Final Report of the MSR Operational Scenarios Definition Team (MOSDT)

Prepared by the MSR Operational Scenarios Definition Team (MOSDT), in response to Terms of Reference received from NASA and ESA

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Prepared by the MSR Operational Scenarios Definition Team (Chairs: Lindsay Hays & Aurore Hutzler; Core Team: J. Nick Benardini, Enrica Bonato, Andrea Harrington, Gerhard Kminek, Richard Mattingly; Experts: Michael Calaway, Francois Gaubert, Alvin Smith; Coordination: Julia Donnerer)

MSR Operational Scenarios Definition Team

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1. Executive Summary

The return of scientifically selected samples from Mars provides a rare opportunity for investigation with the full range of the latest technology available, but to take full advantage of this opportunity, it is important to ensure the pristine nature of the samples upon arrival within the Earth environment until scientific investigations begin. The work presented here is an operational workflow for the Sample Receiving Facility (SRF) that seeks to preserve the pristine nature of the samples while minimizing risk of release of martian material. The Mars Sample Return (MSR) Operational Scenarios Definition Team (MOSDT) was focused on infrastructure purposes, and particular attention was paid to operations that would be most important for these purposes and that could be used as input for facility studies. Thus, the workflow should not be considered a singular science or sample management workflow but merely one workflow that would address the community-based guidance.

The MOSDT produced three deliverables to support and describe this scenario – a Main Sequence of Operations workflow (a visual, flowchart-like description of the sample operations pathway with little to no implementation solution), an Instrument Table (the complete list of instruments identified for sample analyses) and this text Report to support and describe the other two deliverables and suggest some implementation solutions. Because of the limited nature of the task given to the MOSDT, a detailed **List of Assumptions** is included to describe the bounding conditions on the workflow that is presented. These are quite extensive and provide justifications for certain decisions that

were made regarding the workflow; however, they should not be read as requirements. The report also contains a detailed description of the **Main Sequence of Operations** (the visual representation of which is a separate deliverable), and providing written descriptions of each of the steps, as well as the identified side workflows that were not as critical for the main sequence following the samples. A section on **Timing** describes some of the most significant time-relevant steps, including ***how the gas sampling methods may take relatively long times to equilibrate***, and ***how certain elements of the infrastructure may also be a trade between the facility size, the number of particular elements present and the time needed to complete different steps in the workflow***. A detailed section describing considerations for the different **Environments** that an individual sample would move through during the workflow includes much of the focused input and consideration from the NASA HQ Planetary Protection office regarding biosafety and biocontainment of potential Mars organisms for backward planetary protection (PP), to balance the sample science integrity focus of the MSPG2 group. ***To meet planetary protection and scientific requirements the sample receiving facility will have to combine aspects of BSL-4 labs with state-of-the-art cleanroom designs, and this would be the first attempt to combine these two types of facilities.*** Significant changes to requirements beyond MSR Science Planning Group Phase 2 (MSPG2) and Sample Safety Assessment Protocol (SSAP) are described in the **MOSDT added requirements** section. The most significant of these is taking into account the end-to-end process inside the SRF, resulting in an increase in the number of isolators from the number reported from previous reports; a separate section is included to describe the justification for this number. The final section on **Future Work** balances the List of Assumptions at the start by describing the most important next steps that were identified by the MOSDT but that were beyond the scope of this work. This section is broken into three sections presented in rough priority order: Facility Operational Trade Space (of which ***the two most important studies will be focused on 1) the science prioritization of instruments and the option to descope as a trade with SRF timeline and funding*** and 2) the ***implementation of technology within high-containment***) Technology Development (which describes both ***further development of the Double Walled Isolator (DWI) design*** and ***monitoring the capabilities of consolidated instrumentation to meet both science and SRF space and footprint needs***) and Engineering Development (which describes all of the different engineering-specific decisions which will need to be monitored for potential SRF implications).

2. Introduction

2.1. Statement of Task from the Terms of Reference:

The SRF Operational Scenarios Definition Team will use community-defined requirements to conceptualize the sample operations that will inform future architecture teams during the ESA and NASA facility studies planned in the 2021-2022 timeframe. Translating requirements should result in various operational scenarios, with implementation options considered in terms of sample handling, core isolation technologies, and any other possible technical implementation choices. Emphasis is placed on the responsibility of the Definition Team to represent the view of the international scientific community.

Operational scenarios should cover any operations within the facility and any operations to transport samples to other containment and non-containment facilities.

The Definition Team is expected to document their work in the form of implementation options and corresponding trees of operational scenarios (format To Be Determined (TBD)), supported by a written report narrating the reasoning and potential choices made, for accountability and traceability.

Operational scenarios must be in a final state […] to be integrated in subsequent facility studies. It is to be understood that these scenarios will be based on best available knowledge at the time and are intended to be used by ESA and NASA to support the upcoming facility studies in 2021-2022 only. It is expected that updated operational scenarios will be needed to be established in the future to support any development phase of a US or European facility.

2.2. Assumptions from the Terms of Reference

1. The draft requirements for infrastructure activities are comprehensively described by MSPG2 (2021) and SSAP (2021).
2. A strawman instrumentation set can be derived from MSPG2 and SSAP recommendations.
3. Some consideration should be given to costs and risk, but programmatic or political aspects should be left for future activities.
4. This study exclusively considers samples from Mars, and the initial activities for the returned samples.

2.3. Deliverables

The Main Sequence of Operations workflow is the key output of the MSR Operational Scenarios Definition Team. It recommends a "likely path" for the samples starting at Earth landing, through the SRF and eventual release to outside labs. Activities range from engineering operations to curation to science, with science being used here as the science to be done within the SRF. The side workflows (e.g., dust or gas analysis) and potential contingencies are described in additional workflows, but the main sequence is considered the path that focuses on a workflow for the samples in the Returnable Sample Tube Assemblies (RSTAs). The workflow has been created by identifying and ordering the steps and activities to be done in the SRF but refrains at the maximum to show any specific implementation for each step. The MOSDT identified then which steps could be conducted without a change in environment parameters, with environment meaning in this context a set of specific parameters such as temperature, humidity, type of atmosphere, cleanliness level, biocontainment levels, acceptable materials, etc. While a significant discussion of steps has been included for preparing samples to be released from the SRF (including for decision making relative to safety assessments as well as operations relating to samples being sterilized prior to release), specific transportation scenarios for samples outside of the facility were not addressed.

The Instrument Table is the secondary output. It describes the complete list of instruments identified by the MSR Operational Scenarios Definition Team as crucial based on the MSPG2 and SSAP findings and reports as well as standard facility/curation support needs. It contains information about the instrument types, instrument specifics, and where appropriate contains "Significant Instrument Comments" that represent following through the broad recommendations in the reports from earlier groups to a range of instrument implementation possibilities (e.g., the need for three distinct SEMs due to differing

environmental contamination levels, to achieve all the SEM functions as recommended in the MSPG2 final report).

This written report serves to provide additional support and descriptions for the two deliverables described above, as well as providing a list of high-level assumptions key to the reasoning for the materials above, and the future work to move forward. It also provides a narrated description of the Main Sequence of Operations.

A note on the implications of this report: The workflow described in detail in this report should not be interpreted as the best or only workflow that will be used to process samples at the SRF; this should be considered as an operational scenario that will serve the purpose of getting SRF infrastructure studies initiated. The actual operational workflow that will ultimately be employed in the SRF will be different in a multitude of ways from that described here. The primary input that was used in consideration for generating this example operational scenario was the MSPG2 report, and therefore only addresses recommendations that were presented in the final report. Additionally, because the main focus was on infrastructure of the SRF, there was consideration of the infrastructure aspects that would need to accommodate receiving all the hardware that would be encasing the samples, whereas the infrastructure implications of sending samples out of the facility should be minimal and was given less consideration.

3. High-level assumptions

The following high-level assumptions form the basis for reasoning through the sample handling activities and creating the Sequence of Operations. These either reiterate assumptions or findings from previous groups or work or became evident through the course of MOSDT work.

Assumptions drawn from previous work

A1 - Cleanroom capability will be necessary to minimize any form or means of terrestrial contamination (e.g., particulate, airborne, molecular, surface contact from any source being inorganic, organic or biological) that may alter sample integrity and/or affect the analytical results to unacceptable levels that would either confound the sample safety assessment or above the signal to noise ratio of the desired downstream investigations.

A2 - BSL-4 equivalent containment will be required to protect Earth's biosphere from extraterrestrial materials that have not been sterilized or have yet to be determined safe through a sample safety assessment.

A3 - After landing on Earth, the EEV will be encapsulated in two layers of containment, and the exterior layer will be free of Martian material.

A4 - While the contents of each tube will be analyzed individually starting at Basic Characterization (BC) through the rest of the Operational Scenarios, there will be at least eight categories of samples.

A5 - Sample Safety Assessment (SSA) will be informed by activities in multiple stages of Pre-Basic Characterization (Pre-BC), BC and Preliminary Examination (PE), and thus will not require a specific physical area.

A6 - Processing for SSA will need to have biologically cleaned equipment and support equipment that will come in contact with the sample.

