The Relationship of Mars Sample Return Science and Containment

A Report from the Workshop "MSR Science in Containment," January 14th-16th, 2019 in Columbia, Maryland

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EXECUTIVE SUMMARY

Samples returned from Mars would be placed in quarantine, referred to here as containment, until they are deemed safe to release to outside laboratories, either by analysis or by sterilization. A key planning question related to a potential future Mars Sample Return Campaign is "To what extent does MSR science need to be done in containment?" The answer to this would determine the character of the science-sourced requirements on a notional Sample Receiving Facility (SRF), including the number and definition of additional supporting science-related facilities (both within and outside of containment). This question was discussed at a 3-day workshop January 14-16, 2019 in Columbia, MD. Three high-level conclusions were that 1) Where the option exists to conduct MSR investigations within containment or at scientists' home laboratories, the latter is overwhelmingly preferred; 2) For >90% of the MSR science that has been mapped out by the iMOST committee, the investigations appear to be tolerant to at least 1 sterilization method that is used on Mars spacecraft (and might be permitted for use on Mars samples); and 3) Very few of the MSR science measurements are time-sensitive (at the scale of hours/days/weeks). This leads to the derived conclusion that most MSR science could be effectively planned for, using either sterilized samples or samples that have passed the Sample Safety Assessment Protocol, in labs distributed around the world outside of containment, and without time urgency. Science requirements for the SRF, therefore, would fall primarily into the areas of preliminary sample characterization, sterilization-sensitive investigations, and time-sensitive investigations.

SUMMARY OF FINDINGS

FINDING #1: There are three sets of observations that may be beneficial before opening sample tubes: 1) Reconnaissance analysis of dust on the outsides of tubes; 2) Basic physical observations; 3) Micro- and nano-beam x-ray 3-D imaging (e.g., CT, Synchrotron, other).

FINDING #2: Prior to making the samples available to the world's research community, a 2-phase preliminary sample characterization process would need to be completed: Basic Characterization (BC) and Preliminary Examination (PE).

FINDING #3: The Preliminary Examination of MSR samples may be optimized by using different teams of international scientists for different samples (or groups of samples), although this may not be the only way to do it.

MAJOR FINDING #4: **It appears that a large majority (>90%) of the MSR-related science investigations, as identified by the International MSR Objectives & Samples Team (iMOST, 2019), could probably be acceptably performed on sterilized samples, thus potentially enabling the analysis of MSR samples in uncontained laboratories without a dependency on the results from Sample Safety Assessment Protocol (SSAP) testing.**

FINDING #5: It is expected that the properties of the samples would be vulnerable to degradation in at least 4 significant areas as soon as they are removed from the equilibrium environment inside their tubes. Because of the time-sensitivity, these attributes should be measured quickly, or the opportunity may be irretrievably lost. This may require that these measurements be done in containment.

MAJOR FINDING #6: **The scientific community, for reasons of scientific quality, cost, timeliness, and other reasons, strongly prefers that as many sample-related investigations as possible be performed in PI-led laboratories outside of containment.**

FINDING #7: For reasons of optimizing the use of irreplaceable sample mass, consortium sample utilization studies, including those that make use of facility-related sample-preparation procedures, are of high interest.

FINDING #8: Space within containment must logically include functionality for BC+PE, SSAP tests, time-sensitive science, and sterilization-sensitive science. Sterilization-tolerant science can most effectively be planned outside of containment.

CONCLUSIONS & NEEDS FOR FUTURE WORK

Needs for Future Work:

Based on the workshop proceedings and the findings above, the authors of this report strongly suggest that funding, and coordinated work teams, are needed in five high-priority areas:

1) Effects of sterilization processes on geological samples;
2) Effects of x-ray imaging on sample properties;
3) Effects of sample analysis and sample prep on sample properties;
4) Degree of overlap between MSR sterilization-sensitive science and SSAP investigations;
5) Identity and determine the relative importance & degree of degradation with time of time-sensitive MSR science measurements.

Conclusion #1: The iMARS-2 (Haltigin et al., 2018) report provides a good starting point for further discussions and analysis regarding the organization, management and staffing of a notional Sample Receiving Facility.

Conclusion #2: The workshop group was unable to identify any investigations that are sensitive only to radiation (i.e., but not also to heat). This is therefore judged to be a more promising sterilization method, if the metric is preservation of scientific value of the samples.

Conclusion #3: Assuming that isolator cabinets can be effectively cleaned between samples (considered technically reasonable at this time), the number of needed isolators is judged to be less than the number of samples. For planning purposes, a figure of 15 ± 5 isolators may be a reasonable estimate. This number could be influenced by several factors, including the different types of environmental conditions desired for different samples and processes (e.g., vacuum, N_2, He, Ar atmospheres, low temps, etc.), differing contamination requirements for different processes, how many samples might be worked on simultaneously, etc.

Conclusion #4: We cannot see that analysis of the headspace gas would be important for operational decision-making before the regolith and rock samples are extracted from the sample tubes. However, the chemistry of the headspace gas is vulnerable to change with time, and it should be analyzed promptly for that reason.

1. INTRODUCTION

What Question are we trying to Answer?

In order to design the lowest-cost Sample Receiving Facility that is able to meet its requirements, it is important to answer the following question: **To what extent does MSR science need to be done in containment?** We assume that as part of the MSR Campaign, planning needs to be in place to achieve ALL of the scientific objectives of MSR. However, there has been a history of different arguments on how much of that needs to take place in containment.

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 this report, was focused on investigations that need to be performed while under biological quarantine, defined here as "in containment". Two other workshops are planned to address 1) contamination considerations, and 2) the integration of science and SSAP investigations needed in containment. Thus, contamination and SSAP considerations were explicitly excluded from this workshop.

The workshop participants discussed a set of prepared questions during three breakout sessions. Participants were assigned to one of three groups roughly based on the primary disciplines of: astrobiology (Group 1), geochemistry (Group 2) and curation (Group 3). 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 will require follow-up work by somebody. 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 contamination control and planetary protection recommendations, as mentioned above, will be developed in subsequent workshops. After the workshop series is complete MSPG intends to finalize an overall summary of its conclusions.

As part of the process of planning the workshop, we recognized early that the five topics represented by the colored boxes on Figure 1 constitute the essence of the problem. In order to ensure that science and planetary protection inputs to MSR planning are held independent, we did not debate the pink "PP" topic. However, for the other four topics, we constructed discussion prompts to help elicit from our science experts primary planning inputs.

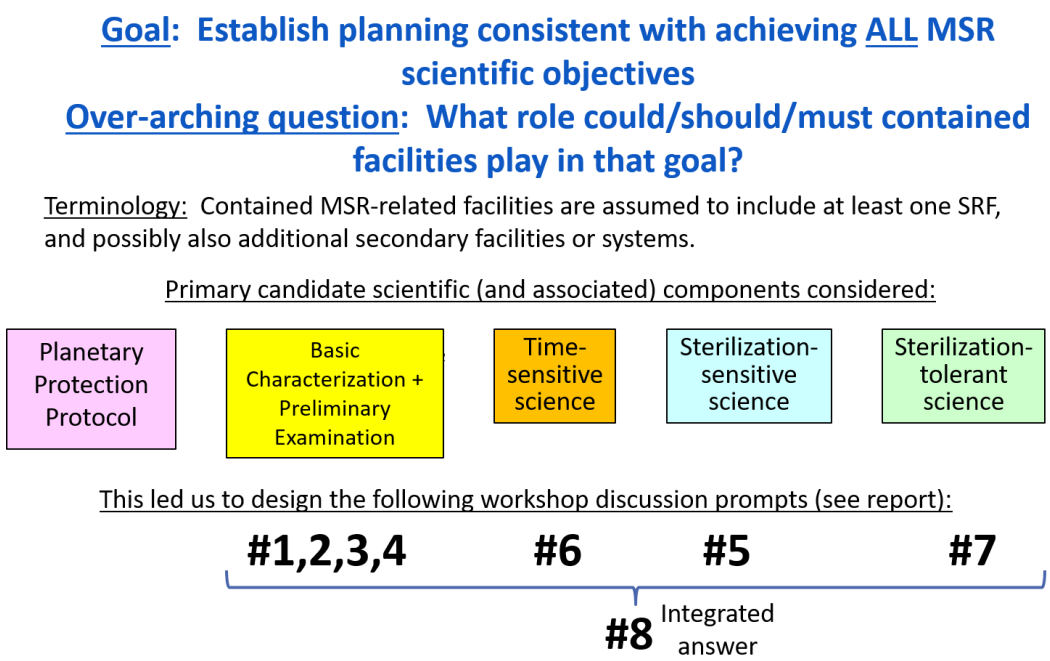


Figure 1*. Schematic representation of the eight topics discussed in the workshop. See report for the specifics of the discussion prompts used.*

SRF and Containment—Terminology. A Sample Receiving Facility (SRF) is considered to be a necessary part of planning the MSR Campaign. This is where the sample-containing returned spacecraft would be received, and samples processed and analyzed under biological containment. The attributes of that facility (or facilities), as defined by requirements, are still under discussion. Some as-yet open questions include the acceptability of using existing facilities, strategies and priorities relating to scientific investigations in containment, strategies for contamination control, the benefits/consequences of using more than one facility, strategies that enable scientists to do their work with excellence, etc. However, the samples could still leave the SCF if the technique is deemed important enough and they remain in containment (e.g., packaging, transport, and/or BSL-4 rated facility) during the analyses. In this respect, we do not use "SRF" and "containment" as synonyms.

This report. This is a report from the first of the series of three planned workshops. It was held at the Universities Space Research Association (USRA) Headquarters in Columbia, Maryland between 14th – 16th January 2019 with 28 participants (see Appendix A). The theme of the workshop was "Science in Containment", and its scope covered the initial examination of samples and formulation of strategies for how much sample science must/should be planned for within containment. The second and third workshops will have the themes of contamination control and incorporating analyses needed for planetary protection assessment, both of which were not addressed in this workshop. Planetary protection assessment is being addressed at this time by a separate COSPAR-sponsored group defining a recommended "Sample Safety Assessment Protocol".

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 future workshops may need to be reconsidered.

1. The scientific objectives of MSR are those described by iMOST (2019).
2. The sample-related facility scenario would be as follows:
    a. Overall sample science and facility management (of any and all facilities that host samples returned from Mars) would operate under a TBD model of international governance.
    b. A "BSL-4"-rated SRF in the U.S. would be responsible for sample containment until such time as they are deemed safe for release or transfer under containment to another equivalently rated facility.
    c. Additional uncontained curation facility(s) in the US and/or Europe would exist. A European facility would 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.
    d. PIs, located around the world in academic institutions, research institutes, government laboratories, and elsewhere, will desire access to the SRF and curation (primary) facilities, and eventually if safe, access to samples distributed outside the curation facilities.
    e. The decision on where to locate the U.S. SRF or a potential European bio-contained facility will 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.

In addition, we make the following technical assumption:

- All material from Mars will be collected as the layers of sample containment in the returned spacecraft are progressively opened, including rock samples, dust on the outside of the tubes, headspace gas inside the sample tubes, bulk atmospheric gas (if present), and hardware that has been exposed to the martian environment.
-

2. PRELIMINARY SAMPLE CHARACTERIZATION

2.1. Measurements on Unopened Tubes

Discussion Prompt #1: What is the priority of measurements made before the tubes are opened? Should or must such measurements be done?

There is a limited set of measurements that could potentially be made on unopened sample tubes. The workshop participants were able to identify only three that were deemed to be potentially beneficial to the sample characterization process: 1) Collection and analysis of dust on the exterior of the tubes (and other exposed surfaces in the OS); 2) Physical measurements of the unopened tube; and 3) micro- and nano-beam x-ray imaging. These are illustrated in Figure 2.

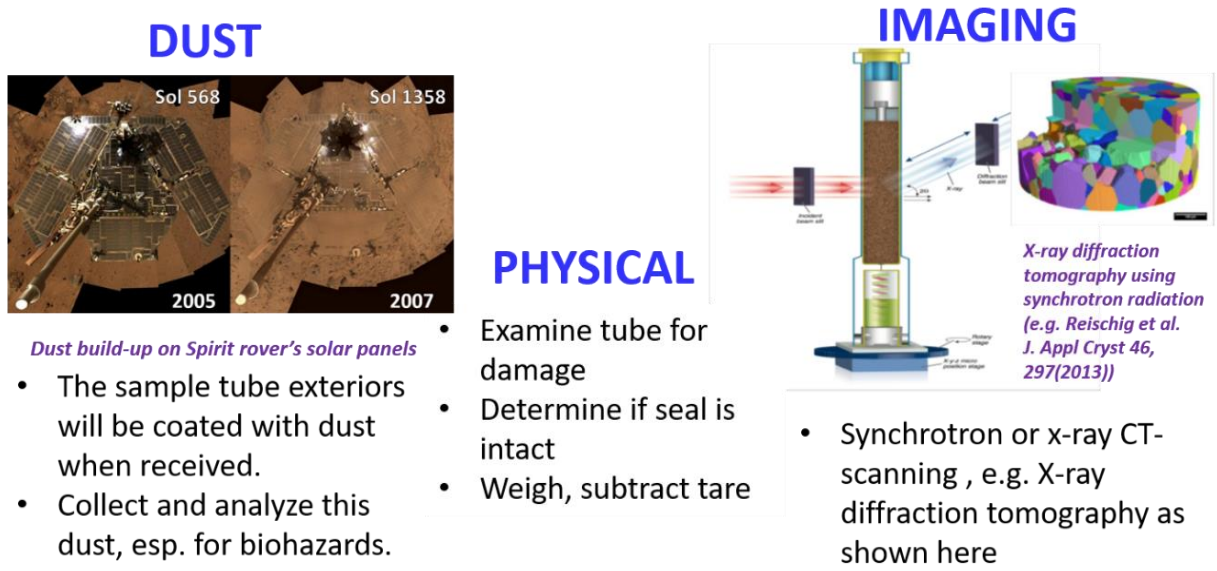


Figure 2*. Summary of three classes of measurements that could or should be done on sample tubes before they are opened. Left) Dust build-up on the Mars Exploration Rover Spirit's solar panels; Center) Description of physical measurements to be done on the unopened sample tubes; Right) schematic of one type of imaging technique that could be performed on unopened tubes*

Examination of dust on the outside of the tube

As illustrated in Fig. 2-left (which is an image of the solar panels on the rover *Spirit* after it had been on the martian surface for a few years), we can expect that objects on the martian surface will become coated with particulate grains ("dust") that settle out of the martian atmosphere. This includes the exterior of the sample tubes and the interior surfaces of the notional Orbiting Sample (OS), which would need to be open during sample tube loading. Because it would be possible to analyze this material before the sample tubes are opened, the question is whether there is a reason to do so, and whether or not the data would have decisional value. If not, the analysis of this sample material could simply be allocated to the research community in the same manner as other sample types would be allocated.

This particulate sample would presumably represent mixed contributions from various areas of the surface of the Mars, including from the local Mars surface environment where the sample tubes were cached. These particulate grains, much like those from the Hayabusa mission, would certainly be of interest to scientists, but this does not mean that they should or must be analyzed right away. These samples would intrinsically be less valuable than the sample material inside the tubes for at least two important reasons: 1) They would lack context, unlike the carefully documented samples inside the tubes, and 2) The exterior surfaces of solid samples are not expected to be as clean as their interiors so the exterior dust particles are subject to higher levels of contamination. One option to consider would be to sterilize a sub-sample of the exterior dust, and send it out of containment for analysis. This could perhaps provide useful input to the design and conduct of the Sample Safety Assessment Protocol (SSAP). Could this potentially help guide biohazard testing and allow for refinement of the test protocols? These samples could play an important role in understanding the contamination state of the exterior of the tubes, which may be an important contribution to interpreting the critical SSAP tests.

Even though analyses of the exterior dust will be of interest for purposes related to traditional planetary science, in addition to the reasons above, we were unable to identify compelling reasons why these measurements need to be done quickly—i.e., before the primary samples in the tubes are extracted.

Physical measurements

A certain set of physical measurements will clearly be needed (see Fig. 1-center): 1) Examine the sample tube for any form of damage; 2) Evaluate the quality of the sample tube seal (one example might be to place the sample tube in an evacuated volume, and watch to see if the pressure increases); and 3) Weighing, and subtracting the pre-launch tare weight, in order to generate an initial measurement of sample mass.

Micro- and nano-beam x-ray imaging

The potential value of penetrative imaging has been recognized for many years. In fact, one of the design requirements for the sample tubes was that they not preclude this kind of investigation. This led to extensive discussion of the potential benefits and consequences of through-the-wall imaging using penetrative energy (e.g., X-ray, or other) or some or all of the sample tubes. Micro- and nano-beam x-ray imaging, using synchrotron radiation for example, can deliver information on the physical state, morphology (e.g., presence of void space), the mineralogical composition and spatial distribution of minerals in samples, as well as the chemical speciation and distribution of (potentially biogenic) elements. The technique can also validate the integrity of the sample tubes, e.g., by checking the seals for potential cracks and leaks. This information may provide important guidance on the sequencing of opening the tubes, the specifics of how to get the samples out with minimal damage, and initial planning for deciding the initial sample subset that would go for SSAP testing. We already know that such imaging is not damaging to most investigations of scientific interest on the kinds of samples we are expecting from Mars. However, it is also known that these techniques are not 100% non-destructive. The essential questions are: 1) how destructive are they, and what would be damaged, and 2) the priority of the measurements affected.

