Report of the COSPAR Workshop on Refining Planetary Protection Requirements for Human Missions

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Introduction

The Committee on Space Research (COSPAR) is maintaining a planetary protection policy with associated requirements as an international reference standard for spacefaring nations to guide compliance with article IX of the UN Outer Space Treaty. Updating the COSPAR Planetary Protection Policy is an iterative process based on new discoveries, new understanding of scientific observations, and in response to needs identified to prepare for future space missions. The latter is the case for preparing human missions to Mars. The current principles and guidelines for human missions to Mars have been part of the COSPAR Planetary Protection Policy since 2008 and are based on a series of earlier workshops and reports. However, in order to define proper engineering requirements and assess technical solutions for future human-rated flight systems, a more detailed level of quantitative planetary protection requirements specifically for human missions to Mars are necessary. Recognizing this need, the National Aeronautics and Space Agency (NASA) organized a workshop in 2015 to identify Knowledge Gaps (KG) related to planetary protection for human missions beyond the Earth-Moon system. Subsequently, COSPAR, NASA and European Space Agency (ESA) organized another workshop in 2016 to focus on ways to address and prioritize the identified KG. This report summarizes the findings of the 2016 workshop.

Workshop Organization

As part of the stepwise planning for future space exploration, COSPAR together with NASA and ESA, organized an interdisciplinary workshop to consider the next steps in the review and eventual formulation of the COSPAR Planetary Protection Policy for future human missions beyond the Earth-Moon system.

The workshop, which took place at the Lunar and Planetary Institute, Houston, Texas, USA on October 25-27, 2016, was attended by 34 invited participants (see Appendix I for a list of participants).

The discussions were arranged in three splinter groups on the following three main areas, each given the task of reviewing the Knowledge Gaps (KG) identified by the 2015 NASA workshop:

- Microbial and human health monitoring
- Technology and operations for biological contamination control
- Natural transport of biological contamination on Mars

This report describes the deliberations and findings of the individual splinter groups. In addition, the workshop organizers identified four high priority **Knowledge Gap Areas** based on the splinter group results.

Splinter Group 1. Microbial and Human Health Monitoring

Splinter group 1 addressed issues around the monitoring of Earth microbial populations and astronaut health status, with an emphasis on using these data to assess the potential for possible biohazards that could be returned to Earth, and/or indications of extra-terrestrial life. There are significant synergisms between these planetary protection interests and issues relevant to assessing the health status of astronauts on these missions.

The presence of microbial populations in habitats and flight systems needed to support human astronauts, and their isolation from the Earth's environment during long-duration spaceflight, creates an ongoing experiment on how microbial ecosystems change in a confined, microgravity environment. The same populations also represent a reservoir of potential pathogens that could harm astronaut performance and result in degraded mission success. For these reasons, capabilities to monitor both microbial populations and human health status will be an important feature of all long-duration human missions.

Although human health and habitat-associated terrestrial microbes are not addressed by planetary protection requirements, information about these topics has the potential to inform planetary protection policy decisions in the context of Earth safety assurance. The possible exposure of human explorers to Mars life will occur in the context of their ongoing exposure to Earth-sourced commensal organisms. Overall, splinter group 1 concluded that comprehensive microbial and health monitoring will be critical on long-duration human missions to Mars, and that these capabilities should be developed on missions to targets of lower concern for planetary protection.

During their deliberations, the group evaluated the previously identified KG and framed them in the context of specific technologies that could or should be developed to address specific needs in microbial and human health monitoring. The group rearranged the questions to address them in the following four open issues (1A-1D), which capture the key KG that were identified during previous work. These can be addressed by a range of investigations, undertaken anywhere from ground-based locations on Earth to spaceflight environments ranging from near-Earth orbit to cis-lunar space to Mars.

Summaries of the discussions around each of these identified four issues are presented below.

1A. How do we systematically provide for microbial monitoring of the environment?

In the context of identifying key KG, this splinter group found it useful to consider environmental monitoring for the purpose of interest predominantly to planetary protection and for monitoring human-associated microbial populations that are likely to be of direct relevance to assessments of astronaut health.

The fundamental goal of environmental monitoring is to assess potential risks during long-duration spaceflight by evaluating how microbial populations change over time. This addresses planetary protection concerns around both 'forward' and 'backward' contamination. Monitoring for potential releases of Earth microbes into microbial contamination-controlled environments (e.g., Mars) would ensure awareness of possible biological contamination events that might affect scientific and other future objectives at Mars, and could also facilitate mitigation of contamination events in accordance with requirements applicable to that mission. To some extent, monitoring and mitigation capabilities could be tested in environments where contamination is not of concern for planetary protection (e.g., the Moon, near-Earth asteroids), which might reduce risk for missions where microbial contamination would be of concern.

It is expected that microbial populations would only display a limited range of alterations during long-duration human spaceflight in response to the environmental conditions present in the isolated spacecraft ecosystems. Exposure to the physical and chemical conditions associated with non-living planetary materials could produce additional, but still identifiable, alterations to microbial populations. Data collected for microbial monitoring would also inform basic scientific understanding of the adaptability of microbial populations exposed to novel environments.