A7 - All instruments listed as part of Pre-BC, BC, and PE will stay under high containment; though some part of the instruments could be kept under lower levels of containment, this would likely have the largest implications for SRF infrastructure and is the assumption used for this work.

A8 - Activities on each RSTA that happen in Pre-BC and BC will occur before PE, and activities that happen in PE will generally occur before science (e.g., time-sensitive or sterilization-sensitive investigations) and before samples are released for dedicated investigations. The analysis workflow (out of scope for this work) would need to be optimized to accommodate time-sensitive measurements and there may be some measurements from earlier activities that can inform later phases.

A9 - Activities will occur in a variety of environments to include non-classed space, cleanrooms, a range of BSL-2 through – BSL-4 laboratories, high-containment prep space, and high containment space. Additional space and capabilities outside of the high-containment laboratory, but within the SRF, and described herein will need to be considered to support sample safety risk and inadvertent release.

A10 - Additional infrastructure in the SRF will be needed to support high-containment activities and compliance with industry best practices and will likely include: A. central services for both front-end preparation (e.g. reagent preparation, autoclave, clean laboratory staging for disposables of common use items, space for cleaning and kitting of equipment), B. decontamination of effluents, and C. sterilization of waste and materials. These will drive additional facility requirements.

<u>Assumptions that were made during MOSDT activities</u>

B1 - The landing site will be remediated at the impact zone; though there are other potential operations at the landing site, this would likely have the largest implications for SRF infrastructure, and is the assumption used for this work. Additional SRF infrastructural requirements should be limited to storage and analyses of contamination knowledge and biological knowledge samples collected at the impact zone (pre-sterilization/remediation).

B2 - When sample dust/particulates, cores, or core subsamples are not actively being worked with, they will be kept in a dedicated (to sample or subsample, depending), rigid, clean container, as compact as possible.

B3 - Distinct categories of sample should not be processed in the same environment. As a balance between timing, space and staff availability, we considered 2-5 parallel lines.

B4 - For each isolator line needed to process a single sample tube, the infrastructure support (e.g., HVAC) will be independent so that decommissioning and recommissioning associated with the deep cleaning (e.g., precision cleaning, sterilization) and cleaning/sterilization validation can be done independently from the other lines. This may require an independent isolated room with independent infrastructure support for each line to enable the continued work on other isolator lines. Though there are other potential operational options for this separation, this

would likely have the largest implications for SRF infrastructure, and is the assumption used for this work.

B5 - The transfer of samples to different environments will be assumed to be as conservative as possible in order to stringently maintain contamination control, though this will likely have the largest implications for SRF infrastructure.

B6 - The choice of environment block types (e.g., airlocks, cleanroom corridors between operations rooms) will be determined by considering optimal workflow and cleanroom ingress/egress protocols.

B7 - For Go/NO-GO decision points, one additional environment was considered sufficient for infrastructure sizing, in case of a NO-GO decision.

B8 - It should be possible to clean and sterilize tools, isolators and equipment to the point that they can be reused for other purposes and/or other materials. Cleaning capabilities utilized within the SRF will be baselined on the current state-of-the-art for BSL-4 laboratory facilities and decontamination as well as contamination control cleaning measures developed for astromaterials and curation.

B9 - PE and Science instruments will need the same level of access and use and may not be physically segregated.

B10 - Sterilization equipment (radiation and/or heat) will be available in the SRF and be accessible for Martian samples at any stage.

B11 - The outside of the Secondary Containment Vessel (SCV) and Primary Containment Vessel (PCV) are expected to be free of unsterilized Martian material unless breached during landing, so no dust gathering steps should be needed, but a contamination monitoring step will likely still be needed. For this contamination monitoring step only, if there is no dust, it should still be done in same isolator as engineering inspection.

B12 - The inside of the PCV and outside of the OS are expected to have limited, but non-zero, quantities of Martian dust. If any dust is collected from these two surfaces, it will be collected together since it is the same environmental space. Any material gathered will be stored in a non-pristine isolator such as the reusable subsample isolator (RSubS) due to the lower contamination control requirements for this part of the flight system relative to inside the sample tubes.

B13 - The inside of the OS and outside of the tubes are expected to have enough Martian dust to be recoverable, and to be used in scientific investigations. The material collected from these two surfaces should be collected together since it will have been in shared environmental space for years during the return missions. Any dust gathered will be stored in a non-pristine isolator such as the reusable subsample isolator (RSubS) due to the lower contamination control requirements for this part of the flight system relative to inside the sample tubes.

The last three assumptions in the section above (B11-B13) are specifically related to the multiple layers of containment vessels that the samples are stored in and are therefore highly dependent on their design; the disassembly and cleaning of these vessels has only a minor effect on the infrastructure but are included for completeness.

4. Sequence of operations

4.1. Main Organization Flow for Mars Sample Handing

The process of retrieving the martian samples from the landing site through PE and Science, as defined by MSPG2, can be broken down into eight main steps:

- Landing Site Ops
- Transport Isolation Container (TIC) SRF Arrival and Disassembly of Earth Entry Vehicle (EEV)
- SCV Disassembly
- PCV Disassembly
- OS Disassembly
- Pre-Basic Characterization
- Basic Characterization
- Preliminary Examination and Science

The steps identified below represent major transition points in process but do not necessarily represent changes in environment. However, within each section, major environmental transitions and proposed instruments and technologies will be described. While the main operational flow will be the focus, separate "side investigations" or other possible options will be mentioned.

4.1.1. *Landing Site Operations*

The scope of the MOSDT was focused on operations within the SRF. In the absence of publicly available reference materials for Landing Site Operations, it was deemed necessary to reflect on these operations, in order to verify that nothing that could majorly impact the infrastructure would be overlooked. The same reasoning applies to the hardware disassembly steps: the specific steps should not be considered as requirements or the optimal workflow, but they are described to keep a high-level of accountability and to support the MOSDT conclusions.

Assumption B1: The landing site will be remediated at the impact zone; though there are other potential operations at the landing site, this would likely have the largest implications for SRF infrastructure, and is the assumption used for this work. Additional SRF infrastructural requirements should be limited to storage and analyses of contamination knowledge and biological knowledge samples collected at the impact zone (pre-sterilization/remediation).

The Martian samples are planned to arrive on Earth via ballistic reentry in the EEV System. When the EEV touches down, a mobile team should secure the area. As part of this process, the team should do a rough cleaning step of the exterior of the EEV and do a preliminary structural engineering inspection. Contamination Knowledge (CK) and Biological Knowledge (BK) samples of the terrestrial soil and material surrounding the EEV, as well as the material used to clean the EEV, should also be collected and secured. The EEV should then be placed within the ERIC, and additional cleaning of the landing site may be conducted. The ERIC notionally represents the initial layer of high-containment protection around the EEV after it returns to the Earth. However, due to projected terrain difficulties, the ERIC design may have to be minimal to spare weight for transport to another area. After the EEV has been secured within the ERIC, the outside of the ERIC and the outside of the containers

Table 1. Storage types described in the workflow			
Storage Name	**Acronym**	**Purpose**	**Duration**
EEV Receiving Isolation Chamber	ERIC	Initial layer of high-containment and Contamination Control (CC) protection around the EEV at landing site. This may include the use of hermetic seals and provides an inert gas or vacuum environment for the EEV during transport (removal of terrestrial atmosphere during transport time period)	Landing site and transportation to the SRF
Transportation Isolation Chamber	TIC	Secondary layer of high-containment and CC protection around the ERIC that contains the EEV during transport	Transportation to the SRF
Double Walled Isolator	DWI	Provides both an inert, clean environment as well as Class III Biosafety Cabinet (BSC-III) equivalence for high-containment	Flight system disassembly (SCV through RSTAs), sample handling and storage, and possible integration with PE/Science instruments
Containment End Cap	CEC	RSTA leak protection measure in case of known/unknown tube seal failure	After RSTAs removed from OS until RSTA physical sample extraction
Sample Tube Isolation Chamber	STIC	Additional layer of containment for CC and PP for the RSTAs	After RSTAs are cleaned and as needed until RSTA physical sample extraction. Possible storage container for empty tubes thereafter.
Temporary STIC Storage	TSS	Clean and contained environment to store the STICs	STICs (with RSTAs) as needed until physical sample extraction occurs. Possible storage container for empty tubes thereafter.
Pristine Sample Storage	PSS	Storage location(s) with the most stringent CC requirements in order to keep the samples in their most pristine state. This could be one location or multiple if the samples are stored by type (e.g., igneous, carbonate-rich).	Location for all pristine samples from BC onward.
Pristine Subsample Storage	PSubS	Storage location(s) with stringent CC requirements to keep the samples in a pristine state. Any sample alternation here is from mechanical separation techniques necessary for PE, SSAP, and/or science. This could be one location or multiple if the samples are stored by type (e.g., igneous, carbonate-rich).	Location for all nearly pristine subsamples from PE onward.
Reusable Subample Storage	RSubS	Storage location(s) with sufficient CC requirements to keep the samples in their current state. Sample stored here are still suitable for scientific investigations but have been geochemically altered due to an analysis or sample mounting technique (e.g., imbedded in epoxy). This could be one location or multiple if the samples are stored by type (e.g., igneous, carbonate-rich) or amount of degradation.	Location for all reusable subsamples from PE onward.

containing CK/BK samples of the surrounding environment should be cleaned. The ERIC and CK/BK samples should then be transported to the TIC.