For the existing list of MSR scientific objectives, and component sample-related measurements (iMOST, 2019), the only measurements of special concern thus-far identified were the measurements of certain organic molecules. The main concern is that organics of interest may be altered or destroyed by micro- and nano-beam x-ray radiation. This was discussed at length by the workshop's astrobiology sub-team, and by the end of the workshop, our pre-workshop fears were at least partially allayed. Available studies of X-ray and gamma ray irradiation of amino acids in meteorites during various microcomputed tomography imaging experiments found that this X-ray exposure (up to ~ 3 kGy) did not noticeably degrade amino acids, nor cause any amino acid racemization (Friedrich et al., 2016, 2019). However, experimental studies have shown that gamma ray exposure of both pure standards and meteorites will lead to increasing amino acid radiolysis as a function of molecular weight at total ionizing doses above 0.5 MGy, although the amino acid enantiomeric ratios were preserved (Iglesias-Groth et al., 2010; Kminek & Bada, 2006). These studies observed evidence of degradation of heavier amino acids by gamma rays at doses of 0.2-0.5 MGy.

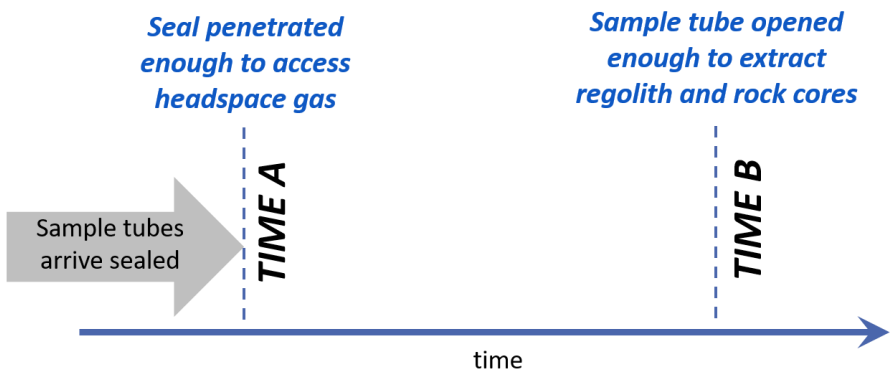
Additional investigations are warranted to study effects on other soluble organic compounds and refractory organic components. Soundwave tomography was mentioned in the workshop, but the workshop attendees do not feel that they have the background to judge its application to unopened sample tubes. Although neutron beam imaging may also provide important information as to the location and abundance of organics within the samples (e.g., Carlson, 2006), there was strong concern expressed at the workshop regarding the known effects of this method on sample geochemistry.

In summary, the potential benefits of imaging the samples through the tube walls before opening are deemed important enough that further evaluation of any possible deleterious side effects is warranted, and is of high priority.

Need for Future Work #2: The workshop attendees concluded that more research is required before committing to applying through-the-tube irradiation techniques to any or all MSR samples, and that this research is a high priority.

Opening the tubes

The term "opening of the tubes" can mean one or both of two actions (see Fig. 3): A) The first penetration of the sealed sample tube, which is likely to be for the purpose of extracting the headspace gas, and B) opening the tube enough to remove the solid samples. For the purpose of this discussion, opening the tubes is interpreted to occur at Time A.



Conclusion: We cannot see that analysis of the headspace gas between Time A and Time B would be important for operational decision-making at Time B. However, the chemistry of the headspace gas is vulnerable to change with time, and it should be analyzed promptly for that reason.

Figure 3*. Schematic illustration of a key question involving the headspace gas.*

Headspace gas

No measurement was identified that could be made on the headspace gas before the tubes are first opened (Time A). However, once the headspace gas is collected, it could potentially be analyzed before Time B when the solid samples are extracted. Although such analyses could be done, this workshop did not discuss this topic enough to determine if they should, or must, be done as part of the sample characterization process. This is an obvious area for future discussion. Some of the measurements that could be made on headspace gases, while not strictly necessary for sample characterization, may be time sensitive for other reasons, and this is addressed further under topic #6 "time-sensitive measurements."

FINDING 1: There are three sets of observations that may be beneficial before opening sample tubes: 1) Reconnaissance analysis of dust on the outsides of tubes; 2) Basic physical observations; 3) Micro- and nano-beam x-ray imaging (e.g., CT, Synchrotron, other).

2.2. Preliminary Sample Characterization (on opened tubes)

Discussion Prompt #2: What data needs to be collected on each of the samples in the Preliminary Examination (PE) stage after opening of the tube in order to make sample management decisions like sample splitting, sub-sampling for Planetary Protection (PP) purposes, sample allocation to science PIs, and other? In PE, what could/should/must be done?

Proposed Terminology

After extensive discussion, agreement was reached on the general terminology shown in Figure 4 (which modifies that used in the discussion prompt above). This terminology is used in the remainder of this report.

We propose to define two primary groups of activities, using the terminology "Basic Characterization" and "Preliminary Examination". The "curation" break-out group at the workshop made the point that this terminology is consistent with modern curation usage. Together these can be lumped under the larger banner of "Preliminary Sample Characterization," but this latter choice of term would probably benefit from more discussion (since sample characterization will also be a significant aspect of the external competed science investigation phase).

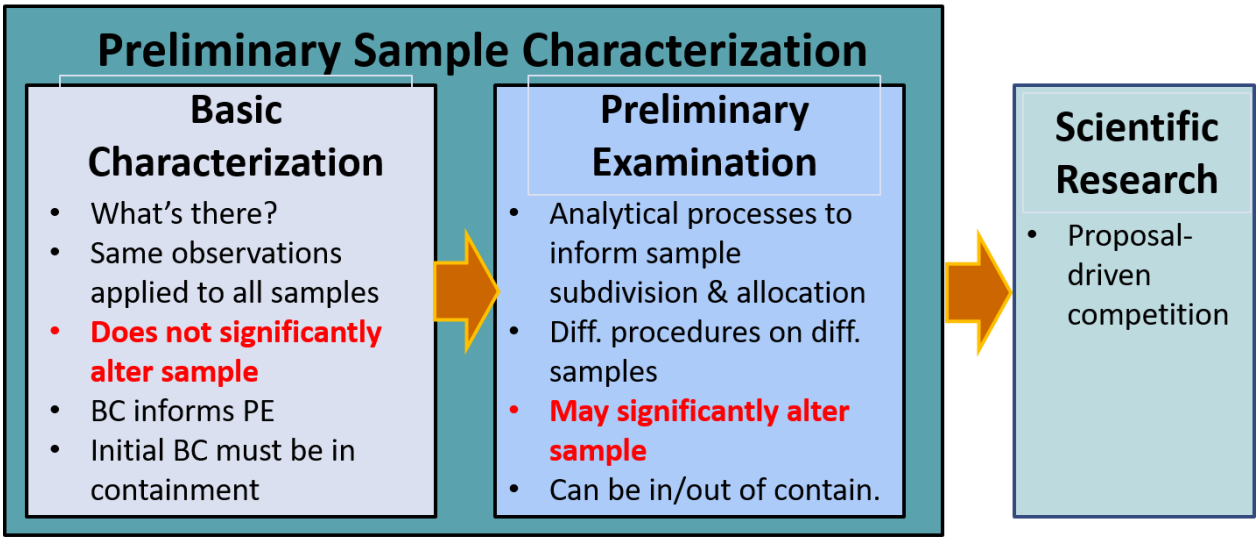


Figure 4: *Relationships between Basic Characterization, Preliminary Examination, and Scientific Research (nominally equivalent to competed scientific investigation).*

Basic characterization

Basic characterization (BC) is a series of data collection steps that do not (or minimize to the greatest degree possible) alter, damage, or induce any change in the sample and its associated properties (physical, chemical, spectral etc.). These steps would include, for example, weighing, photographing, and optical investigation. The BC steps would be applied to every sample in a standard way and would be used to inform the PE and SSAP methods that would be implemented. The BC stage would actually begin before opening the tubes, as rover data and geological setting information should be collated beforehand, including (but not limited to) sample type and likelihood of the presence of organic molecules. This information would be integrated into the BC process and referred to when designing the PE protocols for each sample. In our usage of the term, BC would take place repeatedly in the future,

including after samples have left the SRF, as new sample subdivisions are made. This action would be needed in order to keep an adequately detailed log for each sample and sub-sample before they are subjected to any preparation or analytical procedure. This is a standard curatorial practice. An excellent example is the Apollo samples. The Lunar Sample Compendium is a hugely valuable resource that combines regional geological settings with BC and PE results, along with results gained from later PI-led investigations.

Preliminary Examination

The data generated by BC would be used to design a PE program for each sample. Depending on how significant the differences between samples are, the PE sub-sampling and data collection steps could be significantly different between samples. Note in particular that the PE phase allows for both sub-sampling and sample preparation, both of which make irreversible changes to the sample. For example, the decision may be made to commit one or more samples (or sub-samples) to be extracted for organics testing. The organic extract could be rendered sterile, either as a direct consequence of the preparation technique (e.g., acid hydrolysis) or possibly through sub-micron filtration, and could be transferred outside of containment for analysis. Note that in our usage of the term, PE would happen once, in the SRF, to guide certain critical early decisions, and would not be repeated later.

The primary objective of PE would be to provide enough information for:

- Principal Investigators (with their supporting teams) to submit relevant and specific proposals for the scientific study of the samples.
- The design of consortium sample studies
- A sample allocation committee to make informed decisions about the best use of limited, high-value, and irreplaceable sample mass.

Some examples of PE include creating sub-samples, carrying out the SSAP, and performing specific analytical steps deemed necessary to make the above decisions. In contrast to BC, PE will differ between samples and sub-samples depending on what data are required for each sample. These analytical steps may take place in the Sample Receiving Facility (SRF), elsewhere in containment, or even outside of containment (using either sterilized samples or post-SSAP samples). PE for each sample is expected to last a constrained amount of time and then the samples and sub-samples would be made available for competitive science investigations (see Fig. 4).

The Importance of Scale

A point emphasized in the workshop was that samples need to be characterized in the BC and PE stages at a resolution appropriate for the distribution of the expected sample and sub-sample sizes. This may be at a far finer scale than is normally done for meteorites or for the Apollo lunar samples, for example. The resolution needed for the preliminary sample characterization phase may be dependent on sample type and will require detailed planning by future teams.

WORKING FUNCTIONAL DEFINITION #1: Basic Characterization will rely on simple, relatively inexpensive, non-destructive observations, and all samples will likely be examined in the same way.

WORKING FUNCTIONAL DEFINITION #2: More sophisticated instruments and more complicated sample preparation procedures will be needed for Preliminary Examination, sample-altering observations are permitted, and different procedures are likely to be applied to different samples—this requires more discussion.

FINDING #2: Prior to making the samples available to the world's research community, a 2-phase preliminary sample characterization process needs to be completed: Basic Characterization (BC) and Preliminary Examination (PE).

2.3. Capabilities Needed for the Preliminary Sample Characterization Phase (i.e., Basic Characterization + Preliminary Examination)

Discussion Prompt #3: How many instruments, and of what type, are needed? What sample preparation methods are needed? And what would be their relationship to biosafety cabinets?
Note: this assumes that CT scanning is NOT occurring prior to opening of the tubes; if that were to change, then this section must be revised accordingly

In order to provide early scoping information to support potential SRF cost and schedule estimation, the workshop group did brainstorming about instruments and sample prep activities that might be implied for containment (either SRF or somewhere else). However, there are two important caveats for the lists that follow in this section:

1. It is safe to say that all of the activities described could be done. However, our workshop discussions did not penetrate the issues enough to say that this set of people could conclude that they all should be done. Even more to the point, it would take considerably more discussion, including of cost and other implications, to determine what is required.
2. Since the workshop participants did not include representatives of all sub-disciplines of sample science, it should probably be expected that a small number of additional instruments will appear on these lists when the issue is penetrated more deeply.

For the purpose of this workshop, we assume that BC would take place within containment, as would most/all PE. Some of the instrumentation for PE could be outside of containment at the SRF, and some could be at specialist, external laboratories, to which the samples could be transported in containment boxes, or as sterilized material. The workshop recognized that SSAP requirements would have to be satisfied at all stages of sample interrogation, and that some of the PE investigations would be in support of the SSAP as well as in support of science planning.

Basic Characterization: See discussion related to question #2 above for a full description of the basic characterization phase. Capabilities required to successfully carry out BC include sample handling and manipulation capabilities, imaging and recording.

Suggested instrumentation for BC includes, but may not be restricted to, the following (to be vetted through additional future discussion):

- Curation requirements:
    - Sample handling tools optimized for small samples: tongs, tweezers (of different materials: stainless steel, Teflon, ceramic, etc.) Significant robotics sample handling may be required.
    - Tools and containers to collect and store dust from the external surfaces of the sample tubes
    - Tools and containers to collect and store gas sample, possibly including headspace gas if collection and storage of these samples is deemed possible
    - Tools to open the tubes and remove the samples

    - Tools to collect fine fragments broken from the main samples
    - Sample vials (different sizes and materials)
    - Barcode generator or RFID tags
- Balances for weighing the materials
- Cameras for recording size, shape and other external characteristics (e.g., heterogeneity, lithology, texture, petrology, layering, the presence of veins, etc.)
- Optical microscopes for determination of grain size, mineralogy, porosity, etc.

Preliminary Examination would need a more complex and diverse set of instrumentation than BC and is dependent on the information that is required to complete the two parallel strands of activities that have to be completed by PE. The two strands are (i) the satisfaction of SSAP requirements and (ii) the provision of sufficient information to catalog, sub-divide, allocate, and distribute material for scientific investigation. Although most of the PE instrumentation would be inside the SRF, it would be preferable to allow some material to be transported to laboratories where specialist equipment and personnel are based. This could potentially be allowed if small amounts of sample were rendered sterile *via* sample preparation (e.g., solvent extraction and acid hydrolysis, acid digestion, or sub-micron filtration) (Davidson, 2003; Glavin et al., 1999). Decisions about the final suite of instruments employed for PE would be significantly influenced by whether or not synchrotron facilities are employed; this is a major question that has yet to be resolved. The effects of analytical techniques, in general, upon sample properties or preparation requirements is one that needs urgent investigation.

Several types of measurements were identified as being necessary for the PE phase in order to allow for effective sample description and allocation. For example, mineralogy, mineral chemistry and organics analyses would be required in order to validate inferences made based on M-2020s onboard payload about the samples as acquired, cached and returned to Earth. These fundamental investigations are also required as part of PE to inform sample selection for both complementary SSAP and sample cataloguing and documentation for PI-led sample requests. Depending on the resolution of the instruments selected for inclusion in the PE phase, it would be possible to gather mineralogical and chemical information on individual grains (e.g., in the case of dust, regolith etc.) and 'whole-rock' samples (e.g., layers within cores, individual pebbles etc.). In addition, initial biological assessment would be important for selecting samples for further testing for planetary protection reasons. A preliminary notional list of instrumentation that could be used to achieve the objectives of PE are shown in the section 4.1.1. Table 3.

Note that according to these descriptions, the planetary protection -related Sample Safety Assessment Protocol would best be classified as a component of Preliminary Examination.

<u>Sample Preparation Methods</u> (to be vetted through additional future discussion)

The sample preparation methods which would be needed inside containment would include:

- Tools, of different materials, for sample division and sorting
- Equipment for sample preparation for allocation outside the SRF such as:
    - Solvent extraction for organic analysis
    - Thick/Thin-sectioning for petrographic analysis

- Containment boxes for samples to perform analyses on unsterilized and/or samples not yet cleared for release from containment outside the SRF
- Equipment for sterilization of samples (specific technique TBD)

These sample preparation steps would likely require on the order of 2-3 laboratories, which may or may not be the same laboratories used for time- and sterilization-sensitive measurements detailed in topics 5-6.

Need for Future Work #3: Further research is needed to determine how various sample preparation methods affect sample properties to be measured further downstream.

2.4. Sample Characterization Team: Size, Composition & Organization

Discussion Prompt #4: What would be the optimal size/composition of the science team responsible for PE, how should it be led/organized, and how should its members interact with each other (potentially by remote means), as the samples are progressively made available?

iMARS-2 provided an extensive analysis of the size, composition and structure (including on-site vs remote participation) of the science team that would be responsible for Preliminary Sample Characterization, as well as its technical, curatorial and bureaucratic support. Although there is more than one way to organize the teams, and although many details likely will change as the scale and other aspects of Preliminary Sample Characterization become better defined, workshop discussion suggested that the iMARS-2 analysis is an excellent starting place for such planning. Many of the E2E-iSAG (McLennan et al., 2012) findings also bear on the nature of the Preliminary Sample Characterization science team and it was widely accepted that sections of this report should be revised/updated as appropriate.

iMARS-2 (Haltigin et al., 2018) specifically recommended that organization of science teams be based on the nature of the various sample suites that would be collected during an MSR campaign. The process should take into account that Mars 2020 will collect samples at Jezero Crater, and perhaps later at the Midway site (e.g., sedimentary, igneous, hydrothermal, regolith, gases) (https://www.nasa.gov/press-release/nasa-announces-landing-site-for-mars-2020-rover). It was further recognized that superimposed on any such structure should be a "matrix" of discipline-based (e.g., geochemists, microbiologists, planetary protection) and instrument-based scientists (e.g., Raman, IR, mass spectrometry, magnetometer) (Figure 5). One briefly-discussed alternative organizational structure was to design science teams around high-level iMOST science objectives, however, this was not developed in any substantive manner during the workshop. Because iMOST objectives identified the necessary samples, many iMOST objectives may map onto organization by suites. This should be explored by further discussion.

