to support the proposal-driven MSR science investigations (Figures 8 & 9). This process is essential to meet long term curation objectives for Mars samples. Some physical sub-sampling and handling would need to occur at the PE stage to prepare
samples for analysis, such as making thin sections. But the small loss of material in sub-sampling during PE would minimize sample waste later in the process of allocation of the material. For example, the making of thin sections or rock chip mounts could be used by several investigators working on inorganic mineralogical and geochemical measurements.

**Figure 8.** Definition of Terms: The output of the preliminary examination process, in generic terms, would be a sample catalog.

**DEFINITION:** The fundamental purpose of PE is to enable objective-driven science. The overall objective of the preliminary examination (PE) phase can be defined as the production of a catalog that the scientific research community can use to apply for sample allocation(s).
Figure 9. Schematic integrated draft workflow for the Initial handling/Basic Characterization/Preliminary Examination of the MSR samples. Note that as per the discussions in MSPG (2019), an important option not shown above is CT or synchrotron scanning—this would require further evaluation by a successor committee.

Finding #5: The quantity/type of data that would need to be collected during Preliminary Examination would need to be optimized for each sample depending on the primary scientific objectives, identification of priorities can occur only after samples have been collected by M-2020, such that their contents have been preliminarily characterized in-situ by rover instruments.
4. CONTAMINATION CONSIDERATIONS ASSOCIATED WITH ISOLATOR OPERATIONS

4.1. CC requirements (on Earth) for the MSR sample-intimate tools and isolators

Summary Conclusion: There is not likely to be a one-size-fits-all solution for the materials that would be allowed to touch pristine martian samples within a sample processing cabinet, so the materials used in each of the sample processing cabinets would be tailored to each of the sample(s) that it is going to host. This includes the composition of the interior portions of the cabinets, the processing tools, storage containers, and any atmosphere that would fill the cabinets. However, there may be some materials that have been used in the caching process, and so, would be known contaminants and could be used for sample handling. It may not be possible to finalize decisions on the materials and number of distinct sample processing cabinet types that would be needed until after the samples have been collected by Mars 2020.

Discussion/Analysis:
Any returned sample must be processed before it can be observed or analyzed. The sample processing cabinets, any atmosphere in those cabinets that touch the samples, and any tools used to process samples must be made of something that would not compromise the primary science questions that drove collection of the sample. These processing materials that come into direct contact with the samples in a processing cabinet are chosen so as not to affect their pristine nature. For example, Apollo samples are considered to be pristine if they have come into contact with 304 or 316 type stainless steel, Teflon, dry (<5 ppm H2O) N2 gas, and series 6100 aluminum alloy. All of the processing tools, sample containers and processing cabinet surfaces that come into direct contact with the Apollo samples are composed only of these materials. We would know a little bit about each Mars sample as well as the primary reason that it was selected for drilling. These pieces of information would drive primary science question assignments for each sample. Based primarily on the primary science question assignments, materials would be selected that can come into direct contact with the sample without compromising its pristine nature. These materials would comprise the interior surfaces of the sample cabinets, the tools within the cabinets, sample storage containers, and the atmosphere composition (or vacuum) within the sample cabinet. Selection of these materials would also be driven, secondarily, by minimizing contamination for other scientific stakeholders that may be interested in evaluating that particular sample. After these decisions are made for each sample, the sealed tubes that have had their dust removed would be placed within their designated sample cabinet, and the head gas would be extracted from the sample. After the sample is punctured and the head gas extracted, the sample would be open to the environment within the sample cabinet. The removal of head gas should be done relatively quickly after receiving the samples as the state of seals would not be known.

FINDING #6: The science and sample curation communities are unlikely to accept a one-size-fits-all solution for the materials that would be allowed to touch pristine martian samples within a sample processing cabinet. These materials would need to be tailored to each of the sample(s). This includes the composition of the interior portions of the cabinets, the processing tools, storage containers, and any atmosphere that would fill the cabinets.
4.2. Estimating the optimal number of isolators needed to open the sample tubes while maintaining contamination control requirements.

**Summary Conclusion:** In optimizing the strategy for minimizing sample contamination during opening of the tubes and doing basic characterization, it is considered most likely that it would be possible to group the samples into about 4-8 sets that would have a common set of environmental attributes (i.e., atmosphere and types of materials that can touch the samples). If so, this would define the number of isolators (which would be cleaned between samples) required during the initial set of activities. An important condition would be to have the same environment (gas, temp, humidity) from the beginning of handling (opening the tubes) until long term curation.

**Discussion/Analysis:**
An important planning question is the number of isolators needed within the SRF. This would exert a significant influence on facility sizing. This question was touched upon briefly by MSPG (2019). However, valuable additional perspective can come from contamination control considerations. As per the planning of the Mars 2020 sample-caching rover, we expect the samples in the collection to be different in certain ways, and similar in certain ways. It would make most sense to open similar samples in a common isolation cabinet, so that the samples would be exposed to common environmental attributes (such as atmosphere, temperature, humidity, types of materials that can touch the samples, etc.). This would allow the scientific community to consider whether different types of samples, for which different types of scientific questions would be posed, should be exposed to different environmental parameters. Given what we know about the geology of the Mars 2020 area of landed operations, the judgement of the workshop is that it would likely be possible to group the samples into 4-8 sets for which these environmental parameters should be independently planned. It would be advantageous to have the flexibility to adjust these conditions at a relatively late date. This would in turn imply that at least 4-16 isolation cabinets are needed for preliminary examination.

**FINDING #7:** We expect that it would be possible to group the samples based on the primary scientific objectives for each sample into about 4-8 sets that would have a common set of CC-related environmental attributes (i.e., atmosphere and types of materials that can touch the samples). If so, the number of sample sets could be used to help define the number of isolators required during the initial set of activities. Each isolator would be cleaned between samples, even if the samples were from the same sample set.

4.3. Double-walled isolator prototype

**Summary Conclusion:** The key concept of double walled isolation has been demonstrated with a double-walled isolator (DWI) breadboard, where the working volume is maintained at a negative pressure and interfaces are passed through an intermediate positive pressure volume. In terms of validation, this technique solves many problems associated with contamination control, cleanliness and conducting analysis in isolation (containment) because the direction of inadvertent seal leaks is managed in terms of CC and containment. The current system is a mobile 1500 kg unit with external dimensions of 2540 mm high, 2400 mm wide by 1430 mm and operated in an aseptically managed cleanroom. A technical summary of the DWI BB is given in Holt et al. (2019) and described further in Appendix C with an image of the current DWI breadboard. There was widespread agreement that the DWI isolator implementation,
being pioneered at the University of Leicester, shows good potential, and that its development should continue (See Fig. 10, below).

In addition to SSAP assessment requirements, BC and PE analytical concepts, it seems likely that contamination control for a sample would be managed according to a particular analytical chain. For example, one sub-sample may benefit from testing in argon (rather than nitrogen) or have limited physical contact with metals or other contaminating materials. There is not a “one size fits all” solution to managing CC of Mars material, and flexibility built into the DWI BB is key to addressing this problem.