In anticipation of the difficult terrain at the landing site, the TIC should provide a more robust high-containment protection than the ERIC. Therefore, once the ERIC is integrated within the TIC, other measures could be employed within the TIC to mitigate contamination of the samples in case of a breach, such as pulling vacuum or purging the system with an inert gas if not feasible with ERIC in the field. When deemed safe, the TIC should then be transported to the SRF. For an alternate option for EEV recovery and containment, as well as triage options, see Appendix B.

4.1.2. TIC (EEV) SRF Arrival and Disassembly

Assumption A3: After landing on Earth, the EEV will be encapsulated in two layers of containment, and the exterior layer will be free of Martian material.

When the TIC arrives at the SRF truck receiving area, the ERIC should be moved into containment. Since the outside of the ERIC should be sterile and hermetically sealed, there should be no immediate need for the use of high-containment suits, though other PPE may be necessary.

Alternatively, the TIC could be designed to have an access port that directly introduces the EEV into the SRF to further mitigate Planetary Protection concerns.

Once in the first high-containment cleanroom, BSL-4 type suits should be donned before the EEV is removed from the ERIC in case of possible breach in the EEV. Since the EEV surface will be shedding particles, the EEV should be lifted out of the ERIC and placed into a custom mobile stand that could mitigate particulates from the bottom of the EEV and parts further wrapped in Fluorinated Ethylene Propylene Polymer (FEP) film to mitigate particulate spreading during disassembly. Once the EEV is removed from the ERIC and particulate generation is mitigated, the EEV should be moved into a second ISO Class 7 cleanroom. The ERIC and the landing site environmental CK/BK samples are left in the previous cleanroom. The EEV should then have a preliminary engineering inspection for damage, followed by a cleaning step for terrestrial debris removal, and then a final engineering inspection should be completed. After the inspection is complete, the body of the EEV should remain covered to contain any heatshield that may be sloughing off and the cleanroom cleared of all debris to minimize the amount of terrestrial contamination transfer during disassembly.

After any possible sources of contamination are contained and/or mitigated, the Containment Assurance Module (CAM) lid should be removed to allow access to the SCV. Depending on the design of the SCV, the SCV may be removed, wiped clean and placed in an isolator. The isolator should provide a clean and inert gaseous environment to continue the disassembly process. If a double-walled isolator is employed, the isolator could also be classified as a BSC-III and could become the primary containment mechanism to meet BSL-4 equivalent status for the SRF. The EEV would remain in place to be investigated for engineering failure and Planetary Protection verification.

4.1.3. SCV Disassembly

Assumption B7: For Go/NO-GO decision points, one additional environment was considered sufficient for infrastructure sizing, in case of a NO-GO decision.

The first step for SCV disassembly should be to clean its exterior. The cleaning should be done in stages, the first is to collect all possible debris from outside of the SCV for study (PP verification) and then thoroughly clean the surface (e.g., isopropyl alcohol wipes through CO_2 snow) to prevent the transfer of terrestrial contamination later in the disassembly process. Once clean, an engineering inspection should be performed that will prompt the first in a series of GO/NO-GO decisions. These decisions would be made based on the findings from the inspection, and a GO decision would lead to a nominal disassembly course (i.e. continue on the operational scenario workflow), whereas a NO-GO decision would lead to contingency operations that would be dependent on the findings and therefore are not detailed here; it is not envisioned that a NO-GO decision would mean that the samples would not ultimately continue on the operational scenario workflow, but instead that there may be a delay while additional operations are considered and possibly implemented. If the inspection is satisfactory and a GO call is made, the SCV is moved to a new isolator. If any debris is removed from the outside of the SCV, it should be collected and stored in the initial isolator for future investigation or be transferred to non-pristine storage inside containment.

The SCV should be disassembled within the new isolator that is not only equipped with the necessary tools for PCV extraction but also for CC purposes. Although the process for PCV removal is notional, the steps may include drilling a hole to equalize pressure (if the SCV is under vacuum) and release the SCV lid to lift it away the PCV from the base. When the PCV is free from the SCV entirely, the PCV should be transferred to the next isolator, while the remaining SCV parts remain in place for future investigation.

Alternately, depending on the design of the SCV, most of this disassemble step may be bypassed if the SCV is installed as part of the EEV and not removable. In that case, after the CAM lid is opened, the SCV lid would be opened and the PCV would be removed from the SCV and transferred to the next isolator.

4.1.4. PCV Disassembly

As with the SCV disassembly, the first step should be to clean the PCV exterior in stages. First any dust/debris on the surface should be collected for later study (in place) and then a thorough cleaning should be performed. Since this is the last and primary barrier before Martian dust is expected to be present, it would be important to have an extensive cleaning step before the PCV is punctured. Once cleaning is complete, an engineering inspection should be performed along with the second GO/NO-GO decision. If the decision is GO, the PCV should be transferred to the next isolator for disassembly. The transition to the next isolator should provide an extra level of cleanliness to avoid transferring any terrestrial contamination further within the disassembly process and should allow for the necessary specialized equipment to be accommodated for OS container extraction.

Once secured in the new isolator, a gas sample may be taken when the PCV is punctured to equalize the pressure (if the PCV is under vacuum; see the “Timing” section for additional considerations regarding this step). After equilibration, the PCV should be cut below the braze line and opened to expose the OS container. The OS should then be removed from the PCV (base and lid). All Martian dust inside the PCV and outside of the OS should be collected and stored in RSubS until investigated (see Table 1 for more information about the different storage types). The PCV should be investigated in place and the OS should be transferred to a new isolator.

4.1.5. OS Disassembly

Although it is anticipated that Martian dust will be present at this stage, the stringency of the CC requirements for the OS may require its exterior to be cleaned at this stage to minimize the transfer of terrestrial contamination to within the system. Given that the OS disassembly step is likely to be a key parameter impacting the overall contamination budget the cleanliness of this operation will need to be defined and may require high levels of organic molecular cleaning. Due to the proximity to the RSTA, this cleaning process should be thorough but not abrasive and should focus on macro particulate precision cleaning along with minimizing biological transfer. After this thorough cleaning, an visual inspection should be conducted, followed by a 3rd GO/NO-GO decision point. If the inspection is satisfactory and a GO decision is made, the OS should be transferred to the next isolator for disassembly.

Once the OS has been opened and RSTAs exposed, the priority should be to remove each RSTA one by one. Upon removal, each tube should have a non-invasive macro particulate precision cleaning step with Martian dust collected from their surface (1st tube cleaning), a visual inspection to verify tube/seal integrity, and a visual seal integrity check (a final GO/NO-GO decision point) conducted. The RSTA should then be transferred to a new isolator where the Containment End Caps (CECs) should be installed. The CECs should be designed to protect the samples within the RSTAs in case there is a leak in the seal during handling and cleaning. The RSTAs should then undergo a thorough precision cleaning process (2nd tube cleaning) to remove any inorganic, organic, and biological contamination from the outside of the tubes. The cleaned RSTA should then be placed into Sample Tube Isolation Chambers (STICs) and transferred to the Temporary STIC Storage (TSS) or moved directly to the Micro X-ray Computer Tomography (µXCT) for analysis (see the Pre-BC section below). The STICs notionally provide isolation from the surrounding environment for both PP and CC purposes.

All the dust collected from outside the RSTAs and inside the OS should be placed in RSubS. The OS will remain in place for inspection and further removal of fine Martian dust.

A Note About Instruments: The following sections begin to describe some of the instrumentation that would be needed for Pre-BC, BC, PE and Science investigations. These instruments have a large range of sizes as described in the instrument table, and there are infrastructure implications associated with some of the larger instruments (e.g., reinforcement of the ground floor for instrument weights).

4.1.6. Pre-Basic Characterization

Pre-Basic Characterization activities *"constitute the removal of the dust and checking the sample seals (see also Tosca et al., 2021) as well as measurements that would be performed on sample tubes before they are opened*" (Tait *et al*., 2021).

Pre-BC, as described in the MSPG2 report, should consist of two measurements: µXCT and magnetic properties. The µXCT will also be used for an engineering seal integrity check and information will be incorporated for decision about how to cut open the RSTAs.

MOSDT identified three implementation strategies to accommodate these instruments:

- The instruments are entirely situated outside the SRF high-containment: this strategy implies that the STICs (or another hardware) could provide the necessary PP/CC for

the RSTAs. The hermetically sealed STICs would be removed from a BSL-4 isolator through a rapid transfer port and the outside of the STICs sterilized. Afterwards, the hermetically sealed STICs with the RSTA nestled inside could be introduced to the μXCT using normal methods. This strategy would be possible only if high-fidelity analyses are feasible through the STICs, and that the STICs can be sterilized without impacting the samples.