Mechanisms should be considered to ensure that scientists involved in both Basic Characterization and Preliminary Examination obtain appropriate recognition in the form of publications and conference presentations; indeed the very ability to attract world-class scientists for such roles depends on it. Accordingly, a "Rules of the Road" document[1] that defines the rights and responsibilities of all scientists involved in Preliminary Sample Characterization (including their interactions with follow-on PI-led

[1] "Rules of the Road" is the way agreements inside the science teams of certain NASA and ESA projects (esp. MSL, M-2020 and ExoMars) have been documented regarding data rights, publication policies, etc.

investigations) needs to be developed well in advance. The importance of extensive (and early) training for Preliminary Sample Characterization scientists came up several times at the workshop and has also been discussed in previous studies (Haltigin et al., 2018; Hutzler et al., 2017). The BSL-4 containment environment that would be used for the Sample Return Facility are unlike any that the vast majority of planetary scientists have ever encountered. Nor have many even interacted with high-level containment technical staff. It will thus be important for Preliminary Sample Characterization scientists or technicians to be on site and fully trained well in advance of ever handling samples from Mars.

Conclusion: The iMARS-2 (Haltigin et al., 2018) report provides a good starting point for further discussions and analysis regarding the organization, management and staffing of a notional Sample Receiving Facility.

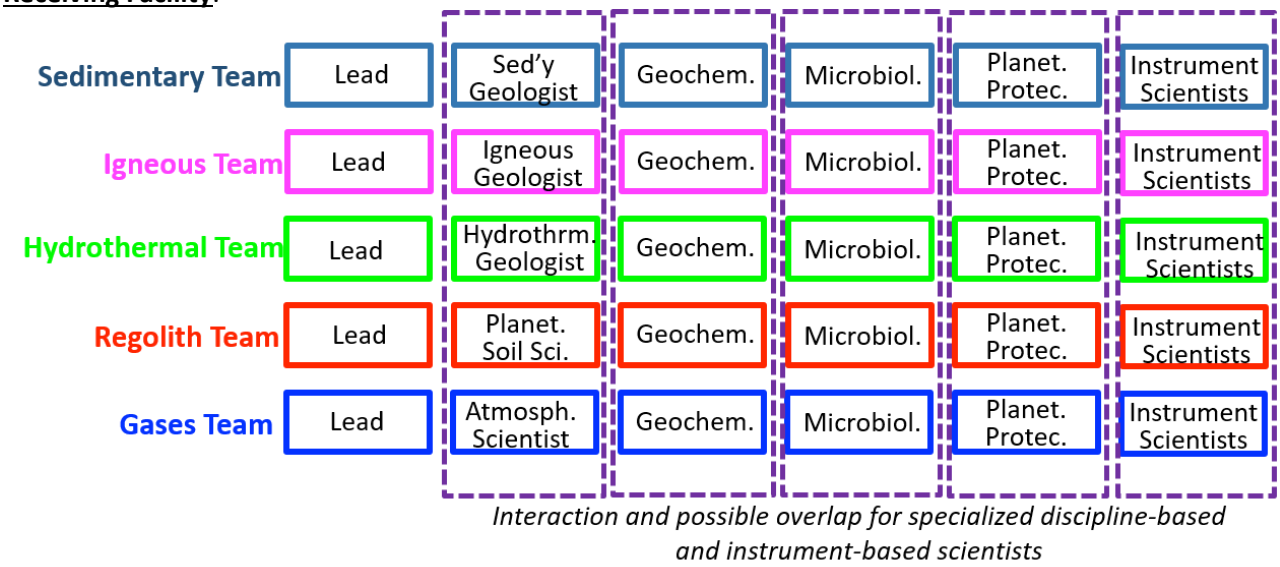


Figure 5*. Strawman "matrix" representation of how sample suite-based Sample Characterization science teams might be populated to include specialized discipline-based and instrument-based scientists. To optimize communications and efficiency, there would be formal interaction (and in some cases, personnel overlap) among the various specialists (vertical dashed boxes). The diagram is illustrative only and not meant to imply any proposals about the exact sizes or compositions of the various science teams.*

FINDING #3: The Preliminary Examination of MSR samples may be optimized by using different teams of international scientists for different samples (or groups of samples), although this is not the only way to do it.

3. SCIENCE INVESTIGATIONS

One of the objectives of this workshop was to estimate how many of the 199 MSR science investigations identified by iMOST are sensitive to degradation by time or by sample sterilization. These are first-order factors in determining how much science needs to be done in containment. This in turn plays a primary role in determining the minimum size/performance/complexity of the SRF.

Figure 6 shows a simple logic matrix, for which the various fields lead to a useful taxonomy. Investigations falling in Fields 1-3 are all time-sensitive, meaning they should be conducted as soon as possible after sample tubes are opened. This is true whether or not they are sensitive to sterilization. As discussed elsewhere in this report, these investigations need to be planned for inside containment. Field 4 contains investigations that can be done effectively on samples that have been sterilized using either high temperatures or radiation doses. Field 5 represents investigations that may be sensitive to either heat- or radiation-induced sterilization, but not both, meaning that a sterilization method could be chosen such that the science investigation could still be done effectively on the sterilized sample. This means that investigations falling in fields 4-5 can be done either inside or outside of containment, independent of the results of the planetary protection protocol. Investigations in field 6 are sensitive to both heat and radiation sterilization which means that they can only be done effectively on unsterilized samples outside of containment after successfully clearing a biohazard assessment. The initial classification of investigations, and the consequences of those classifications, is the subject of topics #5-8 in this report.

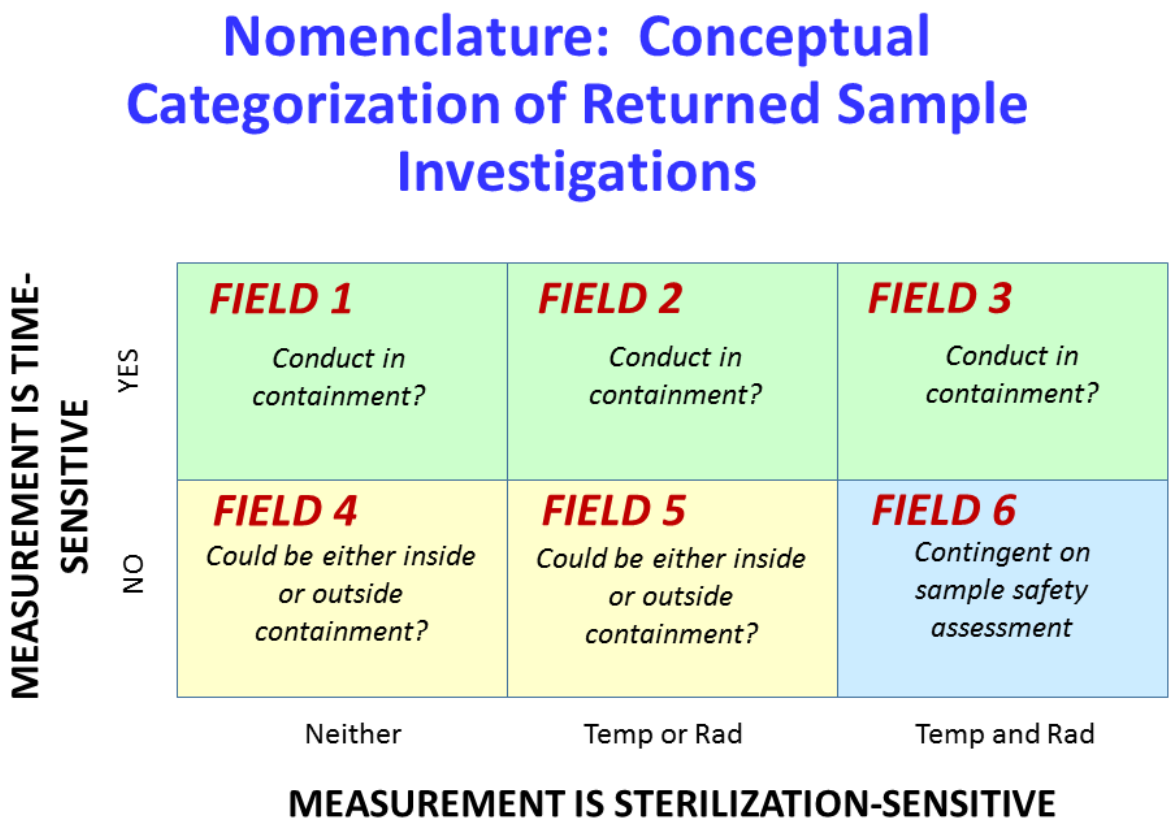


Figure 6. *Categorization of Science Investigations according to whether they are time-sensitive and/or sterilization-sensitive. These fields were used to define the subsequent discussion topics.*

3.1. Sterilization-Sensitive Measurements

Discussion Prompt #5: Is the initial list of sterilization-sensitive measurements complete? Should any investigation not be in the list? Or any other investigation that should be?

Originating with advice from the U.S. National Academies, it has long been assumed that scientific access to the MSR samples could be obtained under one of the following three conditions:

A. Access to unsterilized material within containment.

B. Access to unsterilized material outside of containment only after it has passed rigorous biohazard testing
C. Access to sterilized material outside of containment at any time.

Option A is discussed as part of 4.1 below. Option B has a vulnerability from the point of view of science because the wait for a definitive result on biohazard testing could be very long. This means that we need a careful evaluation of Option C above.

The discussion of Option C has an ambiguity originating in the fact that the parameters of the sterilization protocol have not yet been agreed to. However, some general considerations are worth discussing, and may lead to some valuable planning options that deserve further study. For the purpose of this planning exercise, we assume that one or both of two sterilization modalities are of interest: heat and gamma radiation. Although both are currently approved for the sterilization of spacecraft surfaces, neither is currently approved for rock and soil samples. However, as summarized at the workshop by Dr. Carlton Allen, these two parameters have rather predictable effects, and many geological parameters are relatively insensitive to them.

As shown in Figure 6, this leads to a 3-fold taxonomy consisting of measurements that are sensitive to 1) neither heat nor radiation (at the levels needed for sterilization), 2) those that are either heat-sensitive or radiation-sensitive, and 3) those that are sensitive to both. For the first of these two categories, the samples could be safely sterilized using either sterilization modality, and the samples could notionally be safely transferred out of containment. For the second of these categories, the least destructive of the two modalities could be chosen. The third category is where a potential problem may lie—the samples may need to be investigated in containment, or science may need to wait until the samples have passed the Sample Safety Assessment Protocol, or accept the consequences of a sterilization method with damaging side effects (i.e., diminished science return).

It is therefore important to SRF planning to generate an assessment of the fraction of science that may fit in the third category. iMOST (2019) identified 199 scientific investigations associated with achieving of the seven proposed scientific objectives of MSR (see Appendices C & D). As input to the workshop, one of the planners organized an XLS spreadsheet with all of the iMOST investigations, and assigned each a rating for the approximate impact of sterilization on science measurements. If an iMOST science investigation would be likely be affected by the sterilization method, regardless of the exact methods of measurement, the rating is red. If some science methods for obtaining a given iMOST science measurement would likely be affected, the rating is yellow. These ratings were reviewed and discussed by each of the breakout teams during the workshop, and updates were proposed and compiled. The revised list of investigations and these assignments is presented in Appendix D, and a compilation of the investigations that ended up being classified as “sterilization-sensitive” is in Table 1. Preliminary conclusions are:

- This analysis identified 36 (of 199) investigations as likely being at least somewhat sensitive to heat sterilization (Fig. 8). This is, of course, dependent on the specifics—in addition to the uncertainties of time/temperature thresholds needed, “sensitive” is a gray-scale parameter—how much damage is too much damage? Thus, the currently identified list of 36 investigations could expand or contract in the future, depending on definitions.
- The workshop group could identify only 17 out of the 199 investigations identified by iMOST (2019) that are sensitive to both heat and radiation (Fig. 7; Table 1). This means that >90% of

these investigations could conceivably be carried out on sterilized samples, depending on the specific sterilization methods determined to be allowable by the SSAP and regulatory agencies.

- **The workshop group was unable to identify any investigations that are sensitive only to radiation (i.e., but not also to heat). This is therefore judged to be a more promising sterilization method, if the metric is preservation of scientific value of the samples.**
- In sum, this means that it may be possible for a very large majority of the MSR investigations to proceed using sterilized samples, and without a timing dependency on SSAP testing, assuming regulatory agencies approve release of sterilized samples.

Note that the dose applied when using synchrotron radiation for analysis can be used for sterilization at the same time. This is an option, to be studied further.

Need for Future Work #1: Further research is needed in order to determine the effects of various sterilization parameters on the properties of geological samples.

Table 1: *Summary of iMOST measurements that are sensitive to potential degradation by both heat and radiation sterilization techniques.*

iMOST investigation strategy	Measurements
2.1A	Measure the presence, concentration and characteristics of simple and complex molecules and polymers containing C, H, N, O, P, Cl and S (organic carbon), and characterize organic matter features, including molecular structures (e.g., chirality etc.), abundances and/or molecular weight distributions.
2.1A	Determine co-association of, and context for, organic matter relative to known minerals, especially mineral catalysts that produce organic material from C_1 gases.
2.1C	Evaluate the indigenous nature of any detected carbon and organic molecules. Rule out terrestrial sources of carbon and organic molecules.
2.1D	Identify potential components of pre-biotic chemistry (e.g., prebiotic organic carbon compounds, reactive phosphorous, etc.
2.1D	Assess organic inventory for similarity to known abiotic processes such as Strecker synthesis or Fischer Tropsch type reactions.
2.2B	Evaluate the spatial relationships between organic matter and minerals and volcanic particles, especially such minerals that are compositionally and morphologically associated with biological activity or catalytic activity on Earth (e.g., Fe oxides and sulfides).
2.2B	Evaluate the relationship of potentially biogenic minerals and their associated organic material to the history of the host rock.
2.2B	Evaluate measurements of chemical and isotopic compositions of organic compounds to determine their conditions of formation and to seek evidence of chemical equilibria or disequilibria that are inconsistent with abiotic processes, and thus would be indicative of biological activity. Examples include widespread amino acid homochirality.
2.3A	Measure the presence of biochemical species, especially pigments, proteins, DNA, RNA, lipids etc.
2.3B	Measure the abundance of isotopes, isotopologues and isotopomers.
2.3B	Extract and sequence DNA.
2.3B	Identify and measure evidence for cellular growth, metabolism, and respiration.
2.3C	Measure cell size, shape, and structure.
2.3C	Evaluate morphological indications of replication and specialized features like motility structures.
6A	Identification of the molecular/genetic material within the returned sample(s) (performed in collaboration with Sub-Objective 2.3).
6A	Perform the agreed biohazard assessment protocol, presumably comprising non-destructive characterization (e.g., by CT screening) followed by destructive testing.

6B	Chemical Reactivity (e.g., by ion chromatography and spectroscopy). Characterize soluble ion concentrations, chemical reactions that can occur, and oxidative potential upon humidification.

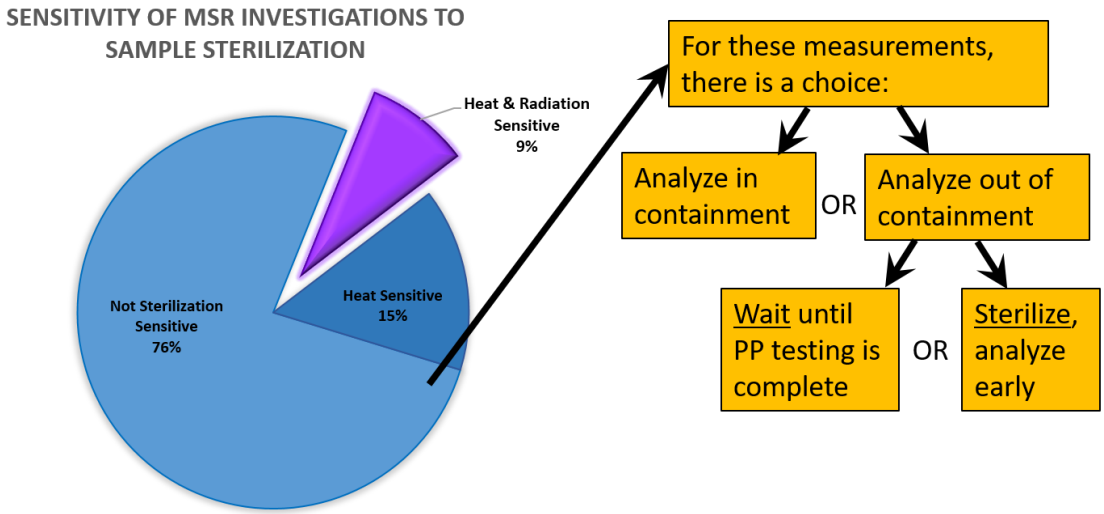


Figure 7. *Of the 199 MSR-related scientific investigations identified by iMOST (2019), very few are sensitive to both radiation sterilization and heat sterilization. For samples that can be sterilized, there is a choice to analyze them within containment, or at external, PI-led laboratories outside of containment.*

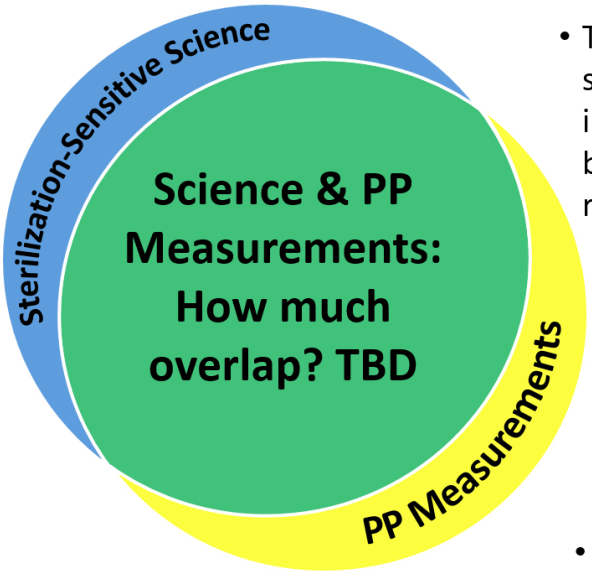
MAJOR FINDING #4:
It appears that a large majority (>90%) of the MSR-related science investigations, as identified by the International MSR Objectives & Samples Team (iMOST, 2019), could probably be acceptably performed on sterilized samples, thus potentially enabling the analysis of MSR samples in uncontained laboratories without a dependency on the results from Sample Safety Assessment Protocol (SSAP) testing.