For planetary protection, the hypothesis underlying the environmental monitoring approach is that the changes exhibited by microbial populations exposed to long-duration spaceflight and non-living planetary environments are likely to be different from changes that would be exhibited in the presence of extra-terrestrial life. By collecting information about changes observed during human missions that do not contact other planetary materials (e.g., International Space Station (ISS) missions), and also human missions in which non-living planetary materials are contacted (e.g., lunar and asteroid missions) it should be possible to establish a 'baseline' of observed and expected changes. If only these predicted changes are observed after exposure to material from Mars, this could be used in support of conclusions that no martian organisms are present. Although certainly not definitive, such data might help to increase confidence that no extra-terrestrial biohazards are present.

Any systematic effort at microbial monitoring can be divided into five steps that are required to ensure useful outputs: sampling, processing, measurement, analysis, and data (and materials) storage. This is true for monitoring microbial populations that are present in spacecraft environments, as well as those more closely associated with humans – although the sample collection and some aspects of the sample processing phases could be different. General considerations are discussed here, with considerations specifically relevant to human-associated monitoring addressed in the next section (1B).

It is of critical relevance to assess what types of monitoring are needed to get good coverage, and which data are needed to identify alterations in microbial population structure that would indicate changes of concern. Essential to establishing the range of microbial populations in relevant environments is determining where to sample, and what to sample. Within human support systems, water and waste processing hardware are obvious candidates for hosting microbial populations that could change over time. Interior surfaces and materials that come into frequent contact with humans are additional sampling targets, as are locations that are relatively inaccessible and therefore challenging to clean and/or disinfect. To understand which locations provide the most return on sampling effort, it could be informative to test candidate sampling targets in analogue environments, both on Earth (e.g., Antarctic overwintering stations; other isolated long-duration habitats) and in existing space systems (e.g., ISS).

Sample processing needs depend both on what is being measured, and how the samples are collected. Microscopy could be very informative, and requires instrumentation that may or may not be available for other investigations. Nucleic acids are an obvious chemical target for monitoring, and it might also be informative to monitor other macromolecules (e.g., proteins, polysaccharides, lipids), to obtain a broader range of information. The collection of the samples is a challenge, because consumables require mass and volume -- one question that should be investigated is the extent to which it is possible to develop sampling tools that also serve other needs (e.g., cleaning wipes that can be processed for DNA). The costs and benefits of monitoring different macromolecules may already be under investigation in other communities' ground-based efforts (e.g., epidemiology, biosecurity) so these, to the extent feasible, should be evaluated early during development of spaceflight capabilities.

Rapid sample processing is preferred over storage and later processing in bulk so that the results can be analysed in the context of risk assurance. For comprehensive analysis, in-situ whole genome environmental sequencing has the potential to address many monitoring questions, although shelf-life and consumables for processing samples, as well as calibration of hardware, are of concern. It is likely that other ground-based communities are making investments in developing technologies for processing, analysis, and data storage that could be of direct use in, or easily adapted for, space exploration.

The overall objective for environmental monitoring is to understand how microbial populations behave over time, with the goal of distinguishing adaptations to spaceflight and isolated closed human support environments, from the potential effects of introduction of planetary materials (e.g., lunar, asteroid, or Mars dust) into the habitats and human support systems. Collecting these datasets, starting with current spaceflight systems, is a high near-term priority required to provide baseline data on environments and enable changes to be monitored as new systems are developed in the future. Major advances are being made in Earth-based biomedical and environmental monitoring, which provides opportunities develop synergisms with other research areas. It will be important to leverage existing

funding opportunities and ongoing work: for example, investigations of how microbial populations on the ISS change in response to resupply missions that regularly introduce a different spectra of Earth organisms.

1B. How do we systematically do microbial monitoring of humans?

Monitoring selected microbes using classical microbiology to assess potential risks to astronaut health has a long history as standard medical practice during human space exploration. The ongoing microbiome initiatives using newer technology are greatly expanding ground-based capabilities that are generating enormous amounts of relevant data. Such ground-based research, as well as research ongoing on the ISS, should facilitate efforts to identify potential human health indicators and infection markers, that might be informative for establishing baseline profiles and understanding the implications of changes observed after exposure to planetary environments.

Analysis of human samples, including blood, skin, excretions, and other materials involves similar concerns to those covered in section 1A for sampling, processing, measurement, analysis, and data storage. Specific capabilities are already being developed for biomedical applications that are good targets for adaptation to use in space. One important aspect of evaluating microbial populations associated with humans is to understand the implications of sample cross-contamination risk, as distinct from mixing of populations in close-contact environments prior to sample collection. This is important since different people's immune systems combat infections with varying levels of efficiency. Given that mass and volume during spaceflight are limited, it will be important to evaluate capabilities for information gained relative to instrumentation/materials needed and shelf-life of required consumables. Testing performed in the near-term is required to inform selection and development of capabilities for long-duration missions to planetary targets such as asteroids and Mars.

The primary objective for systematic monitoring of human-associated microbes is to distinguish potential causes of disease by providing a baseline to identify changes that would inform decisions on treatment. A substantial body of work already exists, from the ISS and other spaceflight experiments, describing changes in selected microbes and small multicellular organisms that suggest there are changes to host-pathogen interactions due to spaceflight, although those changes are not observed to be large. As this research continues, it is a high near-term and ongoing priority to ensure that the potential to address questions relevant to planetary protection is retained as standard protocols and capabilities for assessing astronaut health are developed.