- If high-fidelity analyses are not feasible through the STICs or biosafety does not allow for this type of containment strategy, the Pre-BC instruments will need to be incorporated into the SRF. This could require the analyses to occur through a specialized material compliant transfer sleeve directly connected to an isolator in high-containment. This sleeve would penetrate the wall from the BSL-4 space to a lab space outside of the high-containment area and fitted directly with the instrument situated in a non-classed laboratory. The transfer sleeve would remain BSL-4 sealed/compliant and directly introduce the RSTA to the instrument. This strategy also implies that high-fidelity analyses are feasible through the sleeve material.
- The instruments would all be inside high-containment. Installation within a high-containment suit lab would enable the analyses to be performed on the RSTAs directly and minimize possible interference caused by an isolation container/vessel.

MOSDT recommends conducting a trade-off study for these implementations in the future – see Section 8. Future Work.

It is possible that the STICs can be placed back into TSS and re-retrieved from TSS depending on the timing of the analyses. For example, if an μXCT measurement is complete but the magnetometer is in use, the STICs should be stored within the TSS until the instrument is available.

After both μXCT and magnetometer analyses are complete, the STIC should then be transferred to the next isolator, which should be used for the 3rd and final precision cleaning of the RSTA before the sample tubes are punctured and opened. This precision cleaning step should involve final cleaning to mitigate inorganic, organic, and biological contamination before the opening of the tubes and is vital to the preservation of the sample integrity.

4.1.7. Basic Characterization

After the 3rd RSTA cleaning, RSTAs should be stored or transferred into its designated gas extraction isolation chamber to begin BC. MOSDT made the assumption that from this step, each RSTA should be handled and analyzed separately from the others. The segregation could be defined by sample type (e.g., regolith sample, carbonate-rich sample, non-carbonate-rich/sedimentary samples, or igneous samples as envisioned by International MSR Objectives and Samples Team (iMOST) and MSPG2), or by coring and scooping bits that have been used by the Perseverance rover. Due to cross-contamination concerns, it is also possible that an additional isolation chamber may be designated for blanks, witness tubes, and the drillable blank sample(s). Irrespective of the numbers of categories that will be defined, MOSDT recommends having between 2 and 5 parallel lines of work, as a balance between timing and staff availability.

Before the RSTA is punctured and gas extracted/analyzed, the tubes should be weighed with high precision (+/- 1ug). The weighing balance could be a load cell integrated with the RSTA tube holder for gas extraction. This weight should then be used to determine if there was

solid sample loss during the gas extraction process. The RSTAs should then be punctured, and the headspace gas extracted (see Section 5. Timing for additional considerations regarding this step). Depending on the gas extraction procedure to be used, the installed CEC may be utilized again for the gas extraction process (e.g., integrated into the gas extraction mechanism) or additional CECs may need to be designed if the extraction occurs at the non-seal end of the RSTA. After extraction is complete, the RSTAs should be resealed, or the CEC may continue to function as a new seal. The samples should then be re-weighed before being transferred to the next isolator.

This next isolator should be where the RSTAs are mechanically cut, but not opened. The cutting technique and location should be based on information contained with the M2020 Dossier and the analysis of the µXCT data. Once cutting is complete, the samples should be secured (within the cut RSTA) and transferred to the isolator(s) for opening and further characterization. Due to the expected amount and type of equipment needed to cut the sample tubes, transferring the RSTA to a dedicated isolator for cutting should mitigate risk of contamination from the cutting process and RSTA debris.

The final isolator needed for BC is where the cut RSTA is finally opened to reveal the Martian core or regolith sample. The RSTA should be carefully removed to expose the sample for visual inspection, photo documentation, physical description, weighing, hyperspectral/multispectral imaging, and subsampling of any fractured pieces or regolith. At this stage, samples should not be subdivided by ways more invasive than picking or scooping samples. These isolators should have the highest most stringent CC requirements yet since the samples are in their most "pristine" state.

After this BC is complete, the tube contents should be placed in new dedicated pristine containers for storage and then transferred to Pristine Sample Storage (PSS) for long-term curation and/or PE.

4.1.8. Preliminary Examination and Science

Assumption B9: PE and Science instruments will need the same level of access and use and may not be physically segregated.

Assumption B10: Sterilization equipment (radiation and/or heat) will be available in the SRF and be accessible for Martian samples at any stage.

Assumption A8: Activities on each RSTA that happen in Pre-BC and BC will occur before PE, and activities that happen in PE will generally occur before science (e.g., time-sensitive or sterilization-sensitive investigations) and before samples are released for dedicated investigations. T*he analysis workflow (out of scope for this work) would need to be optimized to accommodate time-sensitive measurements and there may be some measurements from earlier activities that can inform later phases.*

Assumption A5: Sample Safety Assessment (SSA) will be informed by activities in multiple stages of Pre-BC, BC and PE, and thus will not require a specific physical area.

This section covers PE and analyses related to the Sample Safety Assessment Protocol (Planetary Protection), Time-Sensitive measurements, and Sterilization Sensitive

measurements. The Sample Preparation needs and steps are also described in this section. Instead of describing individual workflow activities, the focus will be on optimizing a workflow to maximize efficiency since many of these activities should be able to occur concurrently. Furthermore, unlike the description of the operational scenarios up to this point, there is not a single, universal path forward, as each step will be sample and analysis dependent.

When a pristine sample is removed from PSS, it should be transferred into a new isolator for non-destructive sub-sampling (if necessary), then photo documented and weighed. Due to the possible wide range in sample sizes, isolators for both macroparticle and microparticle processing may be considered. After the processing is complete, the pristine subsample should be placed in a dedicated container for transport to either 1) an instrument for direct measurement, 2) another isolator for mounting, or 3) a laboratory or another isolator for other further preparation (e.g., chemical extraction). The remaining pristine sample core/subsample should be placed back into PSS. The only samples that should be allowed to be placed back into PSS are those that have undergone only minimally invasive subsampling with tweezers or scoops.

If a more invasive subsampling technique is required, the pristine subsample should be moved to another isolator for mechanical subdivision (e.g., dry wire saw, rock splitter, grinding and powdering, etc.). After the desired samples are attained, the pieces should be photo documented and weighed. The remaining sample core/subsample can go back into Pristine Subsample Storage (PSubS). The subsample should be placed into a dedicated container for transport to 1) an instrument for direct measurement, 2) another isolator for mounting, or 3) a laboratory or isolator for further preparation (e.g., chemical extraction).

Once a sample has been analyzed and deemed too contaminated with terrestrial material to return to the PSubS, the sample should be stored in the RSubS. If a further analysis is requested, the sample can move straight to the instrument, or it can be moved into a new isolator for a more invasive mechanical processing technique. Just as with pristine subsamples, the new subsample should be photo documented and weighed and the remaining subsample returned to RSubS. The new sample split should be placed in a dedicated container for transport to 1) an instrument for direct measurement, 2) another isolator for mounting, or 3) a laboratory or isolator for further preparation (e.g., chemical extraction).

MOSDT made the assumption that the instruments identified by MSPG2 for PE, and their anticipated sample processing requirements, should allow for the analyzed sample to be placed back into PSubS (Instrument #s 7-13 in the Instrument table) for future analysis, granted that instruments would be allowing for samples to stay under the same conditions as inside a pristine isolator. However, all other analytical techniques identified are destructive/terminal other than VP-FEG SEM and FIB (#7b in the Instrument table), EPR (#25 in the Instrument table), and BET (#26 in the Instrument table). Of those three, the EPR is the only technique that should allow for the samples to be placed back into PSubS. Due to the resulting sample contamination (VP-FIB) or relative degradation (BET), the remainder of these samples should be placed into RSubS.

While the least destructive subsampling and mounting techniques should be chosen where possible, there are a number of standard techniques that will cause contamination requiring storage in RSubS after processing and/or analysis. Examples of these techniques are cutting

samples by FIB and ultramicrotome, embedding and mounting samples in epoxy, grinding and polishing sample, and sputter coating.

MSPG2 indicated that there should be an ability to store a subset of samples cryogenically (-20°C). In order to preserve volatiles, the samples to be stored frozen should be selected early in the process to decrease time spent at room temperature and minimize handling/processing. There is currently no indication of cold requirements beyond storage. If this changes and sample processing must occur under cold conditions, additional infrastructure will likely be required.

Subsamples (from any storage isolator, independent on their pristine level) should be able to be prepared, sterilized (if necessary), and transported outside of the SRF for laboratory analysis or uncontained curation. Since MOSDT only considered operational scenarios leading up to and within high-containment, the depiction of individual samples and/or the whole collection leaving the SRF and the operational scenarios and storage locations within an uncontained/traditional curation facility are not represented and may be expected as future work (e.g., in the form of a sample management plan).

4.2. Side Operations (SO)

In addition to the main operational flow, ten side operations were identified. For clarity, these operations will be broken down into two main categories, those that should have limited impact on the overall infrastructural requirements and those that may have some impact.

4.2.1. Side Operations with limited impact to SRF infrastructure

This first set of side operations is only related to the flight items that will be disassembled in the process of accessing the RSTAs (yellow arrows in the Main Sequence of Operations). At each of the disassembly steps, while the main operational flow should continue elsewhere, the flight items identified below should remain in place (hence the limited impact to infrastructure) for swab samples to be collected for SSAP and CC verification as well as to perform visual inspections. It is important to note that it may be required to sterilize the flight items and remove them from the facility to perform the highest fidelity engineering inspection. The removal of the items from their designated isolator might also allow for the isolators to be reutilized, after cleaning steps, for other purposes within the SRF.

