**Orbiting Sample Tiger Team Recommendation on Orbiting Sample Cleanliness**

**Abstract:**

The National Aeronautics and Space Administration-European Space Agency (NASA-ESA) Mars Sample Return campaign involves the collection of samples on Mars by the Perseverance (Mars 2020) rover and their return to Earth. To accomplish this, the ‘Orbiting Sample’ (OS) container will be sent to Mars to accommodate the collected samples, launched from Mars and returned to Earth, from which the samples will be removed to be examined in the Sample Return Facility. Crucial to this whole sequence is to decide what level of cleanliness is required inside the OS. In February 2021, NASA Headquarters’ Mars Sample Return Program and Office of Planetary Protection assembled an OS ‘tiger team’ (OSTT) to debate and determine the cleanliness level of the interior of the OS. The team’s remit was primarily focused on making a decision on the trade-offs between cleanliness levels 4a and 4b. These cleanliness levels are determined by Committee on Space Research planetary protection regulations, where 4a requires < 300 bacterial spores/m2 and < 3 x 105 bacterial spores on the spacecraft (in this case the interior of the OS), and 4b mandates the more stringent requirement of < 30 bacterial spores on the spacecraft. This report documents the decision of the OSTT to recommend that the interior of the OSTT be cleaned to a 4a requirement with any feasible effort towards 4b, and it provides the rationale for that decision.

**Orbiting Sample Tiger Team Recommendation on Orbiting Sample Cleanliness**

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***Statement of request***

In February 2021, the Orbiting Sample Tiger Team (OSTT) was requested by NASA Headquarters and the Office of Planetary Protection to discuss and recommend a cleanliness standard for the interior of the Orbiting Sample (OS), the sample return container located in the Mars Ascent Vehicle on the Sample Retrieval Lander. Within the OS, sealed tubes collected by the Perseverance rover and sample fetch rover will be stored for launch into Martian orbit as part of the Earth return phase of the Mars Sample Return (MSR) program.

***Objective of Orbiting Sample cleanliness***

The OSTT discussed the scientific rationale for cleaning the interior of the OS and what was to be achieved by this process. The team recognized that the samples within the sealed sample tubes will not be affected by the differences between 4b vs 4a cleanliness on the interior of the OS. Given that, the team turned its attention to how the cleanliness of the OS interior would impact: 1) material inside the OS and potentially on the outside the tubes (e.g., Martian dust), which will have scientific value, and 2) a sample within a compromised sample tube (i.e., failed seal). **Reducing the quantity and diversity of contamination sources and composition within the OS will enhance the study of materials outside of the tubes or within compromised tubes. This will minimize the downstream chances of contamination compromising Planetary Protection investigations, and the science objectives of the MSR campaign (i.e., the study of Martian organics of either abiotic or biotic origin and life detection).**

A lower biological and/or contamination load within the OS would improve the ability to distinguish contamination from indigenous Martian organics or life signatures, thereby reducing the possibility of false detections. **A second but equally important objective is contamination knowledge through a combination of witness plates, trace-level analyses of clean room contaminants on hardware surfaces, and the archiving of hardware** materials that share the same history of handling and manipulation as the OS from build to launch.

***OSTT Recommendation***

The OSTT considered five possible levels of cleanliness that might be implemented in the interior of the OS:

1. 4b requirement – strictest *requirement*; same as tube interior
2. 4a/b intermediate requirement– split the difference *requirement*
3. 4a requirement – plus *best effort* toward 4b
4. 4a requirement – with any *feasible effort* towards 4b
5. 4a requirement – least strict *requirement*; same as lander, fetch rover, and the Perseverance rover (all hardware other than sample tube interiors)

Each of these was considered by the team.

***The OSTT recommendation*** *is to implement option 4 (category 4a requirement with any feasible effort towards 4b) with four important caveats:*

1) Witness plates within the OS to track contamination and fill a contamination knowledge gap after tube sealing on Mars are essential, preferably with the capacity to provide temporal information on the characteristics/extent/sequence of contamination over the OS lifetime.