Discussion

"Sterilization" in the English language has two definitions: 1) Make something free from bacteria or other living organisms; and 2) Deprive an entity of the ability to reproduce. It is as-yet uncertain what the standard for the samples returned by MSR would be. Regardless, the investigations that are sensitive to both heat and radiation sterilization are almost entirely those involving organic molecules, and are associated with Objective 2 ("Assess and interpret the potential biological history of Mars, including assaying returned samples for the evidence of life") and some of the investigations in Objective 6 ("Understand and quantify the potential martian environmental hazards to future human exploration and the terrestrial biosphere"). Other high-priority objectives related to understanding the geology, geochronology, and volatile history appear to be achievable using sterilized samples. This suggests that only the instrumentation necessary to carry out the sterilization-sensitive science investigations would have to be carried out in containment.

Given that the measurements which are most sensitive to standard types of sterilization procedures are those related to the detection and study of potential biological processes, it seems clear that there is likely to be considerable overlap between the instrumentation needed to carry out the as-yet-to-be-determined biohazard assessment protocol necessary for Planetary Protection and the sterilization-sensitive science investigations.

It was observed in the workshop that some analyses required for satisfaction of SSAP requirements could be conducted on extracts retrieved from samples *via* chemical processes (e.g., acid hydrolysis). Such processes may be inherently self-sterilising, and thus residues remaining following the extraction technique could be identified for 'early release' in order that high priority science in categories 4 and 5 can begin, e.g., geochronology, sedimentology, refractory organics analysis.



- The investigations that are sensitive to sterilization are almost entirely those involving organic molecules & potential biology associated with the objectives related to:
  - The search for biosignatures of extinct or extant Life
  - Understanding potential martian environmental hazards to future human exploration and the terrestrial biosphere
- <u>Major overlap between Science and PP = opportunities for synergy in the SRF</u>

<u>Figure 8.</u> *Potential overlap of sterilization-sensitive iMOST measurements and required Planetary Protection measurements which are still TBD*

Need for Future Work #4: It will be important to work with the SSAP Committee to determine the degree of overlap between measurements associated with science objectives and those required as part of the SSAP. If the same measurements can serve both purposes, it would help to conserve our precious sample mass.

3.2. Time-Sensitive Measurements

Discussion Prompt #6: Is the initial list of time-sensitive measurements complete? Should any investigation not be in the list? Or any other investigation that should be?

<u>Strategic overview.</u> The samples would come from an environment that experiences diurnal and annual fluctuations in temperature, water activity, and other kinds of environmental variations. The rocks at the surface of Mars will vary in response to these regular changes. Thus, when the sample tubes are sealed, they will represent the conditions at a specific point in time. Future science planning should consider

that at the time of sample collection (and sample tube sealing) these conditions will have been measured by the MEDA instrument suite on the M-2020 rover, so we will have knowledge of the initial conditions of each of the samples (which may have been taken at different times during the day, and will certainly have been taken on different days of the year). Most of the investigations described by iMOST are intended to interpret conditions at the time the rocks formed, i.e., much earlier in martian geologic history, and large classes of measurements are insensitive to environmental variations at the time the sample was collected. However, in order to be able to reconstruct our understanding of the environmental context of each sample at the time the sample tube seal was closed, it is important to make measurements of certain ephemeral properties as quickly as possible once the sample tube is opened. Once out of their tubes, the samples would begin to equilibrate with their new conditions.

Some sample properties identified have been documented to change swiftly in samples from all groups of meteorites and mission-returned asteroid samples upon even brief excursions of temperature, and upon even brief exposure to terrestrial moisture and oxidants, during curatorial storage and later in post-allocation work flow in investigator laboratories outside the receiving facilities (see Velbel, 2014, and references therein). We can expect that the properties of the samples would adjust to new ranges of T, and new ranges of vapor pressures and partial pressures of gases involving redox-sensitive elements (e.g., H_2O, O_2, CO_2, H_2, H_2S, SO_2, NO_x, ClO_x) that will affect redox state (Eh), may be expected within containment and post-containment environments as well. Measurements of sample properties that may be unstable in this regard would likely need to be performed as quickly as possible. In order to do so, the best science may come from making these measurements BEFORE the Sample Safety Assessment Protocol can be completed, which means that it would be prudent to plan to make these measurements in containment.

The following list of ideas were consolidated from the workshop, and are considered to be a starting point for more detailed discussion/validation of the specific measurements associated with time-sensitive science investigations. Of the 199 scientific investigations compiled by iMOST (2019), 26 are deemed to have some degree of time-sensitivity (see Table 2). However, these are not all equally sensitive to time, nor are they of equal scientific priority. Further discussion will be needed in order to determine what should or must be planned for within the SRF, and the trade-offs between cost and performance. The ideas generated at the workshop mostly fit within the following five categories:

- Headspace gas measurements. An argument was made that the headspace gas in each sample tube should be analyzed as quickly as possible after puncturing the seal. Since these samples will be small (in terms of number of moles of gas), they will be especially vulnerable to quantitative gas collection, sample transfers, contamination, leakage, etc., all of which have the potential to modify the sample composition. The expected gas quantity could of course be calculated, and an analysis plan derived.
- Hydrated minerals that reflect chemical and isotopic equilibria from Mars. The hydration state of multi-hydrated mineral systems, such as hydrous sulfates, expandable clay minerals, reactive species, etc.) should be analyzed quickly—these sample properties are vulnerable to change once the samples start equilibrating with the curation environment. Phyllosilicates and related amorphous materials are among the main materials targeted for numerous iMOST (2019) investigations focused on environment, climate and habitability of Mars. Many of these properties are highly sensitive recorders of martian aqueous processes, thus if 'contaminated' by terrestrial environmental makes it much harder to conclusively state anything about martian aqueous processes – especially if those are suggested to be recent/current. Note that synchrotron observation of a sealed sample tube before its opening may be used to interpret

mineralogy, and this may reduce somewhat the priority of making these kinds of measurements quickly after the tubes are opened.

- Measurements sensitive to gas-exchange chemistry. All measurements involving redox-sensitive gases (O_2, CO_2, H_2, H_2S, SO_2, NO_x, ClO_x), and the isotopes of redox-sensitive elements in the environment in which the tube is opened, will be different from the abundances of each gas equilibrated with the solids inside the tube after the long time interval expected between sealing the sample tube on Mars and opening the sample tube in the SRF. Even this within-sealed-tube environment will differ from the environment on Mars, but useful constraints on pre-seal (Mars) conditions can be retrieved from thermodynamic equilibrium calculations using the measured abundances of chemical species in the tube. However, evacuation of these gases from the sample tube upon opening (into any environment including the containment isolator environment) will produce vapor-pressure and redox re-equilibration of the contents of the sample tube. This is expected to occur rapidly because reactions involving hydration, dehydration, and redox of gas-hosted species are commonly rapid. Thus, the opening of the tube, even if done in containment, will initiate changes that will make more difficult the challenge of inferring volatile and redox conditions at the place and time of sampling from measurable properties of the returned samples. A reality is that the samples will have experienced a multi-year history between when the samples were first isolated from the martian environment and when they arrive at Earth, and models of this time period will need to be constructed to determine what kinds of changes to the sample are possible/likely/certain.

- Surface chemistry and reactivity of martian regolith or dust samples. As discussed under iMOST's Objective 6, returned regolith samples may be our most important direct representation of the martian surface geochemical environment and its potential hazards for human exploration. Interpreting the data from these samples will be complicated by the fact that the mineral and grain surfaces of these samples will have equilibrated with the head-space gas inside the tube after the multi-year history between when the samples were first isolated from the martian environment, and when they arrive at Earth. Nevertheless, understanding the surface chemistry and reactivity prior to the samples' equilibrating with Earth-sourced gases is important. If such measurements were not performed rapidly, the opportunity to make the measurement at all may be compromised.

- The above arguments presumably also apply to the dust on the outsides of the sample tubes, or on the interior surfaces of the OS.

- Sample Preparation Processes. If solvent extraction is used as part of the PE process there may be time sensitive measurements of reactive or short-lived species in the resultant extracts (e.g., oxidants). This may also result in alteration of other species in the extract over time *via* processes such as the oxidation of organics. For this reason it would be preferred to be able to do some analyses at the SRF (although they may not need to be done in containment if they can be rendered sterile *via* chemical or physical means).

Table 2: *Preliminary list of investigations that may be significantly sensitive to time. The magnitude of the effect is not equal for all measurements, so further discussion is needed.*

iMOST investigation strategy	Measurements
1.2B	Measure bio-essential elements (e.g., C, H, N, O, P, and S, other than compounds containing these elements in the atmosphere), as well as any bio-essential trace elements (e.g., Fe, Zn, Co, Ni), and concentrations of potential electron donors in host rock or soil/paleosol samples associated with hydrothermal activity.
1.2B	Measure mineral suites to determine mixed valence states for redox energy and isotopic proxies of specific redox couples (e.g., $\delta^{34}S$, $\delta^{56}Fe$) expected in hydrothermal systems.
1.3B	Determine the crystal chemistry and valence of redox sensitive elements (Fe, Mn, Cr, S, etc.)
1.3C	Measure H, C, S, O, and metal isotopes of mineral phases in the host rock and in groundwater-altered zones.
1.3C	Determine isotopic signatures from IS 1.3A as function of stratigraphic location and level to determine direction of water flow (top-down vs. bottom up).
1.3C	Measure atmospheric isotope values from coeval systems (e.g., glasses, quenched lavas).
1.4C	Measure the stable isotope compositions of secondary minerals to constrain the composition and history of atmospheric and crustal volatile sources.
1.4C	Determine the oxidation states of minerals to infer position of soils/paleosols relative to the paleo-water table, and exposure to reducing and oxidizing components (including photochemical).
1.4C	Measure variability in the mineralogy, chemistry (including identification and measurement of potential oxidants), mineral proportions and morphology of primary and secondary minerals and amorphous phases as a function of depth and/or degree of weathering, to constrain the duration of water interaction, water chemistry, water temperature, water residence time/drainage, and search for chemical trends that could be biosignatures.
1.4C	Analyze the chemistry and mineralogy of climate-sensitive minerals at nano-, micro-, and macro-scales.
4B	Measure D/H, the oxygen triple isotope composition and O-isotope anomaly ($\Delta^{17}O$) of water vapor in the atmosphere and phyllosilicate species and other hydrated mineral phases in ancient rocks.
4B	Measure the $\Delta\ ^{17}O$ values of sulfate minerals in dust and rocks.
4B	Measure the chlorine and oxygen triple isotopic composition of perchlorate and chlorides on Mars
4B	Measure sulfur quadruple isotopes and S-isotope anomalies ($\Delta^{33}S$, $\Delta^{36}S$) of sulfate in dust, regolith, rock, and soil samples.
4B	Measure the $\Delta\ ^{17}O$ values of hydrated and anhydrous silicates
4B	Analyze compound-specific isotopes of H, C, O, N, Cl, and S in molecular species in the atmosphere and in regolith, sediment, and rock samples.
4C	Analyze volatile species (e.g., H_2O, SO_3, H_2S, CO_2, Cl) preserved either as stoichiometric components of minerals (e.g., carbonates, sulfates, sulfides, chlorides, apatites, perchlorates) or adsorbed onto mineral/grain surfaces or trapped within fluid inclusions for their stable isotopic compositions (e.g., $^{2/1}H$, $^{18/17/16}O$, $^{13/12}C$, $^{15/14}N$, $^{36/34/33/32}S$, $^{37/35}Cl$), including clumped isotopes where possible.
4C	Analyze rocks/minerals/regolith that may have exchanged with the past atmosphere, at specific times in its history, e.g., carbonates ($\delta^{13}C$, $\delta^{17}O$, $\delta^{18}O$, $\Delta^{17}O$), sulfates ($\delta^{33}S$, $\delta^{34}S$, $\delta^{36}S$ and $\Delta^{33}S$, $\Delta^{36}S$) and perchlorates ($\delta^{37}Cl$, $\delta^{17}O$, $\delta^{18}O$, $\Delta^{17}O$) and adsorbed or chemically bound water, especially in hydrous minerals (δD, $\delta^{17}O$, $\delta^{18}O$, $\Delta^{17}O$).
4C	Analyze trapped gases within mineral inclusions and vesicles for the full range of atmospheric species, stable isotopes (especially H-, N- and O-isotopes) and noble gas isotopic and elemental compositions.
4D	Analyze compound-specific isotopes of H, C, O, N, Cl, and S in molecular species.

6A[1]	Identification of the molecular/genetic material within the returned sample(s) (performed in collaboration with Sub-Objective 2.3).
6A[1]	Perform the agreed biohazard assessment protocol, presumably comprising non-destructive characterization (e.g., by CT screening) followed by destructive testing.
6B[1]	Chemical Reactivity (e.g., by ion chromatography and spectroscopy). Characterize soluble ion concentrations, chemical reactions that can occur, and oxidative potential upon humidification.
7A	Identify hydrated minerals and hydration states in multiple samples of martian regolith (to facilitate comparison between different regolith types) and in rock samples.
7A	Characterize the water release profile of these samples with temperature and identify associated contaminants released. Contaminants of particular interest are chlorides and perchlorates which are potential contaminants for water use (e.g., propellant production, life support), but are a potentially useful resource for closed loop life support applications.
7A	Sample probes of at least the depth of the thermal skin depth at sample location, whereby the temperature will remain roughly constant, to characterize the depth of the present day active water layer (e.g., absorption and desorption on diurnal/seasonal time scales).

[1]Note that investigations 6A and 6B in this list are also sensitive to both heat and radiation sterilization.

Need for Future Work #5: Further discussion is needed to identity and determine the relative importance & degree of degradation with time of time-sensitive MSR science measurements in order to determine what should or must be planned for within the SRF, and the trade-offs between cost and science return.

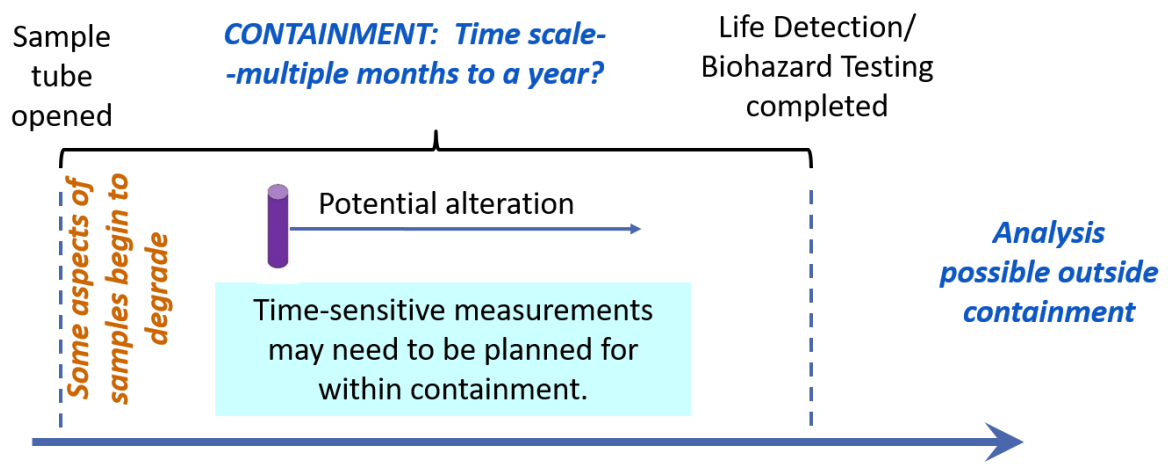


Figure 9. *Schematic representation of the importance of making certain time-sensitive measurements early in the process*.

FINDING #5: It is expected that the properties of the samples will be vulnerable to degradation in at least 4 significant areas as soon as they are removed from the equilibrium environment inside their tubes. Because of the time-sensitivity, these attributes should be measured quickly, or the opportunity may be irretrievably lost. This may require that these measurements be done in containment.