- SO1. EEV
- SO3. SCV
- SO6. PCV Base & Lid
- SO8. OS

4.2.2. Side Operations with potential impact to SRF infrastructure

The side operations identified within this group relate to SSAP, BK, CK, and PP/CC verification, as well as possible science requirements (blue arrows in the Main Sequence of Operations). For most of these operations, the focus should be on the dust or debris removed/collected during the disassembly process. A description of the type of anticipated material and the potential storage location is outlined below.

These side operations have an impact on SRF infrastructure in the sense that to be achieved, they require additional isolators or additional instruments.

- SO2. SCV Exterior Dust. The swab/wipe samples taken from this location should be for immediate analysis or to be stored in RSubS in multiple aliquots. No dust/debris is anticipated to be found in this location due to preliminary requirement for this hardware.
- SO4. SCV Interior/PCV Exterior Dust. The swab/wipe samples taken from this location should be for immediate analysis or to be stored in RSubS in multiple aliquots. No dust/debris is anticipated to be found in this location due to preliminary requirement for this hardware SI5. PCV Interior/OS Exterior Dust. The samples taken from this location should be for immediate analysis or to be stored in RSubS in multiple aliquots. Limited dust/debris is anticipated to be found in this location. If present, it is most likely of Martian origin. In case material is present, dry sample collection should occur first, followed by swab/wipe samples.
- SO7. OS Interior/RSTA Exterior Dust. The samples taken from this location should be for immediate analysis or stored in PSubS in multiple aliquots (or stored in RSubS if the hardware has low CC requirements). Significant dust/debris of Martian origin is anticipated to be found in this location. Dry sample collection should occur first, followed by swab/wipe samples.
- SO9. Atmospheric Gas Process. The samples taken from this location should be for immediate analysis or to be stored in PSS or PSubS in multiple aliquots.
- SO10. RSTA. This side operations should include both the cut tube, the seal cut from the RSTA, and the CEC.
    - Seal and CEC. Once cut from the RSTA, the dust on the hermetic seal should be removed and either taken for immediate analysis or stored in the PSubS (or stored in RSubS if it does not meet CC requirements). After the dry sample collection, the pieces should be put into the STIC and follow the RSTA through the sample removal processes.
    - RSTA. Once the sample is completely removed from the RSTA, wipe/swab samples should be taken (tube, hermetic seal, and CEC). A visual inspection should also be performed to verify tube/seal integrity. This check will be important for understanding any potential contamination signal, as well as an engineering understanding of how well the sample seals performed. Unlike other flight items that can be sterilized out and removed from containment for further study, the possible chemical reactions on the tubes themselves may be studied to understand potential sample alteration. Therefore, the RSTA should be placed back into the STIC and into TSS for a later investigation.

5. Timing

Within the timeline, some steps may be specific drivers for the timing of the overall process, and a number of these time-sensitive and timing-relevant steps are described below.

- Due to specifics about the design of the OS, PCV, SCV, and EEV, and the uncertainties about how these seals will be formed and whether a specific opening mechanism or process will be part of that design, the timing of each of the opening or unsealing steps related to these items cannot be determined at this time.
- Gas sampling methods or technologies were not specified in the input material and most of the current best practices would be expected to have minimal effect on the

infrastructure of the SRF. However, many of the most likely methods to collect the gas in the SCV, PCV, OS, and the sample tubes for analyses (taking into account the small sample amounts and minimizing the risk for contamination) would require relatively long times (on the order of weeks) to ensure that sufficient equilibration has occurred for accurate measurements.

- Some elements of the infrastructure that may affect sizing may also be considered as trades between the number of these elements that are present, the space needed for these elements, and the length of time needed to complete the analyses in the SRF for each sample. Certain rate-limiting steps in the overall workflow may be sped up by additional equipment/instrumentation and space for storage. While the number of DWIs implemented is one of more significant trades (e.g., the total number of DWIs the SRF can accommodate will be important to determine how much space is needed for DWIs in use, how much space is needed to store DWIs not in use, and how much space is needed for maintenance or cleaning DWIs between samples), there should be consideration for general equipment maintenance operations and the replacement of failing instruments and tools.
- If there is a desire to store samples under cryogenic conditions, it may be prudent to identify these samples early in the processes. To minimize alteration due to storage temperature conditions, these samples should be prioritized during BC and PE so they can be transferred into cold storage expeditiously.
- Although samples should be stored in an inert environment, specific Time-Sensitive analyses could still be affected. If time-sensitive science remains a priority, then these samples should be prioritized during BC and PE so they can be analyzed expeditiously.

6. Environments – Contamination Control and Containment

Assumption B5: The transfer of samples to different environments will be assumed to be as conservative as possible in order to stringently maintain contamination control, though this will likely have the largest implications for SRF infrastructure.

Assumption B6: The choice of environment block types (e.g., airlocks, cleanroom corridors between operations rooms) will be determined by considering optimal workflow and cleanroom ingress/egress protocols.

Environments, in the context of this study are defined by a range of environmental parameters for each area, activity or space. Parameters can be temperature, humidity, atmospheric gas, pressure, and levels of cleanliness for all types of contaminants, and levels of biocontainment. The sequence of operation workflow indicates (with dashed arrows) when environment was deemed to change on one or more parameters. There are a variety of possible implementations to move from one environment to another; the MOSDT didn't recommend a single best implementation solution and leaves this work to future groups.

6.1. Contamination Control

Assumption A1: Cleanroom capability will be necessary to minimize any form or means of terrestrial contamination (e.g., particulate, airborne, molecular, surface contact from any

source being inorganic, organic or biological) that may alter sample integrity and/or affect the analytical results to unacceptable levels that would either confound the sample safety assessment or above the signal to noise ratio of the desired downstream investigations.

The long-term science integrity of Martian samples should be a primary program goal. Preservation of these limited natural resources should continue through the SRF and the long-term curation of the samples. Stringent protocols should be incorporated into these operational scenarios to protect each sample from terrestrial contamination and from cross-contamination between sample tubes. Standard storage, handling, and processing methods for samples require rigorous particulate, aerosol and airborne molecular control measures to be in place to mitigate against inorganic, organic, and biological terrestrial contamination, and to mitigate cross-contamination between samples. In addition, samples should always be stored and handled in an inert environment to mitigate against terrestrial atmospheric reactions with the samples, such as the interaction with oxygen and moisture.

In order to maintain each sample in their pristine state for scientific study, the infrastructure should have mitigation strategies. These should include the following:

- Cleanrooms – ISO Class 1 to 7 cleanrooms should be used to control and reduce the particulate and airborne molecular contamination (ISO 14644 standards).
- Isolators – DWI and gloveboxes could be used to provide an inert atmospheric environment (e.g., gaseous nitrogen, argon, helium, or vacuum; and ISO 14644 standards). In addition, isolators can be designed to reduce particulate and airborne molecular contamination.
- Precision Cleaning – the cleaning of isolators, tools, and equipment should be designed for a reduction in inorganic, organic, and biological signatures. In addition, the instrumentation for cleanliness validation should be included to verify that the facility meets and maintains a required cleanliness level.
- Sterilization – the sterilization of isolators, tools, and equipment should be designed to prevent terrestrial biological growth and cross-contamination between samples.
- Adapting protocols to minimize production of fine particles and aerosols. Existing national and international standards and recommended practices on fine particle and aerosols as well as existing protocols (e.g. animal necropsy) may be helpful to start to inform the types of measures that may be necessary.

To preserve the pristine science integrity of the returned Martian samples, the minimum contamination control requirement should be kept at the level of Mars 2020 sample intimate hardware (e.g., at least an ISO 5 cleanroom, aseptic handling of all sterile tools and implements, and review materials in the room as not to exceed inorganic and organic contamination limits for the Mars Samples as defined by the Mars Organic Contamination Report). However, this may require a contamination budget analysis to be conducted since the Mars 2020 project set the sample intimate hardware CC levels with an end temporal date of a few years to only encompass the assembly of the spacecraft. Therefore, for the return samples, if the intent is to keep these samples pristine during long-term curation, a much more stringent contamination control threshold may need to be established in cadence of additive contamination through time.

6.2. Containment

Assumption A2: BSL-4 equivalent containment will be required to protect Earth's biosphere from extraterrestrial materials that have not been sterilized or have yet to be determined safe through a sample safety assessment.

Assumption A10: Additional infrastructure in the SRF will be needed to support high-containment activities and compliance with industry best practices and will likely include: A. central services for both front-end preparation (e.g., reagent preparation, autoclave, clean laboratory staging for disposables of common use items, space for cleaning and kitting of equipment), B. decontamination of effluents, and C. sterilization of waste and materials. These will drive additional facility requirements.

In addition to contamination control measures, biologically controlled high-containment environments should be incorporated into these operational scenarios to protect the terrestrial environment from a potential Martian pathogen. These should include the following BSL-4 equivalent implementation strategies:

- Primary containment and redundant containment
    - Suit laboratory. A protective suit laboratory with negative pressure BSL-4 suit-based laboratory
    - Class III cabinet laboratory – with double HEPA exhaust prior to release outdoors, operated a negative pressure to the surrounding laboratory, and have a dedicated non-recirculating ventilation system. This could be traditional Class III biological safety cabinets or DWIs.
- Sterilization – heat and gamma sterilization capabilities to sterilize the sample as an alternative to containment or sample safety assessment evaluation.