DWI parameters (oxygen and water concentrations), gas type and flow rate, temperature and pressure may be adjusted for a particular operation, providing a means of control over contamination. After initial purging, the DWI gas is recirculated through primary and secondary HEPA filters and regenerated by a molecular sieve. Analytical operations are conducted on a 316 stainless steel tray (Op-tray) that could be surface modified or exchanged for a different material. Isolators tend to have a flat bottom (causing turbulent gas flow and contamination “hot spots”) or a perforated base to achieve uni-directional flow (UDF), over the samples. The strategy of the DWI Op-tray adopts a technical compromise by maintaining UDF over the sample and channeling gas through slots about the perimeter of the tray. During verification tests, in 2018, analogue rock cores were cut inside the DWI BB to mimic a worst case “dirty operation” on the Op-tray. In order to manage the distribution of debris from the sample, the UDF flow was set to zero and low rate nitrogen purging adopted. While it is not expected that a real sample would be prepared in this manner, it identified a management strategy to minimize distribution of sample debris. Similarly, the Op-trays may be configured for a range of analytical operations relevant to SSAP, BC, and PE with appropriate category 1 and 2 instruments (instrument categories are described in appendix C).

As described in Holt et al. (2019), DWI is modular and may be assembled in a chain by using the “instrument Box” (IB) feature with isolating doors and containment provided by the double seal, small intermediate volume (SIV). In addition to system flexibility, to accommodate a range of analytical techniques, this approach allows management of contamination in a configurable facility. For instance, a bifurcating DWI chain facilitates multiple science themes, cascading cleanliness (or environmental modification to manage CC in accordance with a particular PE process/analysis) and prevention of back migration of contaminants that may be introduced during PE (e.g., solvent vapor from an organic extraction). In essence, the IB is a small vacuum chamber that may be used as an isolated pass box to connect DWI’s, accommodation of a sterilizing source or category 2 and 3 instrument interfaces relevant to SSAP, BC and PE. Furthermore, the IB is a small stainless steel chamber (that could include a PTFE surface coating/modification) and may be technically easier and more cost effective for some ultra-clean or specialist operations (e.g., headspace gas sampling). The DWI concept also offers the longer term potential to integrate robotic manipulation and micromanipulation of samples (Vrublevskis et al., 2019).

An ESA-funded 18 month, phase 2 DWI BB de-risk test programme is underway at the time of writing and includes; additional instrument interfaces, an IB with an isolating door to the DWI, further CC assessment (biological, thermal desorption tube/GCMS sampling and particulates below 0.2 micron), integration of a calibrated close up imager and a series of community informed blind science experiments to mimic BC analysis.
CONTAMINATION KNOWLEDGE

5.1. Contamination knowledge (CK)—multiple considerations

Even with the most stringent CC, we must expect that sample contamination would be non-zero. Therefore, rigorous contamination knowledge (CK) is critical to the scientific outcomes of MSR. Returned Mars samples would have the most procedural blank-rich scheme of any extraterrestrial samples. All materials used during the building of the spacecraft are also currently being preserved. This includes all materials the spacecraft are being constructed of and all materials the spacecraft has ever been in contact with on Earth, including all solvent rinses used to check for cleanliness.

Primary strategies for characterizing and quantifying of the contamination associated with individual processes and the integrated (i.e., summed total) contamination contribution of sample processing and analysis include (but are not limited to) the following (below). It is not possible in a report of this scope to describe all of these topics in detail, and we welcome more detailed follow-up analysis by a future successor group (or groups). Short sections derived from the workshop discussions or presentations are presented below for the topics in bold.

- collecting witness plates and procedural blanks from before, during, and after flight,
- the so-called genetic inventory,
• characterization of materials that are allowed to touch the samples after return,
• the application of Bayesian statistics,
• evaluation of internal portions of the sample which have been protected from drill & sample tube contact.
• modeling,
• tests using analog materials,

**Definition of Terms:** Several terminologies were used during the breakout sessions and these are defined here. *Witness plates* are initially clean materials that can either follow samples or stay in a single environment. They can be used to monitor cumulative contamination level in a single or multiple settings (e.g., stationary in a sample storage unit or moving across glove boxes). Witness plates passively record the environment that a sample experiences. *Procedural blanks* are initially clean materials that have gone through a single step (e.g., sawing) or the entire processing procedure that may include BC, PE, and scientific investigation. Procedural blanks are valuable because they give an unbroken record of contamination history of a sample. Procedural and cumulative process blanks must undergo every step that a sample experiences so that there is not a gap in the contamination knowledge record because these gaps in the contamination knowledge record are what has led to false positives in previous high-stakes scientific studies (section 1.2). Ideal procedural blanks are made of materials that mimic sample matrix as closely as possible in order to accurately record contamination transfer and accumulation. Statistical analysis of blanks would allow data that are deemed to be likely false positive results to be discounted. Besides analyzing these blanks, swaps, particle counters and particles trapped in filters are other ways to monitor contamination levels.

**MAJOR FINDING #9:** Effective strategies for contamination knowledge for Mars returned samples are judged to be extremely important due to the inevitable contribution of some level of contamination. Primary strategies include collecting witness plates and procedural blanks from before, during, and after flight, preparing a genetic inventory, modeling, tests using analog materials, the application of Bayesian statistics, and evaluation of internal surfaces of samples which will not have been exposed prior to sample subdivision.

Some specific examples of CK strategies used by the Mars 2020 sample-collecting rover are illustrated in Fig. 11.
5.2. Witness Plates and Procedural Blanks—Examples from Mars 2020

Witness plates are used throughout the Mars 2020 hardware assembly campaign to verify cleanliness levels in all different environments. Such witness plates should also be deployed during sample handling events to inform any contamination events during sample handling. Typical witness plates used for contamination control capture (1) particulate contamination and (2) molecular contamination (organics). Shown below in Fig. 12 is an example of silicon wafer witness plates deployed on Mars 2020 as well as aluminum molecular plates. In some cases, gold foil is also used to capture debris/particulates during Mars 2020 hardware assembly and could be useful for witness coupon materials during sample handling. Biological plates and swab/wipe samples are also deployed for planetary protection verification and knowledge during all hardware builds and should be considered for use in the return facility.

The choice of materials for witness plates and procedural blanks would depend on the science question being asked. Witness plates could be made of materials that the samples are being carried in, although “getter” witness materials have also been suggested, for amplifying signal of contamination record. Procedural blanks should be made of materials that are matrix-matched because different matrix would
absorb different amounts of contaminants from the same environment. However, this may increase the number of blank materials for each sample. The materials and quantities needed for witness plates and procedural blanks are to be determined. The workshop participants agree that there should be some kind of standardization in the production of witness plates and procedural blank materials, and these should be well-characterized and be made available to science PIs. When these choices are determined, tests could be conducted to assess contamination levels caused by materials that may come into contact with the samples (i.e., robotic manipulators and their lubricants, gases, gloves, sample container, tweezers etc).