3.3. Measurements that are Neither Time- nor Sterilization-Sensitive

Discussion Prompt #7: For measurements in Fields #4-5 in Figure 6, what are the general tradeoffs associated with choosing to conduct scientific investigations either inside or outside of containment?

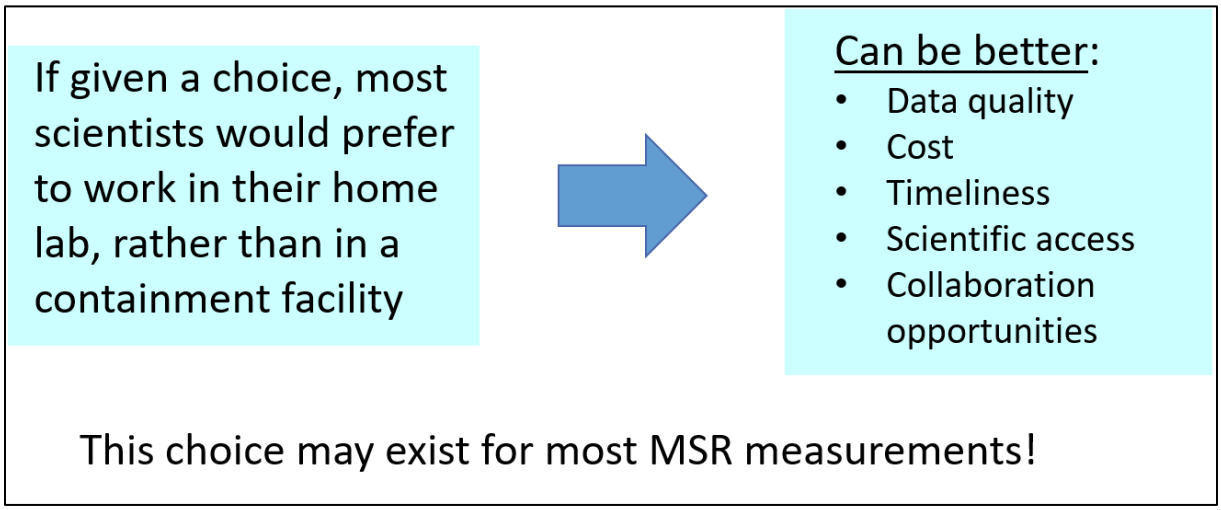
Some considerations might include cost, various kinds of risk, data quality, time, maximizing scientific access to the samples, preservation of sample value, opportunity to use latest equipment, or anything else of importance to one or more MSR stakeholders.

In considering the broad trade space of sample analyses that are not restricted in location (i.e., they could be done either inside or outside containment), key metrics are maximizing data quality, minimizing the time needed to obtain results, and optimizing the preservation of sample quality. The participants in this workshop who are involved in sample-related research would overwhelmingly prefer to do sample analysis in their home institutions rather than in a centralized, contained facility (a similar conclusion was recently reported by the U.S. National Academies (National Academies of Sciences, 2018). While PI-led investigations could be conducted either inside or outside containment, our discussions indicate that there are several advantages (to the science community) for carrying out such work outside of containment when a choice exists:

- Performance A critical factor that affects who wins and who loses scientific competitions that involve the production of laboratory data is analytical quality (most importantly, accuracy, precision, and detection limit). Typically, laboratory geochemists and biologists (among others) put enormous effort into minimizing the blank signal in their home laboratories. Optimizing the performance of a lab requires understanding and compensating for environment-specific factors, setting up effective lab-specific operational procedures and training, quality-control on the reagents (which are sometimes manufactured in-house), effective sample prep procedures (which can involve proprietary steps), etc. Requiring that all geochemists make their measurements in the same facility, and using facility-provided instrumentation, would have the effect of "homogenizing" the community. Eliminating competitive advantages in this area may have the unwanted effect of also eliminating premier data quality. Many scientists and technical staff can produce better results working in their own laboratories, where technical details are well known, procedures have been long-established, and equipment is familiar.
- Cost. The cost of modifying an existing uncontained lab so that is capable of receiving an allocation of precious martian material, and conducting high-quality sample studies, may be significant. However, this cost would pale in comparison to setting up and operating a similar lab inside a BSL-4-equivalent containment facility.
- Instrument development and maintenance. Improvements to and maintenance of analytical instruments can be far more easily done outside a contained environment. This can involve iterative approaches, the use of multiple standards and/or test samples, and physically changing the hardware either by addition, deletion, or configuration. Attempting to do this in containment creates difficulties—moving materials in and out of the restricted, contained volume would at a minimum create significant delays. However, it is acknowledged that certain work can be conducted inside containment with equipment that would have only a limited impact on the size of the contained volume, or the specialist training needed for operators: e.g., petrography, optical mineralogy.
- Timeliness. The time required to produce a large volume of data from returned samples, including making replicate analyses by multiple PIs, would be greatly improved if multiple lab analysts, using multiple labs, can be brought to bear. Restricting laboratory work to the labs that can be held within 1-2 SRF buildings would create a bottleneck that would delay the delivery of eagerly awaited scientific results.
- Replication. As pointed out by E2E-iSAG, the gold standard for laboratory data is to make replicate analyses by different scientists, using different instruments, in different facilities, and if

possible, using different methods. If the same answer is generated using this approach, it can more confidently be accepted as "the truth" than a single measurement.

- Sample prep procedures. Some sample prep procedures are especially demanding on scientific/technical expertise and time (e.g., wet chemistry for isotope analysis). The more of this that is done in containment would end up levying additional requirements on SRF staff and facilities. In addition, for reasons of quality control, sample prep procedures need to be closely connected to the investigation PI—carrying out such activities in containment may require that investigation PIs spend significant time (up to multiple years) in residence at the SRF. Compared to the alternative of doing such work at their home institution, this may become a disincentive to getting the widest spectrum of scientists as possible to work on the MSR samples.
- Collaboration opportunities. It is clear that the opportunity for science PIs to collaborate with their peers on MSR-related scientific investigations would be enhanced by making use of their home laboratories, rather than contained, centralized facilities.
- Special note: Well-coordinated proposals from consortia within the scientific community should be encouraged to ensure the most efficient use of samples (this point also made by iMARS-2; section 4.2.3, p S86).



MAJOR FINDING #6:
The scientific community, for reasons of scientific quality, cost, timeliness, and other reasons, strongly prefers that as many sample-related investigations as possible be performed in PI-led laboratories outside of containment.

FINDING #7: For reasons of optimizing the use of irreplaceable sample mass, consortium sample utilization studies, including those that make use of facility-related sample-preparation procedures, are of high interest.

4. DISCUSSION

4.1. Facility Implications

4.1.1. Facility Considerations for Measurements that must be done in Containment

Discussion Prompt #8: For the research measurements that must be done in containment (e.g., Fields #1-3 and some in Field 6 (e.g., investigation 2.x) in Figure 6), estimate the facility implications (number and size of instruments/laboratories in containment, nature of required sample prep facilities, nature of the supporting facilities outside of the containment barrier, other).

A. What kinds of measurements must (or should?) be done in the SRF because of reasons of **time or sterilization sensitivity** (Fields #1-3 and some in Field 6 on Fig. 6)?

As listed above (Table 2), measurements that are time sensitive (Field #1-3) in Fig. 6 must be done in the SRF. The measurements that are sensitive to both heat and radiation sterilization (Field #6 in Fig. 6) which are mostly associated with Objective 2 in iMOST (2019) (Table 1) may also need to be performed in containment. As a high priority objective, these measurements may be performed together with the biohazard testing or in parallel before the samples can be declared safe. Because the measurements in this objective may overlap with the biohazard testing, the input from the planetary protection group is needed to determine the full suite instrumentation required. One possibility which would reduce the need for organic analysis (and possibly other) instrumentation inside containment would be to do to sample preparation for techniques which utilize liquid extracts inside containment and treat the extracts in such a way as to render them sterile without using heat or radiation (e.g., acid hydrolysis, sub-micron filtration. Analyses on sterilized liquid could then be performed outside of containment. Note that this is contingent on regulatory approval of this type of sterilization method. Future work should be done to investigate these types of options.

B. What instruments are required to obtain the above information?

The following list of instruments were compiled from the workshop notes and **includes those relevant to the measurements called for both BC/PE** (carried over from section 1.3) as well those identified for carrying out time- or sterilization-sensitive measurements. However, even though these are instruments that could be used, further discussion by successor science teams are needed to properly assess priority—which of these should be used, and which must be used.

Instruments (to be vetted through additional future discussion)

A notional list of potential instrumentation needed inside the SRF for PE and for time- and sterilization-sensitive measurements was identified as follows:

Table 3. Notional list of measurements needed at different stages of sample characterization and analysis, along with possible instrumentation types to make those types measurements.

	Type of measurement needed	Notional/possible Instrumentation types
Prelimary Examination	Mineralogy, mineral chemistry, detection of organics	• Optical binocular reflecting microscope and camera with focus stacking (Z focus series) capability (SEM could be used if higher resolution is required); possibly with one or more additional spectroscopic capabilities from among those listed below. • Confocal Laser Scanning Microscope configured for fluorescence imaging • Raman spectroscopy • FT-IR spectroscopy • Micro-XRF • Micro-XRD
	Initial biological assessment	• MinION for DNA sequencing • Fluorescence microscope
Sterilization Sensitive	Organic Carbon analysis[1]	• Solvent Extraction • GC-MS • LC-MS
	Biomolecular Analysis	• MinION • Other (?)
Time Sensitive	Hydration states of minerals	• Raman • Synchrotron (?) • Near/Mid IR
	Surface Chemistry/Reactivity	• Ion Chromatography • Solvent extraction • GC-MS • LC-MS • Fluorescence • SEM-EDS
	Redox Sensitive Gases & Solids	• GC-MS • LC-MS • IC-MS

1. If liquid extracts can be sterilized via methods described above, only solvent extraction systems would have to be present inside containment

C. Facility implications (number and size of instruments/laboratories in containment, nature of required sample prep facilities, nature of the supporting facilities outside of the containment barrier, other).

(This topic was not addressed in the workshop. What follows is some post-workshop discussion by the workshop participants that extends from A & B above).
Some instruments were implied for time-sensitive measurements related to three general categories: 1. mineralogy (and especially hydration state), 2. redox state, and 3. easily exchangeable isotope ratios in volatile species. A generic planning assumption might be that this work could be done within three labs, each of which may contain several instruments. Some sample prep would be required for the measurements in Section A above, and this may imply an additional lab or two (or it may be possible to do the sample prep in the same lab as the measurement). This sample prep lab space may be the same sample prep space used for BC and PE sample prep. Additional lab space might be required if extensive biological and organic testing has to be done inside containment, although these types of instruments may also be present as part of the as yet undefined planetary protection protocol.

4.1.2. How many isolation cabinets are needed?

Discussion Prompt #9: Does science desire/require one isolation cabinet per sample tube, so that substantial activity could happen in parallel, or is isolation cabinet re-use permitted, such that some or all activity is in series?

Background
It was assumed for the purpose of this workshop that isolation cabinets would likely be an important part of the solution to keeping the MSR samples isolated from Earth-sourced contaminants, and in keeping the Earth safe from any potential hazard presented by the samples. As used in many BSL-4 facilities, and also in certain curation applications (e.g., they were used in the Lunar Receiving Lab in 1969, and are still used in the curation of the Apollo samples), isolation cabinets consist of closed volumes, commonly with gloves, and with ports and flanges that allow them to be connected together (i.e., in a cabinet line), and/or connected to instruments or other devices. For possible specific use in the processing of the MSR samples, the prototype of an innovative dual-walled isolator (DWI) has been developed by ESA (e.g., Vrublevskis et al., 2018) and currently located at the University of Leicester (Bridges & Guest, 2011; Holt et al., 2019). However, it was not the purpose of this workshop to debate the role of isolators in overall containment and sample cleanliness strategy, or the specifics of isolator requirements—these important topics are deferred to future study teams. For the purpose of this workshop report, we simply assume, without specific confirmation, that isolators (of some design) will be the focus of much sample-related activity in the SRF. It is also possible that there may be some steps in sample PE that are done outside of isolators on open benches in a biosafety suit lab.

A first-order driver on the size of the containment facility would be how many isolators are envisioned. One end member planning scenario would be to have separate isolators for each sample tube (see Fig. 5). This would minimize cross contamination between Mars samples, and would allow completely parallel processes in the examination of each of up to 31 samples. This would minimize the time required to complete BC + PE. For the activities that would be done inside the isolators, individual humans (for example, people with very specialized expertise) could shift from one sample to another in an unfettered way. This would allow multiple scientists to engage in the PE process simultaneously. A second end-member would be to make use of one (or a small number) of isolators, and to schedule the samples to pass through the isolators for PE one at a time in series (Fig. 5). This would allow a smaller number of scientists to interact with the samples, but each observer may have the opportunity to see them all.

Discussion
Sample suites and workflow

It appeared to be technically reasonable to the workshop participants that it will be possible to clean the isolators between samples to an acceptable level within reasonable cost and time constraints. If that is so (and this needs to be demonstrated), we believe that the number of isolators can be optimized at a figure between the above two end-members. It seems likely to us that samples of similar character would be evaluated by a given PE team. A logical system for organizing the PE activity would therefore be around the concept of sample suites. Based on the planning of the Mars-2020 science team, there is potential for the collection up to about 5-7 sample suites, given our current understanding of the martian landing site, Jezero Crater (and possibly Midway). We could therefore hypothesize that there could be a similar number of preliminary examination teams who are able to work on several samples each, which would mean that the work flow is partially in parallel and partially in series.

Some level of redundancy in the isolators is suggested so that processing work could continue while accommodating cleaning time (thought nominally to take a week) and to allow for repair and maintenance. Each line could possibly consist of 6-7 isolation cabinets, each of which might be used for a different type of BC or PE activity, for example. This would allow different phases of activity to be happening on different types of samples simultaneously.

Sample preparation

The functionality of some of the isolators may be defined by certain sample preparation procedures for which we want to control the spread of contamination. An example might include rock sawing, a notoriously dirty procedure. We may want to have a set of isolators where only a single kind of activity takes place—these would be different than those assigned for suite-related work. Fixed stations would enable consistency across samples and allow for specialized staff needed for each application. Another example might include solvent extraction, a critical organics-related step that may be applied to a carefully-chosen set of sub-samples, but certainly not to the entire collection.

Instruments

It may be most efficient to place some simple kinds of instruments (e.g., balances, cameras, optical microscopes, etc.) inside each of the BC/PE isolators, meaning multiple copies of these instruments would be present. More complicated instruments could be portable, and be mounted such that they could roll up to each PE isolator to make measurements (with the complexity of alignment, recalibration, etc.). Or relatively complex instruments could possibly be mounted inside individual "clean" isolators, and used sequentially for multiple samples. These assets would need to be shared between the multiple PE teams working simultaneously on samples within different isolators. More discussion is needed.

Summary

The nominal number of isolators could be determined by sample suites, types of analyses, sub-sampling processes, risk factors, operational efficiency parameters, and other factors, the details of which are currently TBD. This is a large parameter space with multiple trades to be considered, and there are likely to be multiple solutions. An additional open question is whether isolators could be repurposed for subsequent curation purposes—this may affect the design requirements. If so, more isolators may be useful for long term storage of samples. In order to provide the engineering planners a figure for planning purposes, the group suggested that a preliminary figure might be 15 ± 5 isolators, but the exact number will require further discussion, and will likely depend on the cost and size of the isolation cabinets utilized.

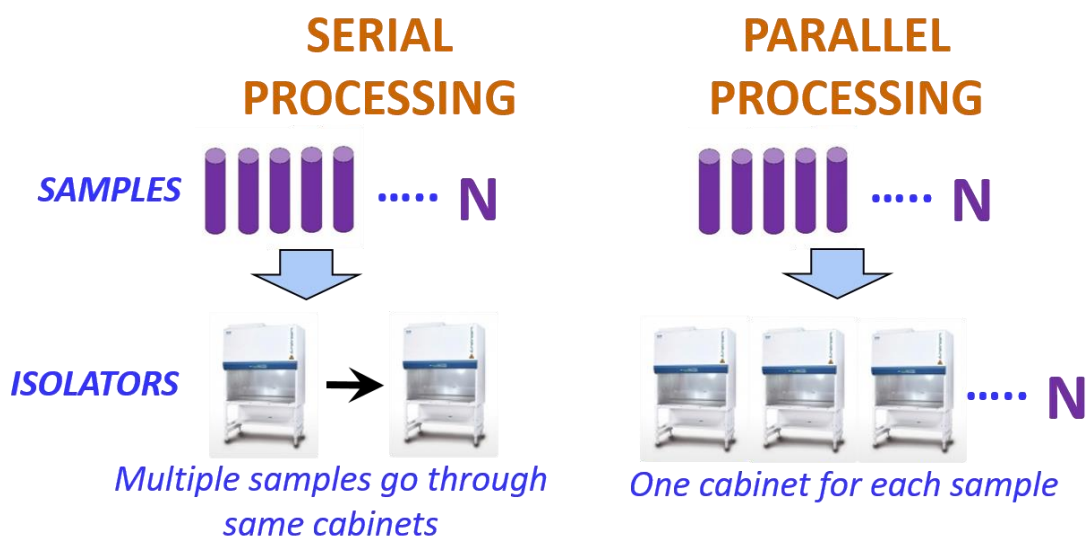


Figure 11: *The preferred number of isolator cabinets to process an assumed set of 31 samples is between the two end-members shown. An initial planning number could be ~15 ± 5 isolators.*

Conclusion: Assuming that isolator cabinets can be effectively cleaned between samples (considered technically reasonable at this time), the number of needed isolators is judged to be less than the number of samples. For planning purposes, a figure of 15 ± 5 isolators may be a reasonable estimate. This number could be influenced by several factors, including the different types of environmental conditions desired for different samples and processes (e.g., vacuum, N_2, He, Ar atmospheres, low temps, etc.), differing contamination requirements for different processes, how many samples might be worked on simultaneously, etc.