6.3. Integration of contamination control and containment principles

The facility should be designed with opposing differential pressures to maintain biosafety and cleanliness requirements. For cleanrooms, a room-to-room positive pressure cascade from the cleanest room (pristine storage and processing) to the least clean room (EEV disassembly) is the baseline. Conversely, the facility perimeter should maintain a negative pressure for BSL-4 high-containment requirements. Therefore, these opposing pressure differentials need to be carefully integrated and evaluated for intent to maintain both the needed cleanliness and biosafety. One consideration in this process is to evaluate the higher risk activities such as the process and analysis of the sample and have the highest risk activity at the lowest pressure. For example, cutting sub-sampling activities would be at the lowest pressure then a positive pressure cascade would be in place up until sample analysis.

For operational egress from high-containment cleanrooms to non-class space, personnel, hardware, tools, and equipment should enter and exit this space with minimal infiltration of contamination and maintain BSL-4 biosafety protocols. The facility should consider the integration of cleanroom standards from ISO 14644 part 1 through 17 along with biocontainment regulations from the BMBL 2020 (NIH CDC Biosafety in Microbiological and Biomedical Laboratories 6th edition). In case a European-based SRF would be envisaged, EU and/or national or local regulations may also apply.

Careful attention should be placed on how personnel are gowning and entering cleanrooms or donning personal protective equipment for BSL-4 as well as how small tools to large equipment are cleaned and prepared to enter and exit the facility. For tools, equipment and hardware, a similar flow to ISO 14644-5 *Section D.2.3 Removing cleanroom packagin*g should be applied, where the protective covering (or hardware in this case) should be cleaned, the outer layer of hardware removed and inner layer unpacked, and then the transfer areas cleaned, before the doors to the new cleanroom are opened for transferring the equipment inside. For redundancy, it is good practice to have a triple protective layer. As a general flow, as the samples are being extracted the cleanroom ingress protocols should provide a means to ensure cleaner hardware in subsequent steps, in terms of cleaning, cleaner environments, and dilution.

7. MOSDT added requirements

While developing the Operational Scenarios, additional requirements were identified that were not explicitly captured in the reference documents but would be necessary to support the overall objectives outlined in MSPG2 and SSAP WG reports. If the goals for the facility are modified, some but not all of these additional requirements may also change.

7.1. Types of isolators

The MSPG2 Curation Focus Group described two types of isolators, “pristine” and “non-pristine”, depending on the type of material that could be used within the isolator. Isolators can be based on various technologies and pressure regime, ranging from positive pressure isolators (such as gloveboxes commonly used in curation), to negative pressure isolators (such as BSC-III commonly used in BSL4), to double-walled isolators offerings a dual pressure regime.

The type of isolators is of course to be determined in future engineering and architectural studies and will impact the overall SRF infrastructure implementation. However, for an estimate for the size of the facility, the type of isolators is not essential, as gloveboxes, BSC-III and DWIs can be considered of the same order of magnitude. For this reason, MOSDT endeavored to not make a distinction between types when other considerations (such as PP or CC) were not considered.

7.2. Number of isolators

Assumption B8: It should be possible to clean and sterilize tools, isolators and equipment to the point that they can be reused for other purposes and/or other materials. Cleaning capabilities utilized within the SRF will be baselined on the current state-of-the-art for BSL-4 laboratory facilities and decontamination as well as contamination control cleaning measures developed for astromaterials and curation.

Assumption B3: Distinct categories of sample should not be processed in the same environment. As a balance between timing, space and staff availability, we considered 2-5 parallel lines.

The MSPG2 Curation document identified the need for approximately 12 isolators during the BC phase (Tait *et al*., 2021). Expanding up from this number to cover other phases, due to the anticipated array of complex equipment/tools required for flight-item disassembly

and PE or Science instrument integration, multiple cleaning and sampling steps, the segregation of the collection by sample type, and the number of storage environments, this number may be expected to increase, with an estimate between 37 and 49 isolators. Additional isolators may be considered for contaminated soil in the event of an off-nominal landing, but since that possibility was not incorporated into the main operational workflow, that number is not included here.

While it may be possible to reutilize isolators from earlier disassembly processes (see assumption B8 regarding cleaning), Pre-BC, or BC for PE and Science, or that the isolator identified may be more similar to a rapid transfer port in practice, the needs of a given isolator will determine its final design. The isolators are likely to vary in size, rapid transfer ports (e.g., scope and number), instrument boxes, and have variable window locations to accommodate viewing, handling, and microscopy (e.g., horizontal vs. vertical vs. 45° windows). Depending on infrastructure and science requirements, isolators may be mobile or stationary, in an interconnected line or independent. Therefore, there may be significant impacts to the infrastructure design to accommodate this increased need and variety of each isolator.

The table below presents a count of the number of distinct environments, as shown in the Main Sequence of Operations. From that number of environment, an estimate of isolators is derived, accounting for the estimated space needed for each phase.

Phase	**Number of environments**	**Numbers of isolators**	**Total of isolators**
TIC (EEV) SRF Arrival and Disassembly	2	cleanrooms	n/a
SCV disassembly (nominal)	2	2	3
PCV Disassembly (nominal)	2	2	2
OS Disassembly (nominal)	1	1	1
Pre-BC	2	2	2
BC per sample tube	3	2	4 to 10
Pristine and Psub sample preparation per sample type	2	2	4 to 10
RSubS sample preparation	3	3	3
PE	--	--	--
Science	--	--	--
PSS per sample type	1	1	5
PsubS per sample type	1	1	5
RSubS	1	1	1
TSS	1	1	1
Additional isolators for blank and witness (from MPSG2)	--	3	3
Additional isolators in case of NO/GO	3	3	3
Total	**24**		**37 to 49**

Table 2: high-level count of environment and suggestion of isolators number, for the end-to-end process within the SRF.

Note that for BC, no redundant inactive isolator has been planned, as is discussed in MSPG2.

For PE and Science, it is considered that the main infrastructure driver will be the instruments, and environments are not accounted for.

Please See Appendix A. Isolator Justification for more information.

7.3. Ancillary Space/Laboratories

Assumption A9: Activities will occur in a variety of environments to include non-classed space, cleanrooms, a range of BSL-2 through – BSL-4 laboratories, high-containment prep space, and high containment space. Additional space and capabilities outside of the high-containment laboratory described herein will need to be considered for contingency planning to support sample safety risk and inadvertent release.

Additional to the analytical equipment for curation, SSAP, and mission science identified by MSPG2 (Carrier, *et al.* 2021), MOSDT identified an array of support equipment, techniques, and space. The additional equipment and space identified for this purpose is listed in the "MOSDT SRF Instrument Integration" file. There was an effort to keep specific support equipment proximal to the main instrument/technique and to group the instruments by purpose (e.g., contamination control/knowledge) within the spreadsheet, as well as to cross reference the equipment within the visual workflow. The spreadsheet provides detailed information concerning the infrastructural requirements for a given instrument, as well as suggestions and potential trades.

- Ancillary capabilities within high-containment spaces
    - Monitoring of cleanrooms and isolators: MOSDT is providing a strawman list for standard real-time monitoring of cleanrooms and isolators within the SRF. The capabilities should be planned and integrated when feasible into the infrastructure itself.
    - Monitoring of organic, inorganic, biological, and particulates contamination in cleanrooms and isolators: MOSDT is providing a strawman list for monitoring of spaces under BSL-4 equivalent level in contact with the Martian samples. Witness plates, swabs and any other contamination monitoring plates should be measured under biocontainment, as any step of sterilization will not keep the Contamination Control and Knowledge (CCK) samples pristine.
- Ancillary capabilities outside of high-containment spaces
    - Monitor organic compounds, inorganic compounds, biological materials, and particulates in the Ultra-Pure Water (UPW) system in real-time: UPW should be available throughout the facility, both in and out of containment. MOSDT suggests that the main production unit to be kept outside of containment, and to setup a loop with a sterilization point after the point of use. MOSDT is providing a strawman list for real-time monitoring of Total Organic Carbon (TOC), organic, inorganic and microorganisms. For ease of utilization, these instruments shall be kept and used outside of containment. Specific CCK measurements at the point of use of UPW can be done using the CCK instruments described above.
    - Cleaning isolators and tools: a dedicated room for precision and ultra-clean preparation of equipment is recommended to prepare all material to be

installed in high-containment spaces and within isolators. The room should be large enough to host several isolators being cleaned at the same time. A trade-off with cleaning in place for isolators specifically could be made but will restrict the type of cleaning methods that can be used. MOSDT suggests that most of the cleaning should be done outside of containment, for tools and hardware that can be autoclaved or fully sterilized. Another cleaning area should be kept under high-containment for tools and hardware that cannot handle repeated sterilization process. UPW should be available in these ancillary laboratories.

- Ancillary capabilities through the biobarrier: MOSDT identified the need to have the full range of sterilization equipment typical of a BSL-4 laboratory for hardware and materials that have come into contact with the Martian samples.