The Mars 2020 mission will also fly witness plate assemblies (WPAs) inside of sample tubes in order to capture contamination knowledge during flight and operations on the surface of Mars. The WPAs are comprised of multiple materials mimicking the materials of the sample intimate hardware such as sample tubes, hermetic seals and bits. All piece parts were cleaned and assembled exactly similar to the sample intimate hardware (i.e. sample tubes, hermetic seals, etc.). The WPAs will fly to Mars inside of sample tubes and can be “activated” to capture contamination at different times throughout the mission. On Mars, the WPAs will be manipulated in exactly similar environments and operations as the sample tube pre and post coring with the exception of physically coring a sample.

Additionally and similar to MSL, Mars 2020 will also fly a drillable blank material. The intent of this material is to capture the contamination knowledge from bit to rock interactions. Similar to the WPAs, this sample can be later deposited on the surface of Mars for potential return with samples.

Proxy hardware was also used for CK throughout the Mars 2020 campaign. These flight-like piece parts and hardware assemblies saw identical machining, cleaning and assembly processes as well as batched with the actual flight hardware itself during these processes. Multiple proxy hardware was collected throughout the Mars 2020 builds for sample intimate hardware cleanliness verification as well as curation for future science useful for sample investigations.

Mars 2020 was also required to collect multiple direct samples on hardware for biological, particulate, and organic contamination knowledge. All of the data and analysis from these direct samples is planned to be archived for future sample investigation science.

CK knowledge of critical processes were also investigated by performing multiple test campaigns using flight-like hardware. For example, contamination transfer from bit to rock interactions during coring were evaluated by coring multiple rock types with flight-like hardware and performing chemical analysis and characterization of contamination transfer to samples. This data is also intended to be archived to help inform future science investigations on samples.

5.3. Genetic Inventory

Complete sterility, that is the absence of all life, is not considered an achievable goal. Further, DNA from dead cells may remain, even after sterilization. Therefore work should proceed under the assumption that some biological material will contaminate spacecraft surfaces and the sample processing pipeline. Though we expect contamination levels will be low, even trace levels may have long-reaching consequences. First, the specialized DNA sequencing protocols for detecting life in low-biomass martian samples may also identify trace contaminants. Second, microbes surviving in clean room environments may be able to metabolize martian substrate, thus altering the sample’s characteristics. A complete
biological (i.e., genetic) inventory may aid to mitigate these problems. A genetic inventory would be obtained by interrogating surfaces that may contact (or are in close proximity to) samples, witness plates, air filters in processing chambers, and reagents used for DNA isolation and sequencing. These samples may be processed (i.e., subjected to DNA isolation and sequencing) or archived for future testing.

The discovery of martian life is most defensible only if it is substantially different from terrestrial life (scientific reasons strongly argue that it is nearly impossible for Mars and Earth life to be the same-RSSB, 2018). If martian life has DNA-based genetic material and if martian and terrestrial life share a common ancestor, one would expect considerable divergence in the genetic repertoire. The most recent common ancestor to all life on Earth dates to ~2 billion years ago. Unless martian life has very recently been seeded from Earth life, martian sequences should be quite different from terrestrial sequences, reflecting the long divergence time. A close relationship between returned samples and genetic inventories would lower the credibility of the discovery. However, the level of resolution achieved by these analyses depends on the completeness of inventories and repositories. Thus, if DNA is obtained from returned samples, a genetic inventory of the most likely contaminants would be critical for validation. Possible scenarios and their conclusions follow (see Fig. 13):

- **Sequences match the inventory from spacecraft surfaces**: This finding would be clear evidence of forward contamination from spacecraft surfaces.
- **Sequences do not match spacecraft inventory, but do match those from processing facilities**: This finding would be clear evidence of contamination from processing facilities.
- **Sequences do not match genetic inventories, but do match other Earth-based organisms**: These sequences would likely represent contaminates from spacecraft surfaces or processing facilities. However, the genetic inventories were not sufficiently characterized. This would trigger a much more detailed set of analyses of the archived swabs, wipes, etc.
- **Sequences do not match genetic inventories, nor do they match other known Earth life**: These data could represent sequencing or analytic artifacts or may be evidence of martian life.

To achieve a complete genetic inventory, NASA’s “Standard Assay Method” is inadequate because it does not detect the majority of potential contaminants. This method involves culturing microorganisms collected from spacecraft surfaces. However, the problems are two-fold. First, 95-99% of microorganisms do not grow under Standard Assay Method conditions. Second, it does not detect DNA from dead cells, which are also an important contaminant. Duplicate swabs/wipes used to generate the Genetic Inventory are also being curated in order to account for advancement in analytical equipment.

We recommend an alternative method, termed metagenomics, which circumvents these issues. In metagenomics, all DNA is extracted and sequenced, negating the need for cultivation. Metagenomic surveys of spacecraft surfaces need not be completed prior to launch. Though this method is common in other environments, it is not yet optimized for spacecraft and cleanroom environments. Instead, swabs/blanks/etc. may be archived and subjected to analyses as protocols are optimized and technologies improve.
5.4. Characterization of Materials Allowed to Touch the Samples

The number of materials that are allowed to touch the samples after they come out of their flight tubes should be minimized, and those materials should be pre-determined and extremely well-characterized. We specifically talked about the possible use of plastic sample-contact tools and trays to minimize inorganic contamination, but we could not see any meaningful advantage. The group was concerned

Figure 13: Importance of a genetic inventory in interpreting DNA found in samples from Mars. In the hypothetical case that the Mars samples have trace Earth-like genetic material (DNA) in/on them, the genetic inventories would be used to interpret the possibility of contamination.

KEY SCIENCE STRATEGY: We recommend using metagenomics in order to construct a complete genetic inventory of spacecraft contamination. This methodology has the benefit of extracting and sequencing all DNA collected from spacecraft surfaces, including that of dead cells and microorganisms which cannot by cultured.

KEY SCIENCE STRATEGY: Contamination knowledge MUST continue after BC and PE. Scientists who may handle MSR samples in their home laboratories should demonstrate sufficient contamination knowledge prior to handling the samples. It is considered advantageous to enforce rules that allow contamination tracking of samples after they have left MSRF, so that full records of possible contamination may accompany the samples to the next science PI.
that defensibly validating the CC and CK requirements would be hard, and significantly more discussion is needed.