4.1.3. Contingency Plans if Unsterilized Samples Never leave Containment: (Sterilization-Sensitive Measurements)

Discussion Prompt #10: For the measurements that are sterilization-sensitive, what are the different contingencies related to sample safety assessment that matter to scientific planning, and what are possible strategies to achieve MSR's scientific objectives for each contingency?

In the event either that evidence of (martian) life is discovered in the samples returned from Mars, or that the safety assessment protocols return an ambiguous result, it may never be possible to release unsterilized samples from containment. We would need to have contingency plans in place to deal with such possible contingencies.

This would involve two primary types of investigations that may need to be done inside of containment: 1) If life is discovered in the samples, scientists would want to have the ability to analyze it fully using multiple instruments and methodologies; 2) investigations for which the sterilization protocol would alter the results. More information regarding the planetary protection protocols and recommended sterilization technique(s) will be required in order to fully determine which measurements fall into category 2. More research into the effects of the recommended sterilization techniques on certain physical and chemical properties would certainly also be needed.

If the samples end up staying at the initial SRF site for a long time, long-term curation needs to be planned for.

We can recognize four categories of contingency plans, each of which has a specific negative aspect:

1. Build a minimalist SRF (or other kind of contained facility(ies), that does not have the capability to respond fully to the discovery of life. After such a discovery, improve the within-containment capabilities, by some or all of the following options
    a. Add more instruments to the existing SRF laboratories. This could involve repurposing labs within containment that are no longer needed, such as those involved in preliminary examination.
    b. Expand the existing contained lab space, for example by adding a new laboratory wing, or constructing an additional building (perhaps in a very different location), to allow for additional analyses/instrumentation. The initial building could, for example, be designed as a modular facility that could be expanded or adapted to fit changing needs.

    DOWNSIDE: THE KEY FOLLOW-UP LIFE DETECTION/CHARACTERIZATION EXPERIMENTS MAY BE DELAYED BY MULTIPLE YEARS
2. Build a more capable SRF (or other kind of contained facility(ies)), that does have the capability to respond fully to the discovery of life.
    a. The implication is to start with a large SRF. The relevant biological characterization instrumentation could either be installed from the outset, or it may be preferable to add it if and when life is discovered, rather than having equipment that may be of the wrong design.

    DOWNSIDE: IF NO LIFE IS FOUND, THE SRF MAY HAVE DESIGN CAPABILITIES THAT ARE NEVER USED
3. As discussed above, current PP policy already allows for the study of sterilized samples outside of containment. However, a crucial detail is missing, which is the sterilization parameters. What if those parameters, once finalized, are found to be so destructive that they are unacceptable to science?
    a. The implication is to start with a large SRF. The relevant biological characterization instrumentation could either be installed from the outset, or it may be preferable to add it if and when life is discovered, rather than having equipment that may be of the wrong design.

    DOWNSIDE: THE SRF MAY BE SIGNIFICANTLY LARGER AND MORE EXPENSIVE THAN CURRENTLY ENVISIONED.
4. Lease space within an existing BSL-4 lab, with an option to be able to expand into adjoining space if needed.

    POTENTIAL DOWNSIDE: EXISTING BSL-4 LABS MAY FIND IT HARD OR IMPOSSIBLE TO MEET MSR'S SUPER-STRICT CONTAMINATION CONTROL REQUIREMENTS.

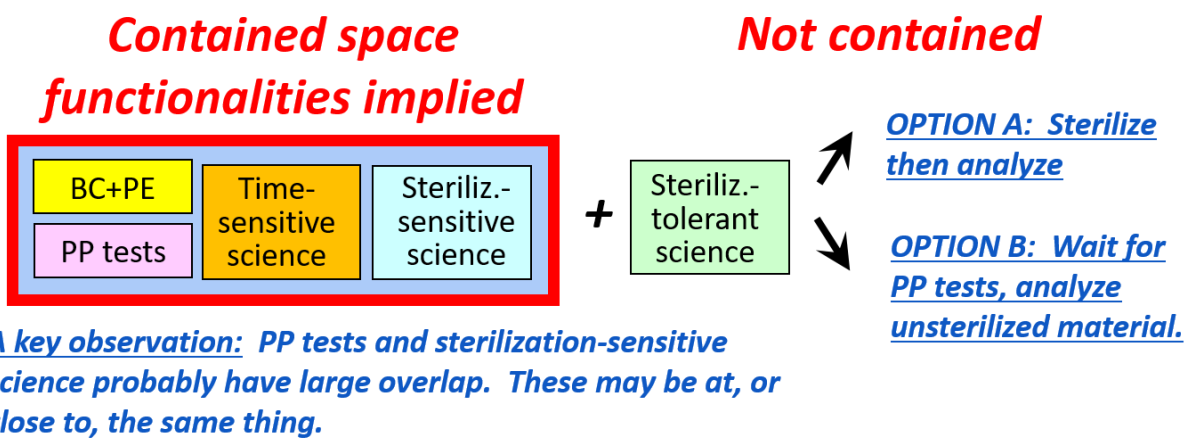


Figure 12. *Summary diagram illustrating the primary science-related functionalities implied for the notional Sample Receiving Facility.*

FINDING #8: Space within containment must logically include functionality for BC+PE, SSAP tests, time-sensitive science, and sterilization-sensitive science. Sterilization-tolerant science can most effectively be planned outside of containment.

4.2. Suggestions for Future Work

The most significant (to science) topics that came up in the workshop that need further action:

Follow-up action needed at high priority

1. The impacts of heat and radiation sterilization on geological samples needs urgent and detailed investigation. Establishing permissible sample sterilization parameters is required.

Discussion: Critical aspects of the planning described in this report are dependent on establishing the experimental basis that the effects of sterilization on different aspects of sample science are acceptable (to science), AND that the physical conditions of sterilization are acceptable to policy-makers. A systematic matrix of such experiments is likely to take some time. Somebody (or perhaps multiple people) need to be funded to do this work.

2. Is the benefit of x-ray imaging through sealed tube walls larger or smaller than the consequences?
3. The effects of analytical techniques and associated sample preparation procedures, in general, upon sample properties is one that needs urgent investigation.
4. How much overlap will there be between the Sample Safety Assessment Protocol and the general category of sterilization-sensitive scientific investigations?

Discussion: It is possible that this analysis will be done in 2019 jointly by MSPG and SSAP-WG.

5. The possible degradation with time of the scientific attributes of martian geological samples in response to exposure to terrestrial environments needs urgent and detailed investigation.

Discussion: As discussed above in connection with Action #1, somebody needs to be funded to perform a systematic set of experiments to constrain the extent of potential modification by terrestrial conditions of scientific signals in Mars samples. SRF activities cannot be planned so as to optimize the science without this information.

RECOMMENDATION: Funding, and coordinated work teams, are needed in five identified high-priority areas: 1) Effects of sterilization processes on geological samples; 2) Effects of x-ray imaging on sample properties; 3) Effects of sample analysis and sample prep on sample properties; 4) Degree of overlap between MSR sterilization-sensitive science and SSAP investigations; 5) Identity and significance of time-sensitive MSR science measurements.

Follow-up action needed at medium priority

- What is the specific process for opening the sample tubes?
- What is the process for cleaning the isolator cabinets? What specific contamination control requirements can be met?
- Would early analysis of the headspace gas be necessary to avoid time-related sample degradation effects?
- Is determining whether the sample tube seals have leaked of such importance that a pressure test is justified?
- Determination of sample masses needed to complete the measurements, and determination of which measurements are destructive vs which can be done in sequence
- What is the necessary resolution for various measurements during PE?
- How can sample prep be optimized for smaller samples?
- [Until the samples are returned, ESA and NASA would need to support instrumentation in PI-led labs as this is where the next generation of scientists will be trained and where protocols will be developed to make optimal use of the returned samples. This is done in the US through various programs (EW, LARS, SSW) and this effort must be sustained].

4.3. Miscellaneous Ideas that may Deserve Further Discussion by Future Groups

- There was some discussion about the possibility of storing the samples in cryostats at low temperature, to slow down/halt deleterious chemical reactions. One could for instance bring the samples to the lowest T that the samples have experienced on Mars and store them under those conditions. This possibility could be discussed again by future planning groups. However, other prior MSR science planning teams have concluded that this is unwise. It would be far better to operate the SRF at “room temperature”, where the performance of instruments, sample prep procedures, reagents, and humans is well known. Prior studies have concluded that once the samples have warmed up (for example, as part of the return/re-entry process), there is little value in cooling them back down.

- It is expected that diverse stakeholder communities (e.g., national governments that apportion funds to NASA and ESA programs and activities; taxpayers from whom NASA and ESA funds originate; and the general public) will be eagerly and impatiently awaiting high-profile early

scientific results about ancient or extant life on Mars (Committee to Review the Next Decade Mars Architecture, 2006).

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Appendix A: Workshop Participants

Name	Affiliation
Allen, Carl	NASA Johnson Space Center (Retired)
Beaty, David	Jet Propulsion Laboratory, California Institute of Technology
Busemann, Henner	ETH Zurich, Switzerland
Calaway, Michael	Jacobs Engineering Group
Carrier, Brandi	Jet Propulsion Laboratory, California Institute of Technology
Chaussidon, Marc	Institut De Physique Du Globe De Paris (IPGP), France
Corrigan, Cari	Smithsonian National Museum of Natural History

Dauphas, Nicolas	University of Chicago
Debaille, Vinciane	Universite´ Libre de Bruxelles, Belgium
Gaubert, Francois	ESTEC, European Space Agency, The Netherlands
Glavin, Danny	NASA Goddard Space Flight Center
Grady, Monica	The Open University, UK
Liu, Yang	Jet Propulsion Laboratory, California Institute of Technology
Martin, Dayl	ECSAT, European Space Agency, UK
Marty, Bernard	Universite´ de Lorraine, CRPG-CNRS, France
Mattingly, Richard	Jet Propulsion Laboratory, California Institute of Technology
McLennan, Scott	Stonybrook University
Meyer, Michael	NASA Headquarters
Olsson-Francis, Karen	The Open University, UK
Sefton-Nash, Elliot	ESTEC, European Space Agency, The Netherlands
Shaheen, Robina	University of California, San Diego
Siljestrom, Sandra	RISE Research Institutes of Sweden
Smith, Caroline	Natural History Museum, UK
Tait, Kimberly	Royal Ontario Museum, Canada
Thieme, Juergen	Brookhaven National Laboratory, New York
Usui, Tomohiro	Tokyo Institue of Technology
Velbel, Michael	Michigan State University

Appendix B: Workshop Presentations

The following prepared presentations were given at the workshop to introduce certain information to the workshop participants. The fact that this information was shared does not necessarily mean that it was accepted as group positions.

Relationship between planning for Science (MSPG) and PP (SSAP-WG)	Sandra Siljestrom
Overview of science objectives for returned Mars samples	Grady, Carrier
Engineering considerations related to the SRF	Gaubert, Mattingly
What have we learned from Mars meteorites that would be relevant for planning for processing of returned samples through the SRF	Bernard Marty
Possible Synchrotron techniques and containment	Thieme
Overview of sterilization techniques/What constitutes a sterilizing dose of heat or radiation	Carl Allen (with Wayne Schubert)
Presentation of preliminary table of measurements (Yang's table)	Yang Liu
iMARS-2: Previous thinking on discussion prompt #4--contingencies related to hazard testing	Caroline Smith

Appendix C: iMOST Science Objectives

iMOST Proposed Objectives			
		Shorthand	**Full Statement of Objective**
Objective 1		***Geological environment(s)***	Interpret the primary geologic processes and history that formed the martian geologic record, with an emphasis on the role of water.
	Sub-Obj. 1.1	***Sedimentary System***	Characterize the essential stratigraphic, sedimentologic, and facies variation of a sequence of martian sedimentary rocks.
	Sub-Obj. 1.2	***Hydrothermal***	Understand an ancient martian hydrothermal system through study of its mineralization products and morphological expression.
	Sub-Obj. 1.3	***Deep subsurface groundwater***	Understand the rocks and minerals representative of a deep subsurface groundwater environment.
	Sub-Obj. 1.4	***Subaerial***	Understand water/rock/atmosphere interactions at the martian surface and how they have changed with time.
	Sub-Obj. 1.5	***Igneous terrane***	Determine the petrogenesis of martian igneous rocks in time and space.
Objective 2		***Life***	Assess and interpret the potential biological history of Mars, including assaying returned samples for the evidence of life.
	Sub-Obj. 2.1	***Carbon chemistry***	Assess and characterize carbon, including possible organic and pre-biotic chemistry.
	Sub-Obj. 2.2	***Biosignatures-ancient***	Assay for the presence of biosignatures of past life at sites that hosted habitable environments and could have preserved any biosignatures.
	Sub-Obj. 2.3	***Biosignatures-modern***	Assess the possibility that any life forms detected are still alive, or were recently alive.
Objective 3		***Geochronology***	Determine the evolutionary timeline of Mars.
Objective 4		***Volatiles***	Constrain the inventory of martian volatiles as a function of geologic time and determine the ways in which these volatiles have interacted with Mars as a geologic system.
Objective 5		***Planetary-scale geology***	Reconstruct the history of Mars as a planet, elucidating those processes that have affected the origin and modification of the crust, mantle and core.
Objective 6		***Environmental hazards***	Understand and quantify the potential martian environmental hazards to future human exploration and the terrestrial biosphere.
Objective 7		***ISRU***	Evaluate the type and distribution of *in situ* resources to support potential future Mars Exploration.

Appendix D: iMOST Investigation Strategies & Measurements

iMOST Investigation Strategies (IS) & Measurements	Measure-ments sensitive to:

IS	Measurements	Time	Temp	Rad
1.2B	Measure bioessential elements (e.g., C, H, N, O, P, and S, other than compounds containing these elements in the atmosphere), as well as any bioessential trace elements (e.g., Fe, Zn, Co, Ni), and concentrations of potential electron donors in host rock or soil/paleosol samples associated with hydrothermal activity.			
1.2B	Measure mineral suites to determine mixed valence states for redox energy and isotopic proxies of specific redox couples (e.g., $\delta^{34}S$, $\delta^{56}Fe$) expected in hydrothermal systems.		2	
1.3B	Determine the crystal chemistry and valence of redox sensitive elements (Fe, Mn, Cr, S, etc.)		2	
1.3C	Measure H, C, S, O, and metal isotopes of mineral phases in the host rock and in groundwater-altered zones.			
1.3C	Determine isotopic signatures from IS 1.3A as function of stratigraphic location and level to determine direction of water flow (top-down vs. bottom up).			
1.3C	Measure atmospheric isotope values from coeval systems (e.g., glasses, quenched lavas).			
1.4C	Measure the stable isotope compositions of secondary minerals to constrain the composition and history of atmospheric and crustal volatile sources.			
1.4C	Determine the oxidation states of minerals to infer position of soils/paleosols relative to the paleo-water table, and exposure to reducing and oxidizing components (including photochemical).			
1.4C	Measure variability in the mineralogy, chemistry (including identification and measurement of potential oxidants), mineral proportions and morphology of primary and secondary minerals and amorphous phases as a function of depth and/or degree of weathering, to constrain the duration of water interaction, water chemistry, water temperature, water residence time/drainage, and search for chemical trends that could be biosignatures.	1		
1.4C	Analyze the chemistry and mineralogy of climate-sensitive minerals at nano-, micro-, and macro-scales.	1		
4B	Measure D/H, the oxygen triple isotope composition and O-isotope anomaly ($\Delta^{17}O$) of water vapor in the atmosphere and phyllosilicate species and other hydrated mineral phases in ancient rocks.			
4B	Measure the D ^{17}O values of sulfate minerals in dust and rocks.			
4B	Measure the chlorine and oxygen triple isotopic composition of perchlorate and chlorides on Mars			
4B	Measure sulfur quadruple isotopes and S-isotope anomalies ($\Delta^{33}S$, $\Delta^{36}S$) of sulfate in dust, regolith, rock, and soil samples.			
4B	Measure the D ^{17}O values of hydrated and anhydrous silicates			
4B	Analyze compound-specific isotopes of H, C, O, N, Cl, and S in molecular species in the atmosphere and in regolith, sediment, and rock samples.			
4C	Analyze volatile species (e.g., H_2O, SO_3, H_2S, CO_2, Cl) preserved either as stoichiometric components of minerals (e.g., carbonates, sulfates, sulfides, chlorides, apatites, perchlorates) or adsorbed onto mineral/grain surfaces or trapped within fluid inclusions for their stable isotopic compositions (e.g., $^{2/1}H$, $^{18/17/16}O$, $^{13/12}C$, $^{15/14}N$, $^{36/34/33/32}S$, $^{37/35}Cl$), including clumped isotopes where possible.			
4C	Analyze rocks/minerals/regolith that may have exchanged with the past atmosphere, at specific times in its history, e.g., carbonates ($d^{13}C$, $d^{17}O$, $d^{18}O$, $D^{17}O$), sulfates ($d^{33}S$, $d^{34}S$, $d^{36}S$ and $D^{33}S$, $D^{36}S$) and perchlorates ($d^{37}Cl$, $d^{17}O$, $d^{18}O$, $D^{17}O$) and adsorbed or chemically bound water, especially in hydrous minerals (dD, $d^{17}O$, $d^{18}O$, $D^{17}O$).			
4C	Analyze trapped gases within mineral inclusions and vesicles for the full range of atmospheric species, stable isotopes (especially H-, N- and O-isotopes) and noble gas isotopic and elemental compositions.			
4D	Analyze compound-specific isotopes of H, C, O, N, Cl, and S in molecular species.			
6B	Chemical Reactivity (e.g., by ion chromatography and spectroscopy). Characterize soluble ion concentrations, chemical reactions that can occur, and oxidative potential upon humidification.			
6A	Perform the agreed biohazard assessment protocol, presumably comprising non-destructive characterization (e.g., by CT screening) followed by destructive testing.			
6A	Identification of the molecular/genetic material within the returned sample(s) (performed in collaboration with Sub-Objective 2.3).			
7A	Identify hydrated minerals and hydration states in multiple samples of martian regolith (to facilitate comparison between different regolith types) and in rock samples.			