2) The initial baseline of contamination on the OS interior hardware post-cleaning with respect to key Tier 1 components (e.g., nucleic acids, organic acids, including amino acids, organo-sulfur compounds, lipids, spores, other common hydrocarbon contaminants; Summons et al., 2014) should be determined, the targets informed based on knowledge from Mars Curiosity and irradiated lab studies.

3) Effort should be made not to clean the OS with solvents or use lubricants and other organic-containing materials that are different from those already used in Mars 2020 sample collection hardware that will come into direct contact with the sample tubes. This will reduce the complexity of contamination as stated in the objective.

4) Materials of the same type and quality from which the OS is constructed and with which it was cleaned (i.e., cleaning solvents) must be archived for contamination assessment purposes.

*The rationale for this decision was as follows:*

The OSTT recognized that the optimum recommendation must be balanced with the cost and logistical difficulties associated with the chosen levels of cleanliness. Although science and planetary protection should not be compromised by taking expedient decisions with respect to cleanliness, it is nevertheless the case that cleaning to 4b and maintaining that level of cleanliness through a Mars landing is a non-trivial task that plays significantly into the decision.

*Option 1: 4b – strictest requirement; same as tube interior*

A 4b cleanliness level would provide the clearest outcome – that the interior of the OS contains the minimum level of contamination achievable. This level of cleanliness may seem illogical given that the outside of the sample tubes being transferred into the OS are cleaned to level 4a. However, there are scientific reasons that would justify higher cleanliness in the OS. The cleaning of the interior of the OS to 4b would localize any contamination discovered later to the exterior of the tubes and remove one ‘tier’ of contamination. From a scientific methodology point of view, cleaning the interior of the OS to a higher standard than the outside of the tubes would reduce the number of sources of contamination and thus the number of variables that must be taken into account when contamination, if it is eventually found, must be accounted for. As the OS will be assembled at a different time and location than the sample tubes, it cannot be ensured that the potential organisms and organic contamination on the interior of the OS would be the same as the outside of the tubes. Hence contamination within the OS interior would likely add an additional level of complexity to the inventory of contamination that would end up on the outside of the sample tubes and potentially within a failed tube.

 However, the countervailing factors in this option are: 1) Cleanliness to 4b involves considerable engineering, logistic and cost implications, particularly in maintaining 4b conditions during transport. The OSTT considered that this effort was not commensurate with the advantages achieved, including minimal benefit to sample material inside sealed sample tubes, 2) The OS is not hermetically sealed. Thus, a 4b requirement for the interior of the OS would have significant implications for the cleanliness requirements of the Earth Return Orbiter, 3) Cleanliness to level 4b inside the OS is not required to achieve a sufficient cleanliness in which the contamination present can be accounted for provided certain steps are taken, such as adequate witness plates to track contamination (see justification for Option 4 below).

*Option 2: 4a/b – split the difference requirement*

The OSTT considered that this option, although not requiring 4b standards, places a *requirement*, and therefore potentially a strong financial and logistic burden on whatever requirement is defined. The team could not identify a specific organic or biological component that needed to be below a specific threshold or what the threshold would be in order to define a requirement between 4a and 4b. Any cleaning beyond 4a is likely to be highly challenging. The establishment of a *requirement* beyond 4a might lead the project into unnecessary difficulties and commitments that are too burdensome with respect to the advantages achieved (see caveats in the option chosen).

*Option 3: 4a – with best effort toward 4b*

This option was considered attractive by the team since it implies a concerted effort to achieve a minimum cleanliness level of the outside the sample tubes, and to attempt, if possible, a cleaner standard.

The problem discussed by the team was that ‘best effort’ cannot be easily defined and quantified and could, in principle, lead to considerable engineering effort and challenges resulting in increased mission costs. Without a well-defined set of requirements which can be formulated in protocols, it leaves an open-ended question about what ‘best effort’ actually means and how that might be apportioned across different types of contamination (i.e., microbial, organics).