The particular concern for planetary protection is to evaluate the consequences of exposure to planetary materials that could contain extra-terrestrial life, because all extra-terrestrial organisms are by definition considered to be biohazards, and release into the Earth's environment is prohibited. Mars is the only proposed planetary target for human exploration where this concern is relevant, so human missions with other objectives can serve as testbeds for protocols that could in future be implemented on human Mars missions. The Apollo Program was the first to implement quarantine procedures for astronauts, and has provided a number of valuable lessons-learned for future missions.

As an exercise, this splinter group developed a notional decision tree for managing infections that could affect the crew of a Mars mission, with an additional goal of using astronaut health as a potential indicator of contamination by Mars biohazards, as distinct from exposure to non-living toxins such as hexavalent chromium, perchlorates, or phosgene (see Appendix II). Similar approaches should be developed and tested in Earth analogues and on near-term missions, to ensure that effective precautions are in place before the first human mission to Mars.

Careful planning, operational analysis, and effective decision making are all essential starting at the early planning stages of the first human mission to Mars, to ensure that both astronaut health and the safety of Earth are assured.

1C. How do we design spaceflight systems to mitigate microbial growth?

This question is intended to address all aspects of spaceflight hardware, including habitats, vehicles, space suits, and other support systems. Materials selection is a critical aspect of reducing microbial growth, and also considering how materials degrade over time from both chemical and structural standpoints. Smooth surfaces without pits and

crannies are easier to clean, by eliminating potential habitats, where microbes can adhere and accumulate or form biofilms. Some microbes are able to derive nutrients or energy from materials that are reactive, releasing volatiles or breaking down over time, so careful materials selection, in combination with effective cleaning practices, should reduce microbe-associated corrosion and biofouling. For components or locations where significant microbial accumulation is expected, repeated sterilization is a common method to control contamination, but has to be balanced with toxicity or damage from the sterilization process. With the advent of 3-D printing and in-situ manufacturing capabilities, innovative solutions could be explored, such as regular re-manufacture and replacement of contaminated parts. Similar challenges are faced here on Earth, so approaches developed for other purposes should be evaluated for utility in spaceflight applications

Minimizing undesirable microbial growth has multiple benefits from normalizing environmental conditions to efficiency of maintenance. Since microbes can release unpleasant volatiles, cleaning and repair of support hardware can impose a significant burden on crew time and effort. Controlling microbial populations can also prevent impairment to astronaut health, and could help to standardize the environmental baselines measured during monitoring. If microbial populations during long-duration spaceflight do not achieve a stable steady-state, then observed fluctuations could be misinterpreted as either false-positive indications of a contamination event, or false-negative 'normal' variation when in fact a contamination event has occurred. Research is needed near-term to understand how biofilms and other microbial assemblages behave in the space environment, so that these results can be used to inform future spaceflight hardware development.

Mars is the only current target for human exploration where planetary protection imposes restrictions on the release of Earth microbes into the environment. More near-term targets for human exploration, such as the ISS, the Moon, and asteroids, can be used to evaluate the extent to which human support systems release microbial contamination, as well as serving as testbeds for approaches to mitigate potential releases (e.g., by ensuring that effluent is sterilized during release) or remediate accidents.

As with the previous topics discussed, the design of support systems to control microbial populations is a high priority issue that needs to be considered early, so investigations should start in the near-term with Earth analogues and existing spaceflight systems, as well as being incorporated into future design and development efforts.

1D. What operational guidelines are needed to understand planetary protection concerns & crew health?

A common mantra within the safety community is that the best protective equipment is only as effective as the people who use it. Standard safe operating procedures, that are implemented correctly by astronauts during both nominal and off-nominal activities, are absolutely essential for effective planetary protection and also crew health maintenance. Detailed operational procedures can only be developed in the context of specific hardware systems, but Earth analogues and early exploration targets can be used to establish guidelines for how to develop operations and hardware that enable clean and efficient activities, while minimizing exposure to potentially harmful planetary materials. In the near term, this should involve education and knowledge capture, so that the experience accumulated over decades to date is retained and supplemented as approaches for clean operations are established. These should facilitate capabilities to distinguish the effects of Earth microbes from potential extra-terrestrial biohazards, and establish criteria for making potentially difficult decisions about crew health. As human missions to planetary targets are developed, attention and awareness will need to be increased to ensure that long-term objectives at Mars are not compromised due to short-term expediency.

Splinter group 1 overall priority rankings

Topics 1A, 1B, and 1C are considered high priority and near-term, due to the potential for synergisms with ongoing work and the importance of incorporating microbial monitoring and control capabilities into the development paths for human support systems.

The aspects of topic 1D that involve mission operations are considered relatively low priority at this time, but that their importance will increase as exploration priorities become better defined.

Splinter Group 2. Technology and Operations for Contamination Control

Splinter group 2 focused on issues associated with technology and operations for controlling and/or mitigating contamination of relevance to planetary protection during future human missions to Mars, including issues such as:

- Cleaning, sterilization, and recontamination prevention technologies for in-situ applications
- Mitigation of spacecraft effluents
- Technologies for contamination control of human surface mobility systems and spacesuits
- Contamination control and localized special region contamination prevention for support systems (In-situ Resource Utilisation (ISRU), power, etc.)
- Human surface exploration operation strategies for mitigating contamination
- Sample containment and breaking-the-chain-(BTC) of contact technologies
- Closed loop life support and extravehicular activity (EVA) support systems
- Other support systems, including those associated with scientific support systems to minimize human contact with the environment of planetary surfaces and sub-surfaces.