7A	Characterize the water release profile of these samples with temperature and identify associated contaminants released. Contaminants of particular interest are chlorides and perchlorates which are potential contaminants for water use (e.g., propellant production, life support), but are a potentially useful resource for closed loop life support applications.			
7A	Sample probes of at least the depth of the thermal skin depth at sample location, whereby the temperature will remain roughly constant, to characterize the depth of the present day active water layer (e.g., absorption and desorption on diurnal/seasonal time scales).			
2.1A	Measure the presence, concentration and characteristics of simple and complex molecules and polymers containing C, H, N, O, P, Cl and S (organic carbon), and characterize organic matter features, including molecular structures (e.g., chirality etc.), abundances and/or molecular weight distributions.			
2.1A	Determine co-association of, and context for, organic matter relative to known minerals, especially mineral catalysts that produce organic material from C_1 gases.			
2.1C	Evaluate the indigenous nature of any detected carbon and organic molecules. Rule out terrestrial sources of carbon and organic molecules.			
2.1D	Identify potential components of pre-biotic chemistry (e.g., prebiotic organic carbon compounds, reactive phosphorous, etc.			
2.1D	Assess organic inventory for similarity to known abiotic processes such as Strecker synthesis or Fischer Tropsch type reactions.			
2.2B	Evaluate the spatial relationships between organic matter and minerals and volcanic particles, especially such minerals that are compositionally and morphologically associated with biological activity or catalytic activity on Earth (e.g., Fe oxides and sulfides).			
2.2B	Evaluate the relationship of potentially biogenic minerals and their associated organic material to the history of the host rock.			
2.2B	Evaluate measurements of chemical and isotopic compositions of organic compounds to determine their conditions of formation and to seek evidence of chemical equilibria or disequilibria that are inconsistent with abiotic processes, and thus would be indicative of biological activity. Examples include widespread amino acid homochirality.			
2.3A	Measure the presence of biochemical species, especially pigments, proteins, DNA, RNA, lipids etc.			
2.3B	Measure the abundance of isotopes, isotopologues and isotopomers.			
2.3B	Extract and sequence DNA.			
2.3B	Identify and measure evidence for cellular growth, metabolism, and respiration.			
2.3C	Measure cell size, shape, and structure.			
2.3C	Evaluate morphological indications of replication and specialized features like motility structures.			
1.1A	Measure mineralogy and chemistry of both clastic and chemical sediments (including fluid inclusions) in a stratigraphic framework.			
1.1C	Determine the minerals formed by weathering processes over a range of distinct parent lithologies.			
1.2A	Detailed examination of mineral fabrics and compositions (e.g., with SEM/EMPA) to evaluate consistency with low-temperature hydrothermal conditions at the martian surface.			
1.2B	Measure geochemical proxies for salinity/composition of fluid inclusions in primary precipitates and secondary pore-filling cements that formed or were modified by hydrothermal processes.			
1.2E	In constructing a paragenetic sequence of the history of a given hydrothermal deposit (see also 1.1C), differentiate cements and other paragenetic events that are pore-filling (i.e., late diagenetic), search for evidence of dissolution and/or extensive replacement, or recrystallization of primary phases, and examine the mineral composition for evidence of suites of hydrothermal alteration minerals.			
1.3A	Examine the mineralogy of host rock and veins/vugs/fractures to identify evidence of chemical interaction with waters and the extent to which primary minerals have been altered by groundwater flow.			
1.3B	Determine all phases present in mineral assemblages in host rock and in groundwater-altered or -precipitated zones.			

1.3B	Examine the petrologic relationships between primary and secondary minerals to determine the chemical reactions taking place in the ground water system and the fluid chemistry.			
1.4B	Evaluate the chemical and isotopic composition, speciation, and abundance of organics, lipids, etc. in sedimentary and mineral precipitate deposits, in particular those with reduced Fe/S minerals and mineral precipitates, in order to determine whether or not organics may be biotic in origin.		2	
1.4B	Analyze the micro- and nano- scale morphology of mineral precipitates to search for textural biosignatures.			
2.1A	Measure the presence, concentration, and characteristics (e.g., redox state) of inorganic carbon including oxidized carbon (e.g., as carbonate) and reduced carbon (e.g., as graphitic or graphite-like carbon).			
2.1D	Identify the association of any organic carbon relative to known mineral catalysts and catalysis pathways.			
2.2A	Determine the presence of phases (e.g., silica, carbonates, phosphates, phyllosilicates, evaporite minerals, etc.) that are conducive to preservation.			
2.2A	Identify evidence for post-depositional diagenetic alteration of sedimentary or hydrothermal deposits.			
2.2B	Measure the abundances of organic macromolecules and smaller molecules and characterize their attributes, including molecular structures, abundances and/or molecular weight distributions.			3
2.2E	Evaluate mounding and layers for indications of past biological activity. Microbial fabrics and mesoscale biolaminated sedimentary structures (e.g., stromatolites) can persist in rocks even after chemical biosignatures have been lost through oxidation, radiation or heating.			
2.2E	Evaluate the possibility of molds or other types of impressions (casts) or associated geochemical signals that may indicate past biological activity or organic matter which may now have vanished, including mineralogically replaced fabrics that may have once been microbially produced.			
2.2E	Undertake hyperspectral analysis of rock sample surfaces to investigate changes in organic carbon content, water content and mineralogy that may point to biological activity.			
2.2E	Undertake high resolution, *in situ* investigation of textures and structures that may be related to fossilized microbial biofilms, colonies and cells.			
2.2F	Fe or Mn redox fronts.	2	2	
2.2F	Fractures, vugs, vesicles, or pore space filled with precipitated minerals (carbonates, silica, sulfates, clays, oxides).			
4B	Measure the C and O-triple isotopes of carbonates and water in rocks, dust and regolith samples.			
1.1A	Provide geologic context by measuring stratigraphy and determining structure of samples from the stratigraphic section.			
1.1A	Capture microscale sedimentary structures (laminations thickness, small ripples, etc.).			
1.1A	Perform provenance and geochronology studies of sediments.			4
1.1A	Measure textural parameters of sediments incl. grain size, grain shape, sorting of sediments.			
1.1A	Evaluate subsidence and accommodation space as part of understanding basin-scale processes.			
1.1B	Determine the mineralogy of sedimentary rocks and evaluate the possibility of secondary minerals that may have formed as cement in the pore network.			
1.1B	Determine the paragenetic sequence, including both overgrowth relationships and mineral dissolution events.			
1.1B	Measure the radiometric ages of the cement with permissible mineralogy (e.g., jarosite by K-Ar, calcite by U-Pb, etc.)		5	3
1.1B	Determine how diagenetic processes may either enhance or obscure possible taphonomy.			
1.1C	Determine the mineralogy and mineral chemistry of the sedimentary grains, and their relationship to the parent lithologies.		6	
1.1C	Determine the proportion of grains derived from igneous, metamorphic, and sedimentary rocks (and associated geochronology measurements, since there are many weathering minerals that can now be dated, such as hematite, goethite, etc.)		6	
1.1D	Determine the mineralogy and mineral chemistry of lithic fragments and mono-mineralic grains.			
1.1D	Determine the crystallization ages of lithic clasts and appropriate minerals, where possible (e.g., within coarse clasts) using radiometric dating.			
1.1D	Determine an integrated surface exposure age of the source regions by measuring cosmogenic nuclides.			3

1.1E	Measure mineralogy and chemistry of both clastic and chemical sediments, capturing different stratigraphic positions.		6	
1.1E	Perform provenance and geochronology studies of sediments.			
1.1E	Measure texture, grain size, grain shape of sediments.			
1.1E	Measure mineralogy and chemistry of both clastic and chemical sediments, capturing different stratigraphic positions.		6	
1.1F	Determine the mineralogy of sand and dust fractions in order to identify source areas.			
1.1F	Determine the grain-size range, sediment sorting, and sand grain shape in order to reconstruct the wind transport.			
1.2A	Identify spatial and temporal variability in the mineralogy, chemistry, texture, grain size and grain shape of each defined facies formed, or modified, by hydrothermal activity. This will delineate the extent of the hydrothermal footprint in time and space.			
1.2A	Measure the stable isotopic compositions of oxygen and silicon in primary rocks and minerals to look for systematic trends with respect to facies and minerals that may reflect thermal gradients.			
1.2A	Spectroscopy/mapping of rock sample surfaces (e.g., Raman), which can be used for paleo-temperature estimates.			
1.2B	Measure the stable isotopic compositions (e.g., O, S, C, N, and Sr) of primary minerals and gases (e.g., CO_2, SO_2, H_2S, CH_4, H_2, etc.) trapped in fluid inclusions.		5	
1.2C	Use multiple methods (e.g., light microscopy, Raman, XRD, EMPA, FTIR and other methods) to study hydrothermal deposits and host rocks to place minerals and microtextures into a broader stratigraphic context; determine the relative age and cross-cutting relationships of minerals, fabrics and structure, linking established outcrop scale to the microscale. Produce a paragenetic sequence of the complete history of the hydrothermal deposit.			
1.2D	Assess system size, and relative fluid volume and duration of fluid flow.			
1.2D	Measure the radiometric ages (Rb-Sr, K-Ar, and $^{40}Ar/^{39}Ar$) of the minerals with permissible mineralogy (e.g., jarosite, calcite, alunite, etc.)		5	
1.2D	Measure cosmogenic nuclides (for example, but not limited to, ^{3}He, ^{10}Be, ^{21}Ne, ^{38}Ar) of surface samples to determine exposure age and erosion rate.			
1.2E	Evaluate evidence for paleo-water table fluctuations, and timing of fractures and history of infilling of fractures to form veins and differential concentration of hydrated sulfate and clay minerals.			
1.3A	Analyze stratigraphy and petrography to determine the protolith of aquifer rocks (igneous or sedimentary and the local stratigraphy).			
1.3A	Characterize the petrology to understand cross cutting relationships and the timing of groundwaters relative to other geological events.			
1.3A	Evaluate the number of distinct episodes of groundwaters by examining rocks with cross-cutting relationships between zones of distinct mineralogy or morphology.			
1.3A	Determine formation ages of the groundwater host rock as well as the fluid-precipitated minerals (e.g., carbonate, jarosite, some phyllosilicates suitable for dating using radiogenic isotopes).			
1.3B	Evaluate flow volumes, fluxes, and water-rock ratios from the mineral assemblages and their relative timings of formation.			
1.3B	Establish the relative timing of each mineral formation to understand the evolution of the groundwater system and reactive flow transport.			
1.3D	Identify zones of mineral precipitation and dissolution.			
1.3D	Determine the sequence of chemical events affecting the rock from petrology.			
1.3D	Determine the stable isotopic values for all phases measured in IS 1.3A-C, sampling those from distinct episodes.			
1.3D	Perform radiometric age dating of samples from the host rock and minerals precipitated during each episode.			

1.4A	Measure variability in the mineralogy, chemistry (including identification and measurement of potential oxidants), mineral proportions and morphology of primary and secondary minerals and amorphous phases as a function of depth and/or degree of weathering, to constrain the duration of water interaction, water chemistry, water temperature, water residence time/drainage, and search for chemical trends that could be biosignatures.			
1.4A	Analyze the chemistry and mineralogy of climate-sensitive minerals at nano-, micro-, and macro-scales.			
1.4A	Measure the micro- and nano-scale morphology, thickness, and chemistry of rinds and coatings			
1.4A	Measure the stable isotope compositions of secondary minerals to constrain the composition and history of atmospheric and crustal volatile sources.			
1.4A	Determine the radiometric ages of applicable secondary mineral phases (e.g., jarosite)			
1.4B	Determine the composition and isotopic signatures of fluid inclusions and associated dissolved gases			
1.4C	Measure radiometric ages of primary and secondary minerals with permissible mineralogy to determine ages of formation and diagenetic events.			
1.4D	Analyze the microscale physical, chemical, and mineralogical properties of sediments to constrain transport history and determine the relative importance of physical and chemical weathering.			
1.4D	Measure cosmogenic nuclides to determine exposure age and erosion rate.			
1.5A	Measure variability in the mineralogy, texture, mineral proportions, and mineral chemistry (major, minor and trace element), and bulk compositions (major, minor and trace element, and radiogenic isotopes) of igneous rocks in order to classify them relative to other igneous rocks from Mars, and determine whether they represent primary mantle melts or melts affected by interaction with other mantle or crustal sources or components.			
1.5A	Measure the compositions of any volatile-bearing minerals or melt inclusions, and the redox states of multivalent elements, in order to determine the conditions of magma genesis and crystallization (e.g., T, P, X, fO_2) for each silicate melt, and to elucidate any changes in conditions during crystallization (e.g., in oxygen fugacity and/or volatile content).		2	
1.5A	Quantify the textures of igneous samples through crystal size distribution analysis and mineral mapping techniques, in order to determine their setting (e.g., intrusive or extrusive) and the conditions during magma ascent, emplacement, and solidification.			
1.5A	Measure radiometric ages (e.g., U-Pb, Ar-Ar, Sm-Nd, Lu-Hf, Rb-Sr) of each igneous rock from as many systems as feasible in order to obtain crystallization ages.		5	
1.5A	Measure stable isotopic compositions (e.g., O, S, Fe, Mg) of minerals and bulk rock samples in order to quantify primary igneous and secondary (e.g., alteration or other modification) processes.			
2.1A	Measure cosmogenic nuclides to determine integrated surface exposure age and erosion rate.			
2.1B	Measure stable isotopic compositions (e.g., of C, H, N, O, P, S, Cl) in organic compounds in context with known isotopic pools.			
2.1D	Measure cosmogenic nuclides to determine surface exposure age and erosion rate.			3
2.2A	Measure cosmogenic nuclides to determine surface exposure ages and erosion rates.			
2.2B	Measure the relative abundances of species containing C, H, N, O, P and S.			
2.2C	Measure patterns of stable isotopic compositions of carbon-bearing minerals and other inorganic phases.			
2.2C	Measure isotopic compositions of bulk organic matter and also patterns of isotopic abundances between organic compounds and within individual compounds.			
2.2D	Detect individual minerals and map the spatial arrangements between minerals in formerly habitable environments.			
2.2D	Determine the relationships between potentially biogenic minerals and the history of the host rock.			
2.2E	Characterize microscale or macroscale rock or mineral fabrics and structures. For example, microbial biofilms can alter the chemistry and physical properties of sediments. Use thin sections and rock chips to search for microscale or macroscale rock, mineral or carbonaceous fabrics and structures that are consistent with formation or fossilization of biological entities (e.g., microbial biofilms and microbialites), but inconsistent with chemical or abiotic processes.			