8. Future work

8.1. Facility Operational Trade Space

8.1.1. Science prioritization

The operational scenarios outlined within the document are driven by the requirements recommended by MSPG2 and represent the minimum instrumentation necessary and sufficient to maximize the scientific value of MSR as per the iMOST report. The descope of any of the instruments from the SRF may, given the current state of the science, represent a permanent loss of that science due to the time-sensitive nature of some of the analyses. However, the implementation of the requirements on an SRF to meet all the goals outlined by MSPG2 report may be challenging given the current timeline and funding. Therefore, operational trades will need to be identified as a high priority for future work that would specifically consider the balance of meeting the highest priority science goals, such as the search for signatures of ancient life, with minimization of the size and scope of the facility.

8.1.2. High-Containment technology implementation

A major implementation consideration will be how to meet the BSL-4 equivalent requirement and what redundancies should be built into the infrastructure. As outlined in the Environments Section within this document, there are currently two different trades to be considered in further work for attaining BSL-4 equivalent containment: 1) a suit-based BSL-4 facility or 2) a BSC-III Cabinet based BSL-4 facility. The DWI design should meet requirements for BSC-III commissioning. Therefore, for the portions of the disassembly, BC, PE, and curation that can occur within a DWI, BSL-4 equivalent status may be able to be achieved by implementing this method. However, there are some disassembly steps that may not be possible within a DWI, and for these a small BSL-4 equivalent space with suits may be required. Furthermore, although there are BSL-4 facilities that rely only on BSC-III cabinets to meet containment requirements, there are others that utilize these cabinets within a high-containment facility to offer redundant protection. Although designed and constructed as a BSL-4 facility, under normal operating conditions, the facility is operated as a BSL-3 facility (personnel in scrubs) but if there is a failure in the BSC-III cabinets (the primary BSL-4 containment method), the facility is pressurized to meet BSL-4 requirements and high-containment suits are donned by the personnel. Given the intended reliance on robotic manipulation within the DWIs to meet CC requirements, any failure of the equipment within the DWI would likely require it to be opened to fix. The implication of this

is that whatever is within the given isolator would need to be completely sterilized before opening the isolator to meet PP requirements. If the sample cannot be contained prior to opening the DWI, then the sample within said isolator would have to undergo decontamination procedures and be rendered contaminated and/or sterilized. Therefore, it would be prudent to design a facility that would allow personnel to don high-containment suits to open the isolators and store the samples to minimize contamination to the samples in that isolator. This same reasoning holds as to why the DWIs should be operated within cleanrooms.

8.1.3. Sample receiving interface

Matured hardware containment designs and ground operation configurations should further evaluate the interface assumptions for this facility. This should include considerations of the size of hardware that is required to start BSL-4 processing when the hardware comes to the SRF. This study assumed that the TIC would arrive at the SRF and the only facility accommodation requirement is that the ERIC/EEV must fit through the BSL-4 airlock before the EEV is removed and disassembly can begin. However, if the disassembly process begins at the landing site due to contamination control concerns (as described in Appendix B), additional SRF interface requirements to accommodate the integration of a mobile BSL-4 trailer/pod into the design may be necessary. Both of these cases present logistical challenges and a cost/benefit analysis of each case should be performed.

8.1.4. Alternative opportunities for sample safety

The current efforts of this team assumed that the necessary and sufficient science be done under containment and that samples would remain in high containment throughout the process until sterile or determined otherwise via SSA. Similar to considerations of science prioritization, further consideration of the workflow should address whether an optimization of the BSL-4 work could ensure that only critical, essential work is conducted in this space for curation and sample safety assessment. This work should consider whether there is a means to get the samples out to the science community in a more expedited manner. Leveraging the processing flow of existing BSL-4 diagnostic laboratories, harmful samples are received and processed with the BSL-4 until the sample can become inactivated through a verified process to ensure absence of sample sterilization sensitivity/inactivation tolerance. Once the sample is inactivated through a verified process and deemed safe, then it can be taken out of the BSL-4 for processing. Similar inactivation approaches through sample preparation should be evaluated at the biochemical level and assessed if the approach could be adopted with Mars samples. This potential could allow for the samples to be analyzed in standard laboratory BSL-2 settings significantly reducing resources required for BSL-4 facility, and opening sample analysis to a broader scientific community.

8.1.5. Landing site remediation impacts

As the landing site remediation flow becomes more developed, this impact on the SRF should be further evaluated, specifically with respect to the necessity of additional BSL-4 facility space. In addition to the contamination and biological knowledge samples taken pre-sterilization/remediation (see Assumption B1), this could include on-site disposables used in the recovery operations, equipment that was contaminated but not remediated at the landing site, and additional soil if removed from the landing site for further assessment or remediation.

8.1.6. Requirements for Uncontained/Traditional Curation Facility

Operational and infrastructural requirements should be defined for samples after they are deemed safe for release from the SRF due to sterilization or an assessment from the SSAF that there is an absence of life present, and the sample would not pose a biological hazard for Earth's biosphere.

8.2. Technology development

8.2.1. Double-Walled Isolator Design and Associated Equipment

Mars samples should be processed in a pristine SRF. Further technology development is critical for understanding technical trades for the facility in concert with sample handling, processing, and storage. The current SRF architecture has these initial steps performed in ultraclean DWIs that serve a dual purpose to ensure BSL-4 equivalent biosafety containment and a pristine environment free of contamination. Traditionally, curated astromaterials are handled and processed in positive pressure inert gaseous nitrogen gloveboxes to maintain a pristine environment, whereas traditional BSL environments (e.g., BSL-3 gloveboxes) are negatively pressured for biosafety concerns. Therefore, the concept of a DWI was formed that combines both positive and negative pressure space to mitigate biohazards as well as maintain pristine space. Unfortunately, traditional glovebox gloves on a DWI represent a risk for direct contamination and breach. While there have been ideas about developing a doubled-walled glove, this has yet to materialize as an option. DWI operations could be performed robotically or by manual mechanical manipulation (e.g., a wobble stick) inside these isolators. It is expected that some robotics will be interconnected or connected directly to a DWI while others would be standalone that use ultraclean methods of introducing and removing samples from a rapid transfer port or an instrument.

The functions of the SRF include inspection, cleaning, and opening M2020 sample tubes; removing head gas; sorting/splitting samples; repacking and storing samples; weighing and multispectral imaging. Some or all of these activities should occur within a DWI to ensure biological containment and sample integrity. Pristine handling and processing hardware including head-gas removal, tube cutting and sample removal from tubes, sorting/splitting, and packaging should be performed robotically as well in DWIs.

8.2.2. Instrument consolidation

Non-traditional instrument/sample transfer interfaces with the DWI may be important for performing sample analyses in an ultra-clean biosafety environment. Future high-level assessment of how different kinds of instruments can interact with the sample will be crucial.

Consolidating and combining instruments may also be necessary to understand trades for SRF space and footprints. MSPG2 developed a list of instruments necessary and sufficient to complete the science goals of the SRF; however, many instruments on the list produced for this work have been combined into one "box" with several different capabilities, but these combinations and trades cannot compromise either science or instrument sensitivity. Coordination with industry technical developers and SRF science teams may be necessary to ensure developed instruments meet the scientific requirements while also minimizing footprint. For example, Raman microscopy and Fourier-transform infrared microscopy are both techniques identified by the MSPG2 group as necessary to complete the science goals

for the Martian samples. Combining these two technologies into one box would provide a significant benefit as it reduces the overall high-containment footprint.

Balancing the sensitivities of multiple technologies will be important when understanding technical trades and scientific requirements.

Additionally, the placement of instruments within BSL-4 environments may have implications for their manufacturer's warrantees and continued maintenance, and subsequently drive decisions regarding the specific placement of instruments within the SRF.

8.3. Engineering and processes Development

8.3.1. Go/No-Go Decision Point

The GO/NO-GO decision points identified in the main sequence of operations generally relate to engineering assessments to assess seal integrity and containment breach. Future work should outline all these decision points and contingency planning pathways should be further developed.

8.3.2. Seal integrity

Further work by engineering and science teams should consider the seal integrity check process, considering what this process would look like; what would constitute a fail or pass; what implications would this check have for downstream processing; what cleaning restrictions might be imposed (e.g., can we have a penetrating gas if the tube may be compromised and only has CEC?); and what contingencies may be necessary if the seal integrity is not as expected. The contamination analysis should also help define the step in which the CEC would need to be implemented to ensure that contamination can be contained and minimize impact on the over contamination budget of the samples.

8.3.3. Definition of contamination control and biological requirements

Facility and support equipment requirements for cleanliness need to be defined throughout the receiving facility. While this was outside the scope of this study, it will have an impact on the types of cleaning and verification protocols as well as the effort and verification laboratories required to support the effort. Future work will have to develop the requirements for all the stages in the sample curation facility.