In discussing the KG identified by the NASA 2015 workshop report, the group agreed in general with the 2015 earlier findings. However, it also considered that rewording was needed to clarify some previous KG descriptions. In the process of revising the earlier wording, the group also chose to eliminate the former KG 8, dividing it into two parts, and inserting the content into existing KG 2 and 3, which covered similar questions and focus. Finally, the group determined to add three new gaps bringing the new list to a total of ten KG.

Based on the splinter group deliberations, the revised KG statements, with respect to the numbering used for the technology & operations for contamination control group during the 2015 workshop and newly added gaps are listed below, with indications of their mission criticality and time priority rankings.

2A. Does the duration of a Mars surface stay affect implementation of planetary protection during the mission?

The splinter group deliberations cantered on understanding two basic questions: How do released levels of microbial contamination and transport depend on short vs. long surface stays and associated operations? And how do subsequent crew stays at the same site impact microbial contamination and transport?

In discussing issues associated with microbial contamination levels, potential contamination and transport, the group acknowledged that the topics were dependent upon data and information from the other two splinter groups. While documentation of actual transport levels and types of microbial contamination will be important, splinter group 2 focused on the variety of issues that will impact engineering solutions. These included, for example:

- The relationship between surface stay and density and spread of contamination
- Consideration of human exploration zones and infrastructure (similar to an Antarctic exploration/research approach)
- How to translate the information from an initial site to other locations
- Understanding impacts over long times into the future
- Studies on generation, distribution and spread of materials
- The impacts of human waste and trash (as well as the human microbiome); the quantity and nature of what's released
- Analysis of how activities (e.g., subsurface drilling) might impact the level and spread of microbial contamination
- Designated 'acceptable' levels of microbial contamination for particular sites
- Strategy to be used in dealing with the levels and accumulation of contaminants.

The group suggested that Knowledge Gap 1 could be addressed and closed by Research & Technology Development (R&TD) on Earth, by:

- Studying analogue missions
- Evaluating trash management protocols and processes
- Developing better Extravehicular Activity (EVA) frequency projections
- Evaluating microbial contamination production and impacts in situations both with ISRU and without ISRU (including whether the ISRU activity uses material from atmospheric or regolith sources).

In addition, the group indicated it would be useful to determine go/no-go point for food production (how long would we have to stay before food production makes sense). Overall, the splinter group assigned this KG a **medium** mission criticality, and a **medium** time priority. Finally, the splinter group reworded the Knowledge Gap as follows:

> **Knowledge Gap 2A (revised wording): How do released levels of microbial contamination and transport depend on short vs. long surface stays and associated operations? How do subsequent crew stays at the same site impact engineering design and operations related to microbial contamination and its transport?**

2B. What level of non-viable microbial contamination escape is acceptable?

The discussions focused on the levels and fate of human microbiological and organic contamination that would vent from extravehicular activities and support systems, as well as whether microbial contamination would be viable or non-viable upon release, and whether there might be dissemination of concern on Mars. They also considered the implications of vented materials for suit materials and cleaning tools designated for Mars.

Viable organisms, non-viable organisms, and organics all have the potential to confound science studies, while viable organisms are of concern relative to the forward contamination of Mars, both now and under future conditions and activities. Concerns about contaminant release(s) must be balanced against natural processes on Mars that may serve to degrade organic signatures and kill microbes. Additional consideration about acceptable releases on future missions clearly depend upon the zone(s) that may be involved—such as habitation vs. exploration areas. For the purposes of splinter group 2 discussions, the focus initially emphasized considerations associated with the presumed Exploration Zone (EZ).

In order to address engineering and technology solutions for future planetary protection needs the following general areas of research were identified as useful.

- There is a need to understand and measure microbial contamination released by EVA suits
- There is need to characterize the different microorganisms that can be released from human systems, as well as determine which ones matter and which don't
- The group suggested the need to expose human forward contaminants to simulated Mars environments (including spacecraft-induced environments) in order to understand if and how terrestrial microorganisms can survive
- Additional R&D is needed to assess microbial reduction options for pressurized craft (space suits, rovers, habitats). Several options were identified including:
  - Attention to microbial vent filters (e.g. can we develop rechargeable filters that can be cleaned/ re-used to minimize consumables mass and limit accumulation of bacterial growth?)
  - Understanding how use of filters impacts system design (pressure drop, power draw, etc.)
  - Undertaking research focused on various UV sterilization methods and other approaches for microbial reduction.

Overall, the group categorized **Knowledge Gap 2B** as representing both **high** *mission criticality* and **high** *time priority,* and noted that research to close the KG could be accomplished on Earth, on the ISS, cis-lunar or the Moon in some cases. Finally, the group suggested revision of the wording for KG 2 as follows:

> **Knowledge Gap 2B (revised wording): What level of microbiological and organic release from humans and their support systems (hab/lab/suits/vehicles) is acceptable?**

2C: Is there need for decontamination and verification procedures after releases (nominal or otherwise)?