5.5. Application of Bayesian Statistics

The objective of scientific measurements is to obtain information that indicates the presence of a target material and Bayesian statistics (Bayes 1763) can help to quantify the probability of a correct detection (Fig. 14). For instance, a major objective of Mars sample analyses is to determine if there is any indigenous carbon (Sephton & Carter, 2014) or biosignatures (Sephton & Carter 2015). Particularly for planetary protection determinations, it is necessary to gain confidence in the absence of a measured target material and Bayesian statistics can be used to determine the required sample size for representative assessments related to organic targets (Carter & Sephton 2013). When the measurement correctly indicates the presence of the target material then this is called a true positive (TP). If a measurement correctly indicates the absence of a target material in the sample then this is called a true negative (TN). Contamination can mimic the signals of a target material and can lead to false positives (FP). Contamination can also obscure the presence of the target material causing a false negative (FN). The ability of particular test to obtain true positives and true negatives can be obtained during instrument development and testing. Laboratory generated samples with user-created contents have been used in the past to determine true positive and true negative rates of instruments with astrobiological objectives (Gordon & Sephton 2016). If instrument development and testing is representative of the real tests to come, then the influence of contamination would be incorporated into the true positive rates (TPR) and true negative rates (TNR). The probability of a sample containing the target material can be estimated either by previous sampling and testing campaigns or, in their absence, by terrestrial analogue materials with comparable characteristics. The probability of the target material being present before any measurements are made is called the pre-test probability. True positive and true negative rates can be used to calculate likelihood ratios for the test. When a signal indicating the presence of the target material is received the positive likelihood ratio (LR+) is used to modify the probability of the target material being present and the more positive signals received the higher the post-test probability becomes. Obtaining confidence that a target material is present is therefore facilitated by the best possible tests (highest likelihood ratios), the samples most likely to contain the target material (highest pre-test probabilities) and repeated numbers of measurements.
6. Suggestions for Future Work

- On Mars, the tube walls will be in contact with partial pressures of volatiles two orders of magnitude lower than on Earth and these will exchange with adsorbed terrestrial volatiles. It will be difficult to assess the extent of contamination, and further tests should be made in conditions analogous to exposure to space at the relevant temperatures and to a Mars-like atmosphere.
- Work with sample analogs on the end-to-end tube opening/BC/PE process to optimize procedures to minimize contamination and loss of sample mass.
- Determine number and optimal materials for witness plates/procedural blanks.
- Determine optimal materials for sample contact tools, and test to determine contamination transferred by these materials using analogs.
- Determine the specific analyses that accompany the first phases of PE for anticipated classes of samples.
- Double walled Isolator Technology needs to be developed, including instrumentation interfaces consistent with preliminary and basic characterisation requirements.
- Investigate how different procedures and analysis techniques contaminate samples.
- Develop biomolecular techniques to detect DNA in minimal amounts or rock samples.

Figure 14. Key equations relevant to Bayesian Statistics. One way to obtain prior-test probabilities for extraterrestrial materials is to use a) terrestrial analogue materials with comparable characteristics, while another is to use (b) data from previous testing campaigns. Prior-test probabilities can then be modified to post-test probabilities by using responses from measurements on Mars and the key Bayesian equations.
7. Acknowledgements

Initial drafts of this report were written by the workshop writing team (listed alphabetically): David W. Beaty, Brandi L. Carrier, Monica Grady, Andrea Harrington, John Holt, Rachel Mackelprang, Bernard Marty, Francis McCubbin, Sandra Siljeström, Corliss Sio, Kim Tait, & Lauren White with editorial support from multiple workshop participants. The MSR Science Planning Group would like to extend our gratitude to our Workshop Participants for sharing their time and expertise both in participating in the workshop and in the preparation of this report and its findings.

A portion of this work was carried out at the Jet Propulsion Laboratory, California Institute of Technology, under a contract with the National Aeronautics and Space Administration.

8. References


## Appendix A: Workshop Participants

<table>
<thead>
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<th>Name</th>
<th>Affiliation</th>
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<tr>
<td>Barbosa Anesio,</td>
<td>Aarhus University</td>
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<td>Alexandre Magno</td>
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<td>Fraunhofer IPA</td>
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<td>Marty, Bernard</td>
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<td>White, Lauren</td>
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## Appendix B: Workshop presentations
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<th>Title</th>
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<td>Rachel Mackelprang &amp; Karen Olsson-Francis</td>
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<tr>
<td>Mars Sample Return: Contamination Control (CC) and Contamination Leverl (CK) associated with Inorganic Contamination: the Case of Transition Metals</td>
<td>Audrey Bouvier</td>
</tr>
<tr>
<td>ExoMars 2020 Ultra Clean Zone Experience</td>
<td>Antonio Saverino</td>
</tr>
<tr>
<td>Mars 2020 RSSC Requirements and Approaches</td>
<td>Lauren White</td>
</tr>
<tr>
<td>Preparation for Mars Sample Return: Contamination Control, Contamination Knowledge, and Advanced Curation Initiatives</td>
<td>Andrea Harrington</td>
</tr>
<tr>
<td>DWI at the University of Leicester</td>
<td>John Bridges &amp; John Holt</td>
</tr>
<tr>
<td>Design Associated with Contamination Controlled Facilities - What is Possible?</td>
<td>Udo Gommel &amp; Guido Kreck</td>
</tr>
</tbody>
</table>
Appendix C: Discussion of the Dual-Walled Isolator (DWI) Prototype

By John Holt and John Bridges

In this appendix, the basic concept of the DWI is discussed and mapped to the scientific requirements of PP, BHP, BC and PE (planetary protection, bio-hazard assessment protocol, basic characterization and Preliminary examination). In terms of analytical instrumentation, the broad interface and accommodation definitions of Category 1, 2 and 3 are used in relation to the proposed curatorial and scientific investigations undertaken inside a DWI or variation of a DWI.

NB: The DWI concept has adopted the instrument interface nomenclature defined in the “Report on the workshop outputs from the Working Group on Scientific Investigations to be conducted in the Mars Sample Receiving Facility, 2015” (ref: E913-010\Working Group Report).

Background:

The concept of a double walled isolator has been around for some time, but the ESA/University of Leicester/TAS breadboard is the first time such a technology has been realized (to the best of our knowledge). Certainly for a Cat V, restricted, sample return mission (like MSR), the technology for safely handling material and performing analysis (inside an isolator within a BSL-4 type facility), does not currently exist. The challenge of safe handling, on Earth, is mandated by international planetary protection requirements relating to article IX of the UN Outer Space treaty; ratified by the US and UK in 1967. This may be updated to provide greater clarity to specific requirements but many of the engineering challenges of conducting science would still remain.

DWI Rationale:

Typical isolator technology generally falls into two main types:

1. **Negative Pressure**
   The gas pressure inside is maintained **negative** (typically a few hundred Pascal’s) wrt the external environment. In this configuration it is possible to contain a material that might be very pathogenic or toxic because the direction of any leak would be inwards (nothing gets out).

2. **Positive Pressure**
   The gas pressure inside is maintained **positive** (typically a few hundred Pascal’s) wrt the external environment. In this configuration it is possible to maintain a high level of cleanliness and contamination control because the direction of any leak would always be outward (nothing gets in).

NB: In reality, definitions and standards of isolators, bio-safety cabinets (BSC) and glove boxes are complex (and costly) subjects and their application must be tailored to a particular set of user requirements.

A Mars sample return mission would have requirements for both negative and positive pressures related to the risk of terrestrial contamination, but at the same time science requires the sample to be maintained at different levels of a “pristine” state. The pressure regime of the DWI is able to accommodate both requirements as illustrated below.
NB: MSPG-2 Workshop – pristine and ultra-pristine was used to propose, as yet, undefined levels of sample interaction.