Nevertheless, *the team recognized the highly desirable goal of cleaning beyond 4a where that might be possible within reasonable engineering possibility, and this informs the caveats the team adds to its selection of Option 4*.

*Option 4: 4a requirement – with any feasible effort towards 4b*

The OSTT chose Option 4 with certain caveats. Option 4 would bring the interior of the OS to *at least* the same required cleanliness level as the exterior of the sample tubes. However, the OSTT recognized that any capability to reduce contamination inside the OS is beneficial in reducing both the absolute quantity and potential complexity of microbial and organic contamination. Thus, any feasible effort to clean beyond 4a without imposing significant mission cost and engineering effort should be attempted.

Any contaminants within the OS could potentially add to the complexity of backing out terrestrial contamination in scientifically valuable material within the OS (e.g., dust) and within a sample tube whose seal is compromised. Therefore, the team proposes four important caveats, or additional requests, to its selection of option 4:

1) Because the interior of the OS will not be cleaned to level 4b, it is critical that the quantity and fate of contaminants in the OS should be tracked. ***Witness plates must be included to track contaminants within the OS****.* Ideally, these should be capable of providing temporal information on the history and characteristics of contamination over different parts of the journey to and from Mars: the journey to Mars, the period on the Martian surface, and the period during orbital capture and return to Earth. Some of the plates should be sealed to prevent contact with Martian dust, providing hardware contamination controls for OS assembly and knowing the fate of these contaminants during the journey to and from Mars. Witness plates should not be hermetically sealed so that they can sample any potential volatile outgassing inside the OS.

2) ***The initial baseline of contamination with respect to key Tier 1 components (e.g., nucleic acids, organic acids including amino acids, organo-sulfur compounds, lipids, spores) must be determined.*** This allows us to know what contamination the OS started out with, improving the capacity to account for this contamination, or any new contamination, identified later. This involves determining the distribution of contaminants on OS interior hardware surfaces and determining quantitative abundances or upper limits in units per surface area and diversity in the case of microbial contamination. We note the importance of characterizing the contaminant load in the metallic ‘crush’ foam, which is expected to occupy most of the volume inside the OS and will serve as mechanical/shock protection for the sample tubes. It is anticipated that sterilization, decontamination, and validation protocols will need to be tailored to the properties of the material (i.e., composition, porosity, permeability) and its mode of manufacturing. The initial baseline of contamination should be determined as close to launch as possible to be representative of the contamination in the OS at the time of launch to Mars.

3) In an effort to reduce the complexity of organics introduced into the OS in addition to those that might already find their way into its interior in the course of MSR, ***the OS should not be cleaned with solvents or use lubricants and other organic-containing materials that are different from those already used in equipment that will come into contact with the sample tubes.***

4) ***Examples of materials and solvents used in the construction of the OS should be archived*** (including flight spare witness materials subjected to the same cleaning processes and assembly environments as the OS hardware) so that they can be investigated later (for example with improved analytical capabilities) to understand the fate of organics and microorganisms on materials and in solvents used to construct and clean the OS.

*Option 5: 4a requirement – least strict requirement; same as lander, fetch rover, and the Perseverance rover (all hardware other than sample tube interiors)*

The OSTT considered this option to be suboptimal. Although it would achieve cleanliness nominally similar to the lander, fetch rover, and the Perseverance rover, if the possibility exists to reduce this contamination (as explained in Option 4), this should be attempted.

Summons, R.E., Sessions, A.L., Allwood, A.C., Barton, H.A., Beaty, D.W., Blakkolb, B., Canham, J., Clark, B.C., Dworkin, J.P., Lin, Y., Mathies, R., Milkovich, S.M., and Steele, A. (2014) Planning considerations related to the organic contamination of Martian samples and implications for the Mars 2020 Rover. *Astrobiology*. 14:969-1027.