The discussions focused on the need for decontamination and verification procedures and protocols after releases, whether nominal or otherwise. Additionally, they considered concerns about monitoring protocols (inside & outside) for remediation after potential releases from humans and their support systems (hab/lab/suits/vehicles).

Work on a variety of research and engineering areas is needed to close this KG, including:

- Research to characterize potential contaminants (organic, in-organic, and particulate) with regard to transport, migration, resistance, and persistence (including mutation). This will contribute to protocols that will improve the design of collection tools, cleaning tools, monitoring technologies and dust mitigation on Mars
- Starting with standard biosafety lab protocols, there is need to test Biological Safety Level (BSL) protocols with space hardware
- Research and development is needed for monitoring both inside and outside pressurised systems to detect releases of different types
- Evaluation of containment options (similar to Hazardous Materials responses on Earth) is needed for space conditions and scenarios
- Development of clean-up protocols for likely mission scenarios; while clean-up on Mars may not be possible after a large release it may be desirable to contain ground spills to prevent contamination to groundwater/ice)
- Development of a regolith simulant that can be used for simulated research of relevance to contamination/decontamination
- Definition of zone boundaries for areas that differ in level of allowable cross contamination (i.e., habitat interior, habitat airlock), and determination of allowable transfer across these boundaries (e.g., if rover and habitat are shirtsleeve environments, what and how many non-shirtsleeve boundaries are crossed to get from one to the other?).

Overall, the group categorized **Knowledge Gap 2C** as representing both **high** *mission criticality* and **medium** *time priority,* and noted that research to close the KG could be accomplished on Earth, but would be substantially informed by the output from a Mars Sample Return mission. The group revised KG 3 wording as follows:

> **Knowledge Gap 2C (revised wording): What decontamination, verification, and monitoring protocols (inside and outside pressurised system) are required for remediation after potential releases from humans and their support systems (hab/lab/suits/vehicles)?**

2D. What considerations should go into design of quarantine facilities and methods (for use to/from or on Mars)?

The discussions considered what should go into the design of quarantine facilities and associated methods for use in various mission phases: on Mars, returning from Mars, and in the Earth-Moon system. They noted that it may not be possible to do a lot of engineering until more is known about human mission operations and architectures, but R&TD can begin by:

- Starting with standard Earth-based quarantine protocols and equipment, consider what will be needed for different scenarios and locations
- Identifying those situations where quarantine is likely to be needed

Overall, the group categorized **Knowledge Gap 2D** as representing both **low** mission criticality and **low** time priority and noted that the work to close this KG could be done on Earth. Wording for KG 4 was revised as follows:

> **Knowledge Gap 2D (revised wording): What considerations should go into the design of quarantine facilities and methods for uses on Mars, returning from Mars, and in the Earth-Moon system?**

2E. How will increased understanding over time about the Martian surface and subsurface modify the definition of Special Regions an associated contamination concerns for human missions?

In order to close this KG, there is need for data and information from Mars robotic precursor missions, specifically related to:

- Understanding the nature of water activity, including seasonal variations on Mars.
- Understanding the nature of and environmental conditions associated with Recurring Slope Linnea (RSL)

Overall, the group categorized **Knowledge Gap 2E** as **high** mission criticality, and **low** time priority. Engineering R&TD to close this KG depends upon research data from Mars. The wording of KG 5 was revised as follows:

> **Knowledge Gap 2E (revised wording): How do the environmental conditions conducive to the support and reproduction of Earth organisms, (Special Regions), vary with time and location on Mars? Both diurnal and seasonal cycles should be considered.**

2F. What research is needed to address assorted questions about testing, ISRU, and habitation?

The group undertook deliberations about cleanliness vs. in situ countermeasures. The main surface activities necessary for human habitation will strongly influence ISRU. For ISRU, extracting atmosphere and subsurface ice creates an opportunity for release of contaminants and microbial contamination into the natural environment while also potentially introducing hazardous chemicals and harmful dust into systems and consumables to be used by the crew. Presently, no technologies have been identified or selected for contamination identification, assessment, monitoring, and control at these interfaces. Also, the failure modes and their potential creation of new unaccounted interfaces between these crew systems and the natural environment have not been pondered since the crew systems have yet to be designed. Definitions are required for the operational constraints for the tools which will be in contact with the subsurface ice (forward contamination).

The splinter group 2 identified the following R&TD areas needed for engineering to close this KG—and noted that future decisions must be informed by relevant human health R&D.

- R&D on how to sterilize Mars resources feeding into ISRU systems (both for planetary protection and to avoid impurities in ISRU processes)
- R&D on how to measure/verify there are no living organisms in ISRU processes
- Analysis and study of potential process/equipment differences between atmospheric ISRU vs. regolith ISRU for oxygen or water production
- Consideration of other resources: perchlorates, iron, etc.

Overall, **Knowledge Gap 2F** was rated both **medium** mission criticality and **medium** time priority. Information to close the KG can be obtained by R&D on Earth as well as verification on Mars. The KG was reworded as follows:

> **Knowledge Gap 2F (revised wording): What research is necessary to understand how to make ISRU processes and planetary protection goals compatible?**

2G. What is considered acceptable regarding waste handling and disposal?