8.3.4. Precision Cleaning methods and procedures

The removal of terrestrial inorganic, organic, and biological contamination (e.g., in the form of particulates, non-volatile residues, TOC, thin-films, etc.) from hardware, tools, and equipment by precision cleaning methods and procedures will be needed in the future SRF. Examples of these include degreasers, surfactants, and solvent cleaning with UPW as the final cleaning agent and the use of Vapor Hydrogen Peroxide (VHP) as the sterilization process. The VHP will also be used for large equipment and rooms inside the BSL-4 space. In addition, cleanliness verification methods and procedures should also be used to validate a pristine environment. Unfortunately, this was outside the scope of this study. An assumption regarding the cleaning capabilities of the facility was described (see assumption #B8), and strawman lists of capabilities have been suggested as part of the ancillary laboratories (section 4.2), however further trade studies should be conducted to better understand cleaning methods and procedures, the flow of cleaning and validation

processes, and the necessary process(es) and compatibilities that should be considered with ground support hardware (e.g., DWIs, facility surfaces) as well as sample handling equipment. This study should consider whether multiple cleaning procedures need to take place to achieve the necessary level of cleanliness and whether these cleaning steps need to occur in a certain order. This study should also evaluate potential residues from cleaning steps (e.g., VHP) and mitigations (e.g., high heat bakeout as a final step) to remove them as necessary. Verification of the cleaning will also need to be established and address whether this will be done with process approval (e.g., time at exposure) or need separate laboratory assessments. Given this study group baselined DWIs being used for multiple stages, additional consideration, and future work will be needed to understand cleaning flow and cleanliness verification.

8.3.5. Biological knowledge processing

To follow the best practices of high-containment laboratories and curation and science facilities, monitoring biological contamination of spaces and equipment that are not in contact with Martian materials should be considered. The measurement of these samples could occur outside the high-containment areas of the SRF, or even outside of the SRF. If so, future work should address what would be necessary to process these samples.

8.3.6. Containment end cap hardware

To secure the end of the sample tubes where the seal integrity may not be fully understood, this working group proposed to generate an additional seal, that would allow for a protective barrier for subsequent cleaning operations to be performed to ensure the sample could be protected. If this seal is required, the design of this seal and associated ground support equipment would need to be conducted as future work with considerations of verification of efficacy and removal.

9. Appendices

Appendix A. Isolator Count Justification

When identifying independent environments that may be necessary to implement within the MSR SRF, MOSDT prioritized the mitigation of risk to the samples and the biosphere and endeavored to maximize short and long-term science priorities outlined within the MSPG2 and iMOST reports. Although it was not a main priority, the MOSDT also considered what may be necessary to minimize downtime for environmental cleaning/sterilization steps in order to ensure samples can be released from the SRF in a timely manner.

In addition to the MSPG2 guidelines for CC and sample suite segregation, the MOSDT had an added consideration for the integration of necessary specialized equipment into isolation chambers. The specialization of this equipment is highly variable in purpose (e.g., COS disassembly, robotic transfer arms, opening RSTAs, processing samples) and as vectors for terrestrial contamination. Therefore, in response to the anticipated stringent CC requirements, an additional environment may be identified to perform the pre-process (e.g., cutting hardware) and then to complete the task (e.g., clean flight hardware, extract the sample). The merging of these environments could put the samples at increased risk for terrestrial contamination and/or increase the time until the samples can be released from the facility due to downtime for additional cleaning/sterilization steps.

Finally, it is also important to recognize that the number of environments identified does not indicate the need for an equal amount of PP/CC isolation chambers or that these isolation chambers should be the same configuration. Given the possible overlaps in some BC and PE functions, it is likely that PP/CC isolation chambers will be reutilized for these later processes. It is also probable that while some of the PP/CC isolation chambers will be similar in size and scope to the current DWI breadboard design presented by the University of Leicester (UK), some may simply be small attachments to these larger chambers (e.g., rapid transfer ports, instrument boxes) or a different configuration completely to accommodate disparate analytical/processing needs (e.g., horizontal vs vertical vs 45° windows).

The environments and associated isolators counts outlined within this report should be seen as the starting point for understanding the potential scope of the MSR SRF if projected CC requirements and all current science goals are prioritized.

See page 4 of the MOSDT Operational Workflow for description of these environments, and attached MOSDT Environment and Isolator Table.

Appendix B. Immediate Triage

While the current notional plan for retrieval and transportation of the EEV from the landing site to the SRF is described in the main organizational flow section, an alternative approach, which includes a Mobile BSL-4 facility, is presented below. This alternate approach differs from the current notional plan in that it offers equal prioritization of PP and CC, instead of just the former. Furthermore, the additional infrastructure required at the landing site for this alternate approach could also be utilized in the event of an off-nominal landing. The current notional plan does not account for this possibility.

Recovering the Earth Entry Vehicle The Martian samples are planned to arrive on Earth via ballistic reentry in the EEV. When the EEV touches down, a team should secure the area. As part of this process, the team should do a rough cleaning step of the exterior of the EEV and do a preliminary structural engineering inspection. Contamination Knowledge (CK) and Biological Knowledge (BK) samples of the terrestrial soil and material surrounding the EEV, as well as the material used to clean the EEV, should also be collected and secured. The EEV should then be placed within the ERIC, and additional cleaning of the landing site may be conducted. If possible, the ERIC will subsequently be hermetically sealed and terrestrial air within ERIC will be replaced with a vacuum or an overpressure of inert gas (such as nitrogen). The ERIC notionally represents the first layer of high-containment protection around the EEV after it returns to the Earth. After the EEV has been secured within the ERIC, the outside of the ERIC and the outside of the containers containing CK/BK samples of the surrounding environment should be sterilized. In the first deviation from the main organizational workflow, the ERIC and CK/BK samples should then be transported to the Mobile BSL-4 facility (MBSL).

EEV Disassembly The MBSL is designed to maintain a negative pressure high-containment space along with a positive pressure cleanroom space inside. Once the ERIC and CK samples are within the MSBL anteroom/airlock, the doors should be secured. Personnel in high-containment suits should open the ERIC and move the EEV, as well as the CK samples, into the cleanroom area. The EEV should then have a preliminary engineering inspection for damage, followed by a cleaning step for terrestrial debris removal, and then a final

engineering inspection should be completed. After the inspection is complete, the body of the EEV should be covered to contain any heatshield that may be sloughing off and the cleanroom cleared of all debris to minimize the amount of terrestrial contamination transfer during disassembly. After any possible sources of contamination are contained and/or mitigated, the CAM lid can be removed to allow access to the SCV.

Securing the SCV Once removed, the SCV should be placed in Mobile Isolator Transport (MIT). The MIT will provide a clean and inert environment for initial SCV cleaning and PP seal integrity verification as well as transportation containment to the SRF. If a double-walled isolator is employed, the isolator can also be classified as a BSC-III and would become the primary containment mechanism for the SCV, making the MIT a redundancy for safety. The first step to securing the SCV should be to clean its exterior. The cleaning should be done in stages, the first to collect all possible debris from out outside of the SCV for study (PP verification) and then thoroughly clean the surface (e.g., Isopropyl Alcohol (IPA) wipes through CO_2 snow) to prevent the transfer of terrestrial contamination later in the disassembly process. Once clean, an engineering inspection should be performed and when satisfied, the SCV should be secured for transport. If any debris is removed from the outside of the SCV, it should be contained and remain in this isolator for further investigation.

Securing the EEV After the SCV is secured, the CAM lid should be reattached to the EEV. The EEV could be fastened in place or moved back into the ERIC for transportation to the SRF for future PP verification and engineering inspection.

MBSL Arrival at SRF When the MBSL arrives at the SRF, it could be integrated into the SRF (if design allows) and/or the MIT isolator/BSC-III could be moved into the facility and integrated into the isolator line. The SCV should then be moved into the main operational isolator flow line (SCV.3.) and progress nominally.

Appendix C. Acronym List

BC	Basic Characterization
BK	Biological Knowledge
BSC-III	Biosafety Cabinet
BSL	Biosafety Level
CAM	Containment Assurance Module
CC	Contamination Control
CCK	Contamination Control and Knowledge
CEC	Containment End Cap
CK	Contamination Knowledge
DWI	Double Walled Isolator
EEV	Earth Entry Vehicle
ERIC	EEV Receiving Isolation Container
FEP	Fluorinated Ethylene Propylene Polymer
iMOST	International MSR Objectives and Samples Team
IPA	Isopropyl Alcohol
MBSL	Mobile BSL facility
MIT	Mobile Isolator Transport
MSR	Mars Sample Return

MOSDT	MSR Operational Scenarios Definition Team
MSPG2	MSR Science Planning Group (Phase 2)
µXCT	Micro X-ray Computer Tomography
OS	Orbiting Sample
PCV	Primary Containment Vessel
PE	Preliminary Examination
PP	Planetary Protection
Pre-BC	Pre-Basic Characterization
PSS	Pristine Sample Storage
PSubS	Pristine Subsample Storage
RSubS	Reusable Subsample Storage
RSTA	Returnable Sample Tube Assembly
SCV	Secondary Containment Vessel
SRF	Sample Receiving Facility
SSA	Sample Safety Assessment
SSAP WG	Sample Safety Assessment Protocol Working Group
STIC	Sample Tube Isolation Chamber
TBD	To Be Determined
TIC	Transport Isolation Container
TOC	Total Organic Carbon
TSS	Temporary STIC Storage
UPW	Ultra-Pure Water
VHP	Vapor Hydrogen Peroxide

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