![Diagram: DWI Concept showing the containment pressure regime.](image)

**Figure C1**: DWI Concept showing the containment pressure regime.

In its simplest form, assume that the DWI working volume (WV) is a totally sealed stainless steel box and is maintained at a negative pressure \(\text{wrt}\) the local environment (e.g. cleanroom lab) Fig. C1. Welded to the side of the DWI is a secondary stainless steel box that is kept at positive pressure \(\text{wrt}\) the inside of the DWI WV. Referred to as the Large Intermediate Volume (LIV), this space is used for the passage of all interfaces connecting instruments inside the DWI to control equipment outside in the lab. This is the basic working principle of the DWI and variations of this configuration can be added to meet specific user requirements. In reality, the breadboard DWI is not a totally sealed box and some parts of the current breadboard use single seals (due to time and cost constraints). However, key interfaces use the pressure regime shown here, provide a double seal and ultra-pure, inert, gas in the LIV to prevent contamination of the sample in the event of a leak. Future iterations of the DWI would adopt the double seal pressure regime at all interface junctions as the technology is matured. Planetary protection takes precedence when considering sample return risk, which dictates the primary function of DWI as providing containment and isolation of the sample. Therefore, DWI has to be an enabling technology providing interfaces for a range of analytical techniques relevant to MSR’s science requirements while maintaining the integrity of its planetary protection role.

Many of the end user requirements (e.g. exact technique, instrument specifications, levels of contamination for a particular analysis) are not yet well defined. In addition, the sequence of events and sample processes in the terrestrial sample chain needs further consolidation. This would pose a considerable risk to the return phase of a MSR mission because of the short period between now and the possible return. The mitigation philosophy implemented in DWI adopts a common architecture and multi-use interfaces that enable both modularity and configuration flexibility. For example, DWI could
be used as a Class III BSC (with remote tele-operations) or the visor replaced with glove ports (this removes the double pressure seal and would need to be risk assessed) or further down the terrestrial sample chain, a Class II air curtain might be added. During the DWI BB design phase a specially designed interface (IF) flange was developed that enables Cat 2 and 3 instruments to be accommodated via an instrument box (IB). In fig C2 below, the main DWI box is shown with four IB’s that have been configured for a particular operation. In this example of a “Super” DWI, the IB is accommodating a Cat 2 instrument (X-ray CT, where the source is isolated in the IB from the main working volume by an x-ray window) and a Cat 3 instrument (ESEM, where the entire instrument can be isolated by a door and the sample placed inside for analysis).

Modularity also allows a chain of DWI’s to be configured for a particular process/operation and assembled into a chain during AIT/ATLO at the SRF. The CDR slide below highlights the initial concept based on available thinking from the scientific community at the time. The exact chain shown here is not important, but the modular capability of DWI is one of the unique selling points in addressing end user needs in a SRF. A chain of DWI’s could enable a sequence of analysis that may change between now and sample return or is not currently defined. For example, after imaging during BC assessment, it might be concluded that a sub-sample is best maintained at -20°C and in pure argon rather than nitrogen. An adjacent DWI in a chain could be configured to argon very quickly and the heat exchanger in the safe-change housing set to a lower temperature. With an IB between the two isolators (configured as an airlock with sealing doors), the sample could be manipulated into the new environment. The example of different gasses and temperatures was an issue raised at the recent MSPG workshop. DWI is able to address such sample chain variations and other containment parameter could be adjusted. The current

Figure C2: DWI 2016 interface Requirements Review showing a variation of a “Super” DWI.
DWI BB is also designed to deplete molecular oxygen and moisture to a reduced level achieving O2 – 372 ppm and H2O – 16 ppm respectively, during a recent test.

In relation to MSPG discussions, the DWI could be used to meet many, if not all, of the scientific and planetary protection requirements consistent with a 2-phase preliminary sample characterization process, as proposed by MSPG-1. Category 1 instruments and RTP interfaces have been tested in the current breadboard while maintaining class 1 containment and contamination control. Initial particulate cleanliness levels inside the breadboard yield zero counts over the range (5, 1, 0.5 & 0.3 µm) with biological swabbing unable to culture a SFC. Based on current designs, the following table summarizes the types of interfaces that may be used during different stages of the terrestrial sample chain if DWI technology was employed inside a sample receiving facility.

<table>
<thead>
<tr>
<th>DWI Purpose</th>
<th>Example Instruments</th>
<th>Instrument Categories (IF’s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reception</td>
<td>Imager, microscope, X-ray CT</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>BHP</td>
<td>GCMS, imager</td>
<td>1, 3</td>
</tr>
<tr>
<td>BC</td>
<td>Imager, microscope, Raman</td>
<td>1</td>
</tr>
<tr>
<td>PE</td>
<td>All inc X-ray CT and ESEM</td>
<td>1, 2, 3</td>
</tr>
<tr>
<td>Single DWI</td>
<td>All, RTP</td>
<td>1, 2, 3</td>
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**Table C1: DWI Interface Categories and example instruments.**

<table>
<thead>
<tr>
<th>DWI Chain</th>
<th>All, RTP, airlock/door &amp; Instrument box</th>
<th>1, 2, 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NB: In reality, each DWI can accommodate any instrument category interfaces including the instrument box, which may be configured to house a Co 60 source or used for temporary storage of samples between analyses.</td>
<td></td>
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</tr>
<tr>
<td>Cat 1 = Analytical instrument inside an isolator</td>
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<tr>
<td>Cat 2 = Analytical instrument where part of the instrument is inside an isolator (eg. sensor head)</td>
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<tr>
<td>Cat 3 = Analytical instrument that is totally outside an isolator</td>
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</table>

Conclusions:

The DWI breadboard should not be seen as a typical microbiological safety cabinet or BSL3/4 facility isolator, but rather as a flexible platform that can be configured to function over a range of containment levels and internal environmental conditions. DWI features flexibility and modularity as a method of meeting the needs of a MSRF and maintaining compatibility with the advancement of scientific methods and technology. Adopting a systems engineering approach in the DWI design allows asynchronous sub-system (instrument) development as well as multi-team contribution and testing of science instrumentation in the longer term. This is partly achieved with a common engineering architecture and a set of ICD managed ports that allow for the addition of COTS and OEM technologies to the DWI system. BSL-4 environments are not typically used for the type of scientific investigations generally associated with planetary materials and that presents a number of significant challenges. With the expected return of restricted samples on the timescale of ten years, coupled with uncertainty regarding the detail and cost of a BSL-4 type facility, highlights an element of risk to the return phase of the mission. As a mitigation strategy in managing that risk, adopting a DWI approach in the SRF has the potential to reduce cost drivers and maximize timely scientific returns on the longer term investment of the mission.

Appendix D: Feasibility of Utilizing an Existing BSL-4 Facility

Andrea D. Harrington and Corliss Kin I Sio

**Context**

Given the focus of the report, a number of containment facility guidelines discussed during the workshop are either not within the scope of CC or given further explanation not specifically discussed at the workshop and therefore are not included in the body of the report. Those concerns, information presented (but not discussed), as well as an expansions of some of the concepts discussed (e.g. existing facility size limitations), are below.