The splinter group determined that a combination of engineering attention and more detailed understanding about the waste will be needed to close this Knowledge Gap. In particular, there is need to identify the likely nature of the varied waste products and their projected quantities in order to fully understand the waste containment needs. A comparison and evaluation of existing waste containment protocols and equipment used on human missions is necessary in order to identify whether any new R&D areas are need. Moreover, it will be Important to factor in recycling options and determine what sterilization might be required in order to recycle materials for various uses.

The splinter group 2 determined that this KG has a **low** priority in terms of mission criticality as well as a **low** time priority. Research and technology development to close this KG can be done on Earth.

2H. What microbial contaminants would vent from an extravehicular activity (EVA) suit?

The deliberations of Knowledge Gap 8 R&TD issues began by focusing on the separate parts of the original wording from the NASA 2015 workshop as follows: What microbial contaminants would vent from an extravehicular activity (EVA) suit, and at what concentrations? What are the implications for suit materials and cleaning tools, designated for Mars?

Overall discussions centered on the need to understand what microbial contaminants - and at what concentrations - would vent from mobile assets such as a manned rover or extravehicular activity (EVA) suits. In addition, the group acknowledged the need a more detailed understanding about the characterization of martian dusts and how it might be transferred to the spacecraft habitable volume from mobile assets. Such information will have implications for suit materials and cleaning tools, collection technology and procedures on Mars.

In discussions of such human associated microbial contaminants and their transfer, splinter group 2 determined that the separate topics had considerable overlap with other identified KG. Accordingly, the group suggested deleting the 2015 Knowledge Gap 8 (2H) entirely, noting that the topics essentially were covered in text and re-wording of KG 2 and 3. Thus, Knowledge Gap 8 (2H), as described in the 2015 report was eliminated in its entirety.

After discussing the original eight KG from the 2015 workshop, splinter group 2 determined that there was need to consider several other areas which lacked information of importance for engineering and planning. The subsequent discussions resulted in the addition of three new Knowledge Gap areas needing R&TD work. Details for the new added KG 2I, 2J and 2K are discussed below.

2I. What approach should be taken to achieve the requirements to "Break the Chain of Contact" with the Martian environment for human missions?

In considering how KG related to current COSPAR Guidelines for Human mission, the group focused on what approach should be taken to achieve the requirements to "Break the Chain of Contact" with the Martian environment for human missions. For example, would it be allowable to leave the EVA suits on the surface?

Clearly, engineering solutions will be important for addressing this KG, and may be obtained from planning efforts and implementation on other missions (e.g., sample return missions; evolvable Mars campaign missions which Include the prospect of multiple changes of crew vehicles before returning to Earth, and the prospect of leaving used spacesuits on Mars.) In a worst-case option, the group noted that it might be possible to consider diverting samples and/or crew to cis-lunar locations (e.g., an orbiting outpost; lunar base) if there is concern during a mission about bringing crew/samples back to Earth.

Overall, the new **Knowledge Gap 2.9** was determined to have **low** mission criticality as well as **low** time priority, although the WG Indicated the criticality may change to “high” depending on involvement of international regulatory agreements. In the meantime, R&TD to close **Knowledge Gap 2I** may be done on Earth.

2J: What is the global distribution and depth of the subsurface ice and does it contain evidence of extant life?

Another area of concern about microbes on future Mars human missions relates to the potential for the presence of viable organisms in the ice and their transport mechanisms e.g., to the atmosphere in the context of surface operations. Clearly, such information will be important for selection of landing site locations and maximization of the safety of the crew (and also for back contamination concerns).

In order to develop appropriate engineering solutions for future missions, an increased understanding about possible extant life in ices and its transport in the atmosphere will be required, drawing on information linked to splinter group 3 KG. The group indicated that addressing this KG must be closed on Mars, for example during the upcoming ExoMars mission. The WG assigned a new **Knowledge Gap 2J** to a category of **high** mission criticality as well as **high** time priority.

2K. Do planetary protection measures need to evolve for precursor missions to human exploration zones, and how?

In order to understand how planetary protection concerns and implementation will impact on technology and operations, splinter group 2 discussed the need to know more about planetary protection implementation for precursors and human missions. For example, with the pre-emplacement of hardware for benefit of later missions; will that be done under the robotic or human paradigms for planetary protection? The concern here is that current requirements are based on spacecraft delivering ~1 ton of hardware to the surface. It is unclear whether these criteria can be met for systems like e.g., habitats that are intended to operate in crewed configuration as an extension of the terrestrial environment, and weigh in at around 20 tons per landing event.

Accordingly, a KG was identified that specifically focused on the question of whether and how planetary protection measures might need to evolve over time. This new **Knowledge Gap 2.11** was deemed to be of **medium** mission criticality and **high** time priority, and would need to be addressed on Mars based on new knowledge about the ability of Mars to cope with the bigger numbers of terrestrial contaminant organisms, obtained in conjunction with the work of Splinter Group 3.

Splinter group 2 overall priority ranking

The numbering of the KG has been modified after the workshop by the workshop organisers to be in line with the numbering of the two other splinter groups.

Former Knowledge Gap 2.8 has been removed because it is covered by Knowledge Gap 2B and 2C.

Four KG were found to require at least some of the effort to be performed at Mars, with implications on future mission activity, if these KG are to be addressed in a timely way to support the arrival of the first crewed mission to the martian surface.

While no KG require work in cis-lunar conditions, it might be possible to address some KG there if opportunities became available.