2.2E	Characterize mineral surfaces and interiors to search for physical evidence of metabolic activity (e.g., pits and trails), especially where associated with redox gradients. Such features can indicate the former presence of endolithic microorganisms and communities (e.g., Friedmann, 1993; Reid et al., 2000; Foucher et al., 2010). Trace fossils (e.g., movement trails) could indicate microscale dissolution textures due to "mineral mining" by bacteria. Use microscopy to image mineral surfaces and interiors to search for physical evidence of metabolic activity (e.g., pits and trails), especially where associated with redox gradients.			
2.2F	Fe oxide or Fe sulfide precipitates (e.g., framboids).			
2.2F	Zones enriched in minerals formed by leaching or *in situ* transformations.			
2.2F	Crystallographic structures and major- and minor-elemental abundances of individual phases.			
2.2F	Abundance patterns of minerals and other phases plus their elemental and chemical compositions.			
2.2F	Example methods include XRD, XRF, XAS, XCT, Raman spectroscopy, NMR, and TEM.			
2.3D	Measure expected contamination levels and calculate their subsequent transfer on returned samples.			
3A	^{40}Ar-^{39}Ar measurements		5	3
3A	U-Pb measurements, especially of zircons or monazites		5	
3B	Isotopic age dating (^{40}Ar-^{39}Ar and U-Pb)		5	3
3C	Coupled magnetic and geochronologic measurements		5	
3C	Magnetic paleointensities and orientations		5	
3D	Oxygen isotopic composition, REE in phosphates, Li isotopes			
3D	Appropriate dating analyses for each sample (e.g., REE in phosphates, Li isotopes, U-Pb, Re-Os, K-Ar, ^{40}Ar-^{39}Ar or Rb-Sr methods)			
3E	^{182}W-^{142}Nd measurements			
3E	Hf-W measurements			
3F	Sm-Nd dating			
3F	Hf-W dating			
3F	Cosmogenic nuclide (^{3}He, ^{21}Ne, ^{10}Be, ^{26}Al, ^{36}Cl) dating of surface rocks		5	3
3F	Isotopic compositions of nuclides with high neutron capture cross sections (e.g., B, Cd, Sm, Gd) in soil, regolith, and other rocks			
3F	U-Th-He dating of U-Th-rich accessory minerals			
3F	$^{40}Ar/^{39}Ar$ and $^{4}He/^{3}He$ thermochronology, as well as cosmogenic ^{38}Ar, $^{21,22}Ne$ and ^{3}He		5	3
4A	Measure the noble gas elemental abundance in the present martian atmosphere.			
4A	Measure the oxygen triple isotopic composition, C isotopes and clumped isotopes in igneous rocks.			
4A	Measure the sulfur quadruple isotopes of oxidized and/or reduced minerals in igneous rocks.			
4A	Measure the indigenous noble gas signature of a geologically well-defined igneous sample.			
4A	Measure the volatile content in unaltered apatite from a Noachian igneous sample.			
4B	Measure the carbon and oxygen triple isotopic composition of CO_2 gas from atmosphere samples, and evolved from solid samples.			
4C	Analyze atmospheric gas implanted into impact melt.			
4D	Determine the seasonal variability of the elemental and molecular composition of the atmosphere, including, but not limited to, carbon dioxide, noble gases (particularly Kr and Xe), H_2O, oxychlorines, H_2O_2 and other oxidizing species and methane.			
5A	Measure variability in the mineralogy, texture, mineral proportions, and mineral chemistry (major, minor and trace element), and bulk compositions (major, minor and trace element, and radiogenic isotopes) of all igneous rocks and igneous clasts within brecciated samples, or as xenoliths in basaltic samples in order to classify them relative to other igneous rocks from Mars, and determine whether they represent primary mantle melts or melts affected by interaction with other mantle or crustal sources or components.			

5A	Measure radiometric ages (e.g., U-Pb, Ar-Ar, Sm-Nd, Lu-Hf, Rb-Sr) of each igneous rock from as many systems as feasible in order to obtain crystallization ages and ages of source regions. (Note that although the Hf-W system would be of interest, this may or may not be possible using samples collected by M-2020, because of potential contamination from the drill bit).			3
5A	Measure stable isotopic compositions (e.g., K, Zn, Rb, O, H) of minerals and bulk rock samples in order to quantify primary igneous and secondary (e.g., alteration or other modification) processes.			
5A	Measure the HSE abundances of all breccia clasts and each igneous rock type sampled in order to gain insights into the original composition of Mars and core-mantle segregation and mantle differentiation processes			
5B	Measure the absolute direction and intensity of magnetization in oriented martian bedrock materials as a function of time.			
5B	Determine the major mineral carriers of martian crustal magnetization by measuring their rock magnetic properties.			
5B	Measure the ages of rocks that contain geomagnetic anomalies to constrain the age of the dynamo and martian magnetic history.			
5C	Measure variability in the mineralogy, texture, mineral proportions, and mineral chemistry (major, minor and trace element) of rocks that have been modified by water/rock interactions.			
5C	Identify cross-cutting relations, and measure radiometric ages of primary (igneous) and secondary (alteration) minerals with permissible mineralogy.			
5C	Measure stable isotopic compositions (C, H, O, N, S) of minerals from veins and cavities.			
5C	Determine the composition of fluid inclusions and reconstruct trapping temperatures from phase relationships within those inclusions.			
5C	Determine the conditions of (metamorphic) equilibration of rocks, including temperature and pressure.			
5D	Measure variability in the mineralogy, texture, mineral proportions, and mineral chemistry (major, minor and trace elements) of rocks that have been formed or modified by impact-induced hydrothermal activity.			
5D	Determine cross-cutting relations, and measure the radiometric ages of the primary and secondary minerals with permissible mineralogy.			
5D	Measure the isotopic composition (e.g., Cr) of rocks that have been formed by impact in order to determine the origin of the meteoritic material impacting Mars.			
5D	Determine cross-cutting relations, and measure the radiometric ages (e.g., by Ar-Ar, Rb-Sr, etc.) of the primary and secondary minerals with permissible mineralogy.			
5D	Constrain the shock pressures and temperatures experienced by the minerals and melts during impact.			
6A	Ecotoxicity tests (TBD selected exposure tests on representative species).			
	Physicochemical Characterization			
6B	Bulk Elemental Composition (e.g., by EMPA) and mineralogy determination (e.g., by XRD) to help understand the origin of any toxicity.			
6B	Particle Size Analysis. Determine particle size distribution within the samples as well as confirm size fraction for exposure studies.			
6B	Total Surface Area and Pore Space (e.g., by BET). Understand the amount of total surface area available for geochemical reactivity and direct interaction with biological systems.			
	Toxicological Assessment (in order of priority)*			
6B	Morphology (e.g., by SEM). Analyze the shapes of martian dust grains with a grain size distribution sufficient to assess their potential impact on human soft tissue (especially eyes and lungs)			
6B	Assess martian dust pulmonary toxicity (adverse impacts to the respiratory system) relative to the known toxicity of lunar dust and well characterized reference dusts (e.g., titanium dioxide and quartz).			
6B	Assess martian dust cardiovascular toxicity (adverse impacts to the cardiovascular system) relative to the known toxicity of lunar dust and well characterized reference dusts (e.g., titanium dioxide and quartz).			
6B	Assess martian dust ocular hazard (Adverse impact to the eye) using representative unfiltered martian drift surface dust. Particle size is not a practical limitation.			

6B	Assess martian dust dermal hazard (Irritation/Abrasion of skin) using representative unfiltered martian drift surface dust. Particle size is not a practical limitation.			
	* Ideally, Permissible Exposure Limits (PELs) should be determined utilizing primary martian material whereas broad toxicological assessments can utilize high-fidelity simulants			
6C	Analyze the particle grain shape, surface area and size distribution to understand how dust will be "kicked up" by large descent engine plumes or large rovers, as well as to validate fluid system component performance, such as filters.			
6C	Abrasivity testing will help evaluation of pressure seal degradation, hatch leakage, optical surface degradation (windows, visors, instruments), damage to protective coatings and rotating equipment (such as bearings or pumps), and wear on spacesuits and flexible insulation materials.			
6C	Determination of electrical and magnetic properties to assess potential problems, such as charged dust particles resulting in static shock equipment damage, as well as to evaluate potential useful properties, such as electrostatic dust cleaning techniques.			
6C	Determination of thermal and optical properties to evaluate dust-coated radiators, solar arrays, electrical cables, and light fixtures.			
6C	Chemical characterization for crop growth experiments (including perchlorate uptake assessment), hardware corrosion assessments, membrane function, and chemical process hardware interactions (such as ISRU or water extraction)			
6C	Surface area analysis to determine how much the sample can adsorb; may also be important for developing simulants used in plant growth studies.			
6C	Physical and mechanical properties characterization to assess the interaction of structural elements with the Mars environment.			
6D	Determine the extent of protection that different thickness layers of regolith provide from different types and dosages of radiation.			
6D	Determine the extent of damage produced by irradiation of the surface, and assess the potential for biologically-significant species to be produced.			
7B	For martian regolith, characterize the geotechnical properties such as regolith particle size distribution and shape, densities, and strength/cohesion properties. This information will influence simulant design and material handling technology (e.g., excavation & transfer).			
7B	For (hydrated) rock samples, characterize the strength and other properties to understand excavation and comminution of rock materials.		7	
7B	Characterize the thermal properties of the samples to understand heat transfer for resource extraction and system thermal management purposes.			
7B	Analyze regolith properties that may be important for its use in construction applications including as a potential building or shielding material, or as a feed stock for additive manufacturing processes.			
7B	Measure grain size distribution in regolith sample that are comprised of airfall dust in order to understand how small particles might damage catalyst function.			
7C	Identify minerals that are high in elements typically used in fertilizer (N, P, K) in multiple samples of martian regolith (to facilitate comparison between different regolith types).			
7C	Identify compounds that could potentially be damaging to food crop production processes.			
7C	Characterize the presence of metals that may be important for soil microbial metabolism.			
7D	Identify minerals that are high in metals (such as Fe, Ni, Al, Cu, Cr, Au, PGM (Platinum Group Metals), KREEP (Potassium, Rare Earth Elements, Phosphorus)) in multiple samples of martian regolith (to facilitate comparison between different regolith types).			

Red: measurement would be affected by the sterilization method regardless of the exact methods of measurement
Yellow: certain measurement methods for this measurement would be affected

1: sample tube interior is likely in an equilibrium state, balanced by the interior atmosphere, opening tube to either dry N2 or any other lab atomsphere may alter the chemistry and mineral. Investigation needs to be done as soon as possible
2: possible depending on fO2 in containment
3: possible depend on radiation type and dosage
4: possible, depends on what are the geochronology studies
5: possible if T is above closure T or T > T_Curie or break fluid inclusions
6: possible if minerals are carbonate or hydrated
7: chemical comminution or strength change by heating

Appendix E: Effects of Sterilization

Sterilization of Geologic Samples Returned to Earth from Mars – Effects and Recommendations

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Planetary Protection: NASA planetary protection strategies have long called for samples returned to Earth from Mars to be immediately placed into biological containment in a Sample Receiving Facility. The samples would then be tested for signs of present or past life and biological hazard (1). The Space Studies Board of the National Academy of Sciences (2) recommended that "Controlled distribution of unsterilized materials from Mars should occur only if rigorous analyses determine that the materials do not constitute a biological hazard. If any portion of the sample is removed from containment prior to completion of these analyses it should first be sterilized".

Mars samples returned by spacecraft may prove to be biologically sterile, in which case further sterilization will not be necessary. If sterilization of a subset of samples is required, however, the desired results are twofold. First, any viable organisms must be killed. Second, to the greatest extent possible, the isotopic, chemical, and physical characteristics of the rock and soil samples must be preserved.

Mars 2020: The Mars 2020 rover will be equipped to characterize and collect Mars rock and soil samples capable of being transported to Earth by future missions. These rocks and soils are potentially the first extraterrestrial samples to be returned to Earth from known locations on Mars.

Drilled samples of rock and soil will be encapsulated and sealed in metal tubes, which will be left on the Martian surface pending possible return to Earth by future missions. The tubes measure approximately 1 cm in diameter and 7 cm long. Current designs envision the return of as many as 31 samples, including blanks.

The Mars 2020 mission is targeted to land in Jezero crater. The crater contains a fan delta deposit rich in clays, and this deposit is likely to be a prime target for sampling. Magnesium carbonates and olivine have also been identified in the crater using orbital spectroscopy.

Sterilization by Dry Heating: No space agency has adopted a specification for heat sterilization of geologic material. However, starting with the Viking landers in 1976, Dry Heat Microbial Reduction (DHMR) has been required to minimize forward contamination of spacecraft and spacecraft components involved in specific missions to Mars.

NASA has updated specifications for Dry Heat Microbial Reduction on spacecraft components designed for robotic extraterrestrial missions (3).

- At a temperature of 125 °C, 5.0 hours would be the "time required to destroy 90 percent of the nonhardy microbial spore population encapsulated in nonmetallic spacecraft material". A time of 18.75 hours at 125 °C is required to reduce the population of heat-resistant spores embedded in materials to 0.1%.

- A temperature of 500 °C for ½ second constitute "conditions at which all organisms will be completely destroyed".

Sterilization by Gamma Irradiation: No space agency has adopted a specification for radiation sterilization of geologic material nor spacecraft components. Medical device companies use doses of

2.5×10^4 grays* for sterilization purposes.

*1 gray = 1 joule of radiation energy per kilogram of matter; 1 gray = 100 rads)

Deinococcus radiodurans, one of the most radiation-resistant bacteria known, survives doses of 5×10^3 grays when active; less when dry and dormant. *D. radiodurans* exhibits exponential decline at doses above 6×10^3 grays, with concentration in growing cultures falling to <0.1% at a dose of 10^4 grays (4). Doses of 7×10^3 grays inactivate *Ebola, Lassa, and Marburg* viruses to the 0.1% level (5).

Effects of Dry Heating Microbial Reduction on Rocks and Minerals: Gooding (6) summarized effects on possible Mars minerals at temperatures between 0°C (approximate maximum Mars surface temperature) and a microbial reduction temperature of 125°C:

- Salt-Mineral/Water Isotope Exchange
- Maghemite/Magnetite transition
- Clay-Zeolite/Water Isotope Exchange
- Zeolites Dehydrated
- Clay-Mineral H_2O(-) lost
- Oxidants measured by Viking landers decompose
- Salt-Mineral H_2O(+) lost
- Mg-Carbonate Transition
- Igneous minerals (pyroxene, plagioclase, olivine) would not be affected

Note that clays and Mg-carbonates, identified spectroscopically at Jezero crater, would be altered by the effects of 125 °C heating. Olivine, also identified at Jezero crater, would not be affected.

Effects of Gamma Ray Sterilization on Rocks and Minerals: Allen et al. (7) performed experiments to determine the effects of sterilizing doses of gamma radiation on Mars analog rocks and minerals:

<u>Experimental Conditions:</u>

- ^{60}Co in a commercial irradiator
- 1.17 and 1.33 MeV gamma photons
- Shielding from 1 cm of basalt ~ 6%
- Estimated temperature 49 °C
- Dose rate 3.15×10^2 grays/min
- Total Doses
    - 3×10^3 grays
    - 3×10^4 grays
    - 3×10^5 grays
    -

<u>Rocks and Minerals Irradiated:</u>

- Plagioclase
- Olivine

- Pyroxene
- Quartz
- Chert
- Clay (Na-montmorillonite)
- Halite (NaCl)
- Aragonite ($CaCO_3$)
- Gypsum ($CaSO_4 \cdot 2H_2O$)
- Basalt
- Mars soil simulant
- Carbonaceous chondrite (CM2) meteorite
- Water (monitor boiling)
- Tin (monitor induced radioactivity)

Summary of Results:

- No induced radioactivity
- No changes in isotopic composition
- No changes in radiometric dating
- No changes in chemical composition
- No changes in crystal structures
- No changes in thermal infrared spectra
- No changes in Raman spectra
- No changes in surface areas
- No changes in fluid inclusions
- Darkening of quartz and halite
- Changes in thermoluminescence

Specifically, there were no measured changes in samples of clay, carbonate or olivine, the minerals spectroscopically identified at Jezero crater.

Conclusions:

- No current standard exists for heat sterilization of geologic samples.
- Dry heat at 125 ^{o}C for hours is the accepted standard for reduction of microbes embedded in spacecraft components.
- This temperature would result in alteration of the clays and Mg-carbonates known to exist at Jezero crater.

- No current standard exists for radiation sterilization of geologic samples.
- Absorbed doses on the order of 10^4 grays are sufficient to destroy most terrestrial microbes and inactivate most viruses.
- Doses as high at 3×10^5 grays have minimal effects on the nuclear, crystallographic, and physical properties of Mars analog rocks and minerals, including those known to exist at Jezero crater.

Recommendations [from this author]:

- Establish standards for heat and radiation sterilization of rocks and minerals.
- Document the effects of heat and radiation sterilization procedures on rocks and minerals known to exist at proposed sample return sites.
- Include sterilization equipment and protocols in the initial design of the Sample Return Facility.
- Establish sample handling procedures that specifically include sterilization of a subset of the samples, for distribution to research labs outside of the Sample Receiving Facility.

References: (1) DeVincenzi D. L. et al. (1998) *Journal of Geophysical Research, 103*, 28,577 - 28,585. (2) Space Studies Board, *Mars Sample Return Issues and Recommendations* (1997) National Research Council. (3) *NASA Procedural Requirement 8020.12*, 2011. (4) Battista J. R. (1997) *Annual Reviews of Microbiology, 51*, 203 – 224. (5) Richmond and Walker (1982) *American Association of Veterinary La*boratory Diagnosticians Annual Proceedings, 25th, 433 - 440. (6) Gooding J. L. (1990) NASA Technical Memorandum 4184. (7) Allen C. C. et al. (1999) *Journal of Geophysical Research, 104*, 27,043 – 27,066.

Appendix F: Acronyms and Abbreviations

BC	Basic Characterization
BH	Biohazard
BHP or BAP	Bio Hazard Assessment Protocol
BSC	Biosafety Cabinet
BSL 3 & 4	Laboratories Or Facilities With Bio Safety Level 3 And 4 Rooms
DWI	Double Walled Isolator
EDS	Energy Dispersive X-Ray Spectroscopy
FT-IR	Fourier-transform infrared spectroscopy
GC	Gas Chromatography
GC-MS	Gas Chromatography-Mass Spectrometry
IC-MS	Ion Chromatography-Mass Spectrometry
LC-MS	Liquid Chromatography-Mass Spectrometry
LD	Life Detection
M-2020	Mars 2020 Rover mission
MinION	Nanopore DNA Sequencer
PE	Preliminary Examination
PP	Planetary Protection
SEM	Scanning Electron Microscopy
SSAP	Sample Safety Assessment Protocol
XRD	X-ray powder diffraction
XRF	X-ray fluorescence