**Making a Dirty Space Clean**

A Fraunhofer presentation indicated that it is possible to build a cleanroom within a dirty space (see Figures D1 and D2 below).
Figure D1: Repurposing of pre-existing “dirty” space. BEFORE: Starting Point: Basement Vault. (Source: Udo Gommel, Fraunhofer IPA)

Figure D2: Example of repurposing of pre-existing “dirty” space. BEFORE: Starting Point: Basement Vault. AFTER: Realized LED research & production facility (Source: Udo Gommel, Fraunhofer IPA). This example is from Bucharest (Voluntari), Romania, and the work was done from 09-2011 to 02-2012 (6 months).
Structural Constraints

To our knowledge, there is no facility in existence with both the required bio-safety containment level (e.g. BSL-4) and clean room standards (e.g. ISO Class 5 or better). Therefore, any existing containment facility would undergo renovations, possibly extensive, in order to meet CC standards. During this process, the facility would need to be decontaminated and shut down for a significant amount of time. Furthermore, it is unknown if an existing containment facility exists that would be compatible with the needed modification for CC or could meet the space requirements.

- Infrastructure Clearance
  - Ceilings. The current heights of the room within containment facilities may be insufficient to construct suitably classed cleanrooms (e.g. ISO 5 and below) let alone allow for the installation and required mobility of DWIs. In order to allow for proper airflow within the laboratory and to accommodate internal airflow systems (e.g. plenums), a minimum of two feet is required above the cleanroom and approximately one foot of space is required if a raised floor system is utilized. The current design of the DWI stands approximately 9 feet high (although this is likely scalable). If the final design requires the measurements to remain the same, in order to ensure proper airflow and spacing the internal height of the cleanroom should be at least 10 feet high. This would bring the required containment facility room height to 12-13 feet.
  - Points of entrance/egress and pass-throughs. Given the features of doorways for entrance/egress from and within known containment facilities, it is improbable that large pieces of equipment (e.g. DWIs or analytical instrumentation) would fit into existing space. Feasibility of completing final assembly within the facility would need to be evaluated.

- Available Capacity, Flexibility and Expansion Capabilities
  - Capacity. Since the notional plan is to perform the bulk of sample handling and processing within a DWI, determining the number of isolators needed is vital in order to properly calculate space requirements. Given the necessity to avoid cross-contamination, possibly accommodate multiple micro-environments (e.g. nitrogen, argon, helium), and an array of functions, 10 DWIs is a reasonably conservative estimate. Since the square footage of the DWIs is approximately 63 ft² (9 ft x 7 ft) plus 45 ft² for open working space/safety (9 ft x 5 ft), the total square footage required for the DWIs alone is over 1,000 ft². Desiccators for storage, a small amount of table space, and any analytical equipment within the laboratory would require another 300+ ft². Plus another 300+ ft² cleanroom for cleaning is required for items of this size and CC/PP requirements this stringent.
  - Flexibility and Expansion Capabilities. Given the number of unknowns surrounding the Mars sample return (e.g. duration of quarantine, range of analytical equipment within containment) the facility should have the ability to accommodate an array of (possibly) rotating equipment and capable of expansion, particularly if samples are not deemed safe for release. Therefore adaptability and modularity is important for both short-term (~<2 years) and long-term (~2-50+ years) use.

- Integration of CC and PP Requirements
  - Structural modifications. It is uncertain whether new inert gas lines can be installed which may be required given the range of possible microenvironments proposed and the limited pristinity recirculation filters offer relative to single-pass inert gas (e.g. filters can cause silicon contamination due to their material properties).
Existing materials. Some types of contamination (e.g. silicon) can be very difficult to remove and represent a risk to sample pristinity (even if DWIs are within a cleanroom).

**Implementation of Cross-Facility Rules and Procedures**

It is unknown how ongoing work performed by other tenants may affect martian samples. As with any shared space, the facility manager or co-tenant may have to impose rules and procedures not required for Mars samples or Mars processor and scientist. Given the hazards associated with the studies performed in a BSL-4 laboratory, Mars sample processors and scientists would be bound to follow imposed restrictions, even if they may negatively affect samples. Conversely, even if there are initial requirements that the rules for each co-tenant are propagated between groups, depending on the situation, it may not be possible for them to take the same precautions required to keep the martian samples safe.

- **Sample and personnel safety**
  - Cleanliness. BSL-4 facilities typically have negative pressure, meaning they are made increasingly dirty with time; even if one can be made clean with an internal clean volume, the starting conditions work against cleanliness. It may not be possible to get an existing BSL-4 facility clean enough, especially when shared.
  - Containment Breach. Possible breach of containment of hazards under the purview of facility co-tenants may affect the security of samples and the integrity of science performed.
  - Scientific Integrity. There is a question of whether the integrity of the data, particularly related to life detection, is less credible in a shared space (even if the spaces are technically separated).

**Facility Fair Use**

Given the international nature of planned Mars sample return efforts, it is important to ensure all scientists are able to have access to the samples. There are a number of concerns related to sharing a facility with a different government agency (e.g. DOD). While some of these can be alleviated by utilizing a university facility (e.g. facility access), other concerns (e.g. sharing space) are not mitigated. Existing BSL-4 facilities have access restrictions for foreign nationals.

- **Government Facility Access**
  - Foreign Researcher. Guaranteed long term foreign access to existing BSL-4 facilities could be difficult. Currently, some foreign researchers need to be escorted which is not conducive to productivity or inclusivity. However, with additional initial screening, foreign nationals are usually/mostly able to work autonomously in United States government facilities. As long as the information is not deemed as classified (which Mars would not be deemed). Currently existing bsl4 facilities accommodate international teams. Any facility must be EASILY accessible by international teams (see Euro CARES for more information).
  - (Inter)National Crisis. In the case of an (inter)national crisis, Mars sample scientist and curation leads could lose control over the security of the samples contained in a facility needed for biohazard assessment.

**Cost Effectiveness**

Depending on the duration of which the containment facility would be needed (~1-5+ years), a new building may be more justifiable in terms of cost.

- Modification and maintenance of a pre-existing facility could end up being more costly than a new facility designed specifically for MSR samples.
• If the containment facility is unable to expand in size and capabilities, a new facility may be required to accommodate evolving needs, particularly if the samples are never deemed safe for release.

Conclusions
Facility planning and implementation need to be started as soon as possible given the proposed Mars Sample Return Campaign timeline. However, the numerous unknowns related to Mars sample return do not allow for specific guidelines for a containment facility. It would be important to minimize costs related to either new construction or adapting an existing facility (provided the concerns above are addressed) while maximizing the credibility of the sample analyses. Reusing existing BSL-4 laboratory space is theoretically possible since it is likely that initial contamination control requirements can be met. More studies are necessary to make a final evaluation. However, the workshop consensus is that a new fully-integrated supranational facility is the best option.