The three tables below present an overview of splinter group 2 assessment of the identified KG, their prioritization by *time* and *mission criticality*, as well as possible *locations* for R&TD.

	2A	2B	2C	2D	2E	2F	2G	2H	2I	2J	2K
	Stay Duration	Acceptable Release	Mitigation Measures	Quarantine Measures	Special Regions	ISRU compliance	Waste Handling	Deleted	Break Chain of Contact	Ice Distribution and Life	Evolution of Goals
Time	Med	High	Med	Low	Low	Med	Low		Low	High	High
Criticality	Med	High	High	Low	High	Med	Low		Low	High	Med
Where?	Earth	Earth	Earth	Earth	Mars	Earth Mars	Earth		Earth	Mars	Mars

Table 1: Time priorities, mission criticality and location to address the KG identified by splinter group 2

		Priority by Time		
Priority by Mission Criticality		High	Medium	Low
	High	2B,2 J	2C	2E
	Medium	2K	2A, 2F	
	Low			2D, 2G, 2I

Table 2: Analysis by time and mission criticality, with the expectation that this will represent a potential priority ranking for space agencies future activities to close the KG

		Priority by Time		
Gap Closure Location		High	Medium	Low
	Mars	2J, 2K	2F	2E
	Cislunar/ISS **			
	Earth	2B	2A, 2C, 2F	2D, 2G, 2I

Table 3: Analysis of locations where work to close R&TD KG may be accomplished. As noted previously, the group felt that the majority of KG could be closed through testing, design, or analysis on Earth; and while no KG *required* cis-lunar exploration activity, the group suggested ** that that some KG *could* be addressed by missions in cis-lunar space/LEO if those options were available.

Splinter Group 3. Natural Transport of Contamination on Mars

Splinter group 3 addressed issues of transport of viable microorganisms and organic contaminants from the EZ to the general Mars environment, with eventual emphasis on the probabilities of reaching possible Mars Special Regions. Natural transport consists of movement by aeolian processes, including abrasion/deflation, suspension and deposition of small particles (i.e. dust, microorganisms and organic molecules), as well as long-distance transport of larger particulates by intermittent saltation events.

Relative to other needs of atmospheric science to understand the present martian climate in terms of global circulation, including extrapolations backwards and forward in time, the concerns for planetary protection also require greater depth of knowledge of meteorological processes at or near the surface of Mars. Adequately understanding the efficiencies, frequencies, and durations of entrainment processes requires measurements at the actual EZ location to set limits on acceptable contamination by subsequent human missions.

The following eight key KG (3A to 3H) represent the key KG in the natural transport of contamination on Mars.

3A. Measurements and models needed to determine atmospheric transport of contaminants

The highest priority in understanding the natural transport of contaminants on Mars is to understand how they are dispersed by the wind, as this drives the requirements for equipment design and operations in the field. The first step in overcoming current KG is to develop and apply atmospheric dispersion models to one or more high-priority candidate sites for a future human EZ. These preliminary dispersion models can be used to conduct sensitivity analyses with a variety of input conditions in order to determine both the degree and transport range of contamination. The modelling results will inform preliminary recommendations for reducing the risk of forward contamination during robotic and human operations within an EZ. Ultimately, the nature of boundary layer physics requires empirical measurement of the boundary layer. In particular, measurements of the atmospheric state (e.g., pressure, temperature, humidity) and direct measurement of atmospheric forcing (e.g., turbulent fluxes) are needed to adequately characterize the lower atmosphere (MEPAG, 2015). Moreover, the properties of the planetary boundary layer are seasonal, daily, and location-specific. *In-situ* measurements are critical to accurately describing natural transport of contaminants. Long-term high frequency meteorological measurements are needed at multiple fixed concurrent locations within the specific EZ to enable assimilation into atmospheric dispersion models. The minimum number of concurrent locations will be dictated by the size and topographical variations of the areas within an EZ where the humans would have their base and be exploring with their rovers. Ideally future surface assets should therefore incorporate the capability to make meteorological measurements at the caliber needed to begin building this dataset.

Refinement of the preliminary aeolian dispersion models will require *in situ* measurements at multiple key sites within an EZ of the processes controlling the local climate near the surface, particularly those relating to the entrainment, transport, and deposition of airborne particulates and saltating grains. Although details of requirements will require further study, especially after selection of an EZ, it is anticipated that at a minimum they will include measurement of the turbulent fluxes of heat and momentum; basic measurements of air temperature, pressure, humidity and wind velocity; and finally, the concentration and atmospheric column abundance, deposition and erosion rates, and physical and chemical properties of mobilized grains. The latter measurements can be used to establish the biocidal properties of the dust, sand, and soil, which will help determine microbial survival rates.

In addition to making these measurements by one or more precursor missions, it will be important that the weather stations be long-lived or replaced/reactivated once humans arrive and begin to operate within the EZ. Because martian weather patterns are not strictly repetitious year-to-year, these measurements must be made over one or more annual cycles, preferably during both intense dust storms and relatively dust-free, quiet conditions (i.e., over a broad range of L_s). Some of the *in situ* measurements listed above have not been made to date by any landed spacecraft on Mars (e.g., turbulent fluxes), but because they have been listed as high priority investigations by MEPAG (Rafkin *et al.*, 2009; Mischna *et al.*, 2009; MEPAG (2015), *Goal II, Objective A.1, Investigations 1, 2, and 3 (GII:*

A1.1-3); Goal II, Objective A.4, Investigation 1 (GII: A4.1)), they are of enormous interest to the scientific community and would undoubtedly result in substantial scientific advances beyond that required for planetary protection.

In addition to the *in situ* requirements listed above, a number of Earth-based investigations are also needed to ensure that dispersion models produce realistic results. For example, one important task is to integrate the killing kinetics of microorganisms into the dispersion models, with parametric control over assumptions on efficiency and probability of occurrence of protection by fomite formation (see KG 3C and 3E below). Further, wind tunnel testing of contaminant removal from spacecraft and equipment surfaces, as well as its subsequent transport, is critical to determining realistic source terms for the atmospheric dispersion models.

3B. Measurement and models needed to determine subsurface transport of contaminants

In general, subsurface transport is not expected to be a difficult issue for a Mars base. Even when the surface might be above the freezing point of water during the day, a spill of liquid would result in instant boiling until the residual liquid froze and then ultimately sublimated into the air. Any portion that sank beneath the surface would encounter sub-freezing temperatures at very shallow depths (a few mm to few cm, depending on location and season). Thus, horizontal transport beneath the surface would be kept highly localized. Although brine may form and depress the freezing point, such brines are highly viscous at these cold temperatures and would spread only very slowly, if at all. In addition, the temperatures are typically -45 to -55°C at depth, well below the minimum temperature for growth and reproduction by terrestrial life. It should be noted, however, that *in situ* data of the EZ subsurface properties will be required before any robust modelling of subsurface contamination transport capability can be accomplished.

3C. Effects of biocidal factors on survival, growth and adaptation of microorganisms on Mars

Numerous biocidal factors persist in the martian subsurface, surface, and aeolian environments. Some biocidal factors are ubiquitous on Mars (e.g., high UV irradiation and desiccation), while other factors might be spatially or temporally constrained (e.g., heavy metals or high salts in diverse soils). The top 7 most biocidal factors for inactivation of Earth microbes arriving on spacecraft were identified as follows: (1) solar UVC and UVB irradiation, (2) extreme desiccation (i.e., low a_w), (3) solar UV-induced volatile oxidants (e.g., O_2^-, O^-, H_2O_2, O_3), (4) high salt concentrations in some soils (e.g., $MgCl_2$, NaCl, $FeSO_4$, and $MgSO_4$), (5) acidic conditions in many soils, (6) solar particle events, and (7) low-pressure (2-12 mbar), In addition, the top 7 inhibitory conditions (i.e., that suppress growth but may not kill cells) were identified as : (i) anoxic CO_2-enriched atmosphere, (ii) low pressure, (iii) low surface temperatures (global average of -61^{o}C), (iv) high salt levels, (v) perchlorate salts, (vi) extremely low concentrations of nitrogen, phosphorus, and fixed carbon, and (vii) a paucity of identified redox couples for microbial metabolism (Cockell *et al.*, 2016; de Vera *et al.*, 2010; Rummel *et al.*, 2014; Schuerger *et al.*, 2012; 2013).

Of the parameters listed above, solar UV irradiation is clearly the most aggressive biocidal factor on the surface of Mars with inactivation times for > 6-log reductions for most Earth bacteria per sol on fully exposed spacecraft surfaces under equatorial conditions (Schuerger *et al.*, 2006). However, solar UV irradiation only works if the cells are fully exposed to the downwelling irradiation. As little as 0.5 mm of martian fines can attenuate > 5 orders of magnitude biocidal UV irradiation (Mancinelli and Klovstad, 2000).

Although there are numerous biocidal and inhibitory factors on Mars that will impact landed Earth microorganisms, there are few studies that have looked at synergistic effects of the parameters listed above on microbial survival, metabolism, growth, and adaptation (Nicholson *et al.*, 2010; Wadsworth and Cockell, 2017). Thus, a key Knowledge Gap is the development of multi-factorial experiments with combinations of biocidal or inhibitory factors under conditions relevant to Special Regions on Mars. For example, if cells are assumed to be fully protected against solar UV irradiation, can Earth bacteria metabolize trace amounts of *in situ* organics assuming the presence of transient conditions of high a_w in some sites?

Splinter Group 3 identified 10 Research and Technology Drivers (R&TD) relevant to the interactions of biocidal or inhibitory factors that will affect survival, growth, and adaptation of Earth microorganisms on Mars. The 10 R&TD priorities are as follows (not in priority):

1) What are the individual and combined effects of (for example) UV, desiccation, oxidants, regolith and aerosol geochemistry, low pressure, temperature, and gas composition on microbial survival?
2) What are the biocidal effects of (1) on the survival of bacteria, archaea, fungi, & other eukarya present on spacecraft?
3) What are the kill-curve kinetics of (1) & (2) relative to dosage and time?
4) What are the effects of direct, diffuse, and reflected UV fluence rates on microbial survival?
5) Do kill-curve kinetics change when spores are in the air column vs attached to aerosols vs on spacecraft surfaces?
6) Continue to characterize microbial diversity and microbial contamination on robotic and human spacecraft.
7) Develop a quantitative Mars microbial survival model(s) for robotic and human surface operations.
8) Repeat (1) to (5) for microbial growth and adaptation (as opposed to just survival) under conditions found in Special Regions.
9) Validate Mars