Appendix E: Acronyms

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<tr>
<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>AIT</td>
<td>Assembly Integration &amp; Test</td>
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<tr>
<td>ALARA</td>
<td>As low as reasonably achievable</td>
</tr>
<tr>
<td>ATLO</td>
<td>Assembly Test &amp; Launch Operations</td>
</tr>
<tr>
<td>BB</td>
<td>Breadboard (prototype)</td>
</tr>
<tr>
<td>BC</td>
<td>Basic Characterization</td>
</tr>
<tr>
<td>BHP</td>
<td>Biological Hazard Assessment Protocol</td>
</tr>
<tr>
<td>BSL</td>
<td>Bio-safety Level</td>
</tr>
<tr>
<td>CC</td>
<td>Contamination Control</td>
</tr>
<tr>
<td>CDR</td>
<td>Concept Design Review or Critical Design Review (depending on context)</td>
</tr>
<tr>
<td>CF</td>
<td>Con-flat</td>
</tr>
<tr>
<td>CK</td>
<td>Contamination Knowledge</td>
</tr>
<tr>
<td>CT</td>
<td>Computerized Tomography</td>
</tr>
<tr>
<td>DWI</td>
<td>Double Walled Isolator</td>
</tr>
<tr>
<td>ESEM</td>
<td>Environmental Scanning Electron Microscope</td>
</tr>
<tr>
<td>GCMS</td>
<td>Gas Chromatography Mass Spectrometry</td>
</tr>
<tr>
<td>HEPA</td>
<td>High Efficiency Particulate Air</td>
</tr>
<tr>
<td>IB</td>
<td>Instrument Box</td>
</tr>
<tr>
<td>IF</td>
<td>Interface</td>
</tr>
<tr>
<td>MSPG</td>
<td>Mars Science Planning Group</td>
</tr>
<tr>
<td>PE</td>
<td>Preliminary Examination</td>
</tr>
<tr>
<td>RM</td>
<td>Remote Manipulator</td>
</tr>
<tr>
<td>RTP</td>
<td>Rapid Transfer Port</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning Electron Microscope</td>
</tr>
<tr>
<td>SRF</td>
<td>Sample Receiving Facility</td>
</tr>
<tr>
<td>SSAP</td>
<td>Sample Safety Assessment Protocol (SSAP) working group</td>
</tr>
<tr>
<td>UDF</td>
<td>Uni-directional Flow</td>
</tr>
<tr>
<td>wrt</td>
<td>With respect to</td>
</tr>
</tbody>
</table>
## Appendix F: Mars 2020 L1 and L2 Return Sample Contamination Control and Planetary Protection Requirements

Table 1: Mars 2020 Level 1 Planetary Protection, Biological and Organic Contamination Requirements

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Object Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>NPR 8020.12D</td>
<td>The Mars 2020 Project shall comply with requirements for the outbound portion of a Planetary Protection Category V Restricted Earth Return mission as defined in NPR 8020.12D and as clarified in Section 6.8 of this PLRA.</td>
</tr>
<tr>
<td>Potential Contamination Characterization</td>
<td>The project shall identify, quantify, document, and archive potential pre-launch terrestrial contamination sources, both organic compounds and organisms, and provide mechanisms to support characterization of round-trip terrestrial contamination.</td>
</tr>
<tr>
<td>Viable Organisms</td>
<td>The Mars 2020 landed system shall be capable of encapsulating samples for return such that each sample in the returned sample set has less than 1 viable Earth-sourced organism.</td>
</tr>
</tbody>
</table>
| Organic Carbon             | The Mars 2020 landed system shall be capable of encapsulating samples for return such that the organic contamination levels in each sample in the returned sample set are less than:  
  - Any Tier 1 compound (organic compounds deemed as essential analytes for mission success): 1 ppb  
  - Any Tier 2 compound (organic compounds not categorized as Tier 1): 10 ppb  
  - Total Organic Carbon: 10 ppb Baseline, 40 ppb Threshold |
Table 2: Mars 2020 Tier 1 Compound Requirements on Sample

<table>
<thead>
<tr>
<th>Contaminant class</th>
<th>Examples</th>
<th>Potential measurement methodology</th>
<th>Comments/Justification</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleic acid</td>
<td>DNA</td>
<td>Interception dye and hanging drop fluorimeter</td>
<td>DNA is the universal signature for terrestrial life and, therefore, terrestrial contamination</td>
<td>Liu et al. (2013)</td>
</tr>
<tr>
<td>Spores</td>
<td>Dipicolinic acid</td>
<td>Fluorescence</td>
<td>Bacterial spores are the most recalcitrant form of terrestrial biota</td>
<td>Krísný et al. (2013)</td>
</tr>
<tr>
<td>Bacterial and fungal cell walls</td>
<td>N-Acetylglucosamine</td>
<td>LC-MS</td>
<td>Bacterial and fungal cell wall components may be detectable after the cell is destroyed.</td>
<td>Schleifer and Kandler (1972) Barriañez-Garcia (1968)</td>
</tr>
<tr>
<td>Amino acids</td>
<td>Glycine</td>
<td>LC-MS</td>
<td>Glycine is the most abundant amino acid in nature; abundant in fingerprints.</td>
<td>Salazar et al. (2012)</td>
</tr>
<tr>
<td>Lipids</td>
<td>Alanine</td>
<td>GC-MS</td>
<td>Alanine is chiral and abundant.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Palmitic acid</td>
<td>GC-MS</td>
<td>Most common fatty acid in bacteria and eukarya.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Squalene</td>
<td>GC-MS</td>
<td>Lipid common to all life; abundant in fingerprints</td>
<td></td>
</tr>
<tr>
<td>Hydrocarbon biomarkers</td>
<td>Pristane</td>
<td>GC-MS</td>
<td>Common component of petroleum and, therefore, petroleum-derived aerosols</td>
<td></td>
</tr>
<tr>
<td>Martian organics</td>
<td>Chlorobenzene</td>
<td>GC-MS</td>
<td>Need at least one likely Mars-derived organic compound. Chlorobenzene is a reaction product of aromatic carboxylic acids (e.g., benzoic, phthalic) with perchlorate.</td>
<td>Biemann et al. (1977) Navarro-González et al. (2010)</td>
</tr>
<tr>
<td></td>
<td>Dichloromethane</td>
<td>GC-MS</td>
<td>Identified by both Viking and MSL. May be terrestrial and/or martian contaminant</td>
<td>Biemann et al. (1977) Navarro-González et al. (2010)</td>
</tr>
<tr>
<td>PAHs</td>
<td>Naphthalene</td>
<td>GC-MS</td>
<td>Most abundant and readily detectable PAH. PAHs have been detected in ALH 84001 and DaG 476 and appear to be part of the aromatic inventory of martian igneous and possible biogenic processes. Should be monitored to avoid false-positive measurements</td>
<td>Clemett, et al. (1998) Steele et al. (2012)</td>
</tr>
<tr>
<td>Nitrogenous compound</td>
<td>Urea</td>
<td>LC-MS</td>
<td>Important to prebiotic chemistry</td>
<td>Esther et al. (2008) Hu et al. (1994)</td>
</tr>
<tr>
<td>Short-chain carboxylic acid</td>
<td>Acetic acid</td>
<td>GC-MS</td>
<td>Simple organic acid relevant to both biological and industrial contamination sources</td>
<td></td>
</tr>
<tr>
<td>Polyhydroxy compound</td>
<td>Glycerol</td>
<td>GC-MS</td>
<td>Simple polyol relevant to both biological and industrial contamination sources</td>
<td></td>
</tr>
<tr>
<td>Hydroxy carboxylic acid</td>
<td>Pyruvic acid</td>
<td>LC-MS or GC-MS</td>
<td>Metabolite of sugars and important metabolic intermediate</td>
<td></td>
</tr>
<tr>
<td>Linear hydrocarbons</td>
<td>n-Heptacosane</td>
<td>GC-MS</td>
<td>Common industrial hydrocarbon contaminant</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Mars 2020 Level 2 Inorganic Contamination Requirements

<table>
<thead>
<tr>
<th>Requirement</th>
<th>Object Text</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inorganic Contamination at 0.1%</td>
<td>The PS shall limit contamination of rock samples with Earth-sourced inorganic contaminants to no more than the contamination mass limits listed in Table IOC for the following 12 elements: K, Rb, Sr, Sm, Nd, U, Th, Re, Os, Lu, Hf, W</td>
</tr>
<tr>
<td>Inorganic Contamination at 1%</td>
<td>The PS shall limit contamination of rock samples with Earth-sourced inorganic contaminants to no more than the contamination mass limits listed in Table IOC for the following 23 elements: Zr, Nb, Ta, La, Ce, Eu, Gd, Li, B, Cs, Sc, Mn, Y, Mg, Zn, Ni, Co, Cl, Br, P, S</td>
</tr>
<tr>
<td>Inorganic Contamination: Lead</td>
<td>The PS shall limit contamination of rock samples with Earth-sourced Pb contaminants to no more than the Pb contamination mass limit listed in Table IOC.</td>
</tr>
</tbody>
</table>
Table IOC (see Mars 2020 Inorganic Contamination Requirements)

<table>
<thead>
<tr>
<th>Elements</th>
<th>Target % of SNC</th>
<th>(g/g)</th>
<th>absolute quantity</th>
<th>unit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>1</td>
<td>1.75E-03</td>
<td>15.7000</td>
<td>mg</td>
</tr>
<tr>
<td>K</td>
<td>0.1</td>
<td>1.66E-07</td>
<td>2.49</td>
<td>μg</td>
</tr>
<tr>
<td>S</td>
<td>1</td>
<td>1.18E-05</td>
<td>168.0000</td>
<td>μg</td>
</tr>
<tr>
<td>P</td>
<td>1</td>
<td>2.20E-05</td>
<td>327.0000</td>
<td>μg</td>
</tr>
<tr>
<td>Cl</td>
<td>1</td>
<td>6.30E-07</td>
<td>9.4600</td>
<td>μg</td>
</tr>
<tr>
<td>Br</td>
<td>1</td>
<td>4.00E-09</td>
<td>0.0593</td>
<td>μg</td>
</tr>
<tr>
<td>Li</td>
<td>1</td>
<td>2.10E-08</td>
<td>0.3140</td>
<td>μg</td>
</tr>
<tr>
<td>B</td>
<td>1</td>
<td>1.00E-08</td>
<td>0.1500</td>
<td>μg</td>
</tr>
<tr>
<td>Sc</td>
<td>1</td>
<td>3.90E-07</td>
<td>5.9100</td>
<td>μg</td>
</tr>
<tr>
<td>Mn</td>
<td>1</td>
<td>3.70E-05</td>
<td>559.0000</td>
<td>μg</td>
</tr>
<tr>
<td>Co</td>
<td>1</td>
<td>5.80E-07</td>
<td>8.7500</td>
<td>μg</td>
</tr>
<tr>
<td>Ni</td>
<td>1</td>
<td>2.70E-06</td>
<td>39.8000</td>
<td>μg</td>
</tr>
<tr>
<td>Zn</td>
<td>1</td>
<td>6.30E-07</td>
<td>9.4650</td>
<td>μg</td>
</tr>
<tr>
<td>Rb</td>
<td>0.1</td>
<td>3.40E-10</td>
<td>0.0050</td>
<td>μg</td>
</tr>
<tr>
<td>Sr</td>
<td>0.1</td>
<td>3.30E-08</td>
<td>0.4990</td>
<td>μg</td>
</tr>
<tr>
<td>Y</td>
<td>1</td>
<td>1.40E-07</td>
<td>2.1100</td>
<td>μg</td>
</tr>
<tr>
<td>Zr</td>
<td>1</td>
<td>2.10E-07</td>
<td>3.2120</td>
<td>μg</td>
</tr>
<tr>
<td>Nb</td>
<td>1</td>
<td>2.50E-09</td>
<td>0.0370</td>
<td>μg</td>
</tr>
<tr>
<td>Cs</td>
<td>1</td>
<td>1.60E-10</td>
<td>0.0020</td>
<td>μg</td>
</tr>
<tr>
<td>La</td>
<td>1</td>
<td>3.00E-09</td>
<td>0.0450</td>
<td>μg</td>
</tr>
<tr>
<td>Ce</td>
<td>1</td>
<td>1.10E-08</td>
<td>0.1580</td>
<td>μg</td>
</tr>
<tr>
<td>Nd</td>
<td>0.1</td>
<td>1.50E-09</td>
<td>0.0230</td>
<td>μg</td>
</tr>
<tr>
<td>Sm</td>
<td>0.1</td>
<td>9.74E-10</td>
<td>0.015</td>
<td>μg</td>
</tr>
<tr>
<td>Eu</td>
<td>1</td>
<td>4.50E-09</td>
<td>0.0680</td>
<td>μg</td>
</tr>
<tr>
<td>Gd</td>
<td>1</td>
<td>1.80E-08</td>
<td>0.2660</td>
<td>μg</td>
</tr>
<tr>
<td>Lu</td>
<td>0.1</td>
<td>1.80E-10</td>
<td>0.0030</td>
<td>μg</td>
</tr>
<tr>
<td>Hf</td>
<td>0.1</td>
<td>9.10E-10</td>
<td>0.0140</td>
<td>μg</td>
</tr>
<tr>
<td>Ta</td>
<td>1%</td>
<td>1.38E-10</td>
<td>0.002</td>
<td>μg</td>
</tr>
<tr>
<td>W</td>
<td>0.1</td>
<td>4.10E-11</td>
<td>0.6150</td>
<td>ng</td>
</tr>
<tr>
<td>Re</td>
<td>0.1</td>
<td>1.70E-11</td>
<td>0.2480</td>
<td>ng</td>
</tr>
<tr>
<td>Os</td>
<td>0.1</td>
<td>1.10E-10</td>
<td>1.6200</td>
<td>ng</td>
</tr>
<tr>
<td>Pb</td>
<td>0.1</td>
<td>2.00E-10</td>
<td>3.0000</td>
<td>ng</td>
</tr>
<tr>
<td>Th</td>
<td>0.1</td>
<td>2.40E-11</td>
<td>0.3800</td>
<td>ng</td>
</tr>
<tr>
<td>U</td>
<td>0.1</td>
<td>7.00E-12</td>
<td>0.1050</td>
<td>ng</td>
</tr>
</tbody>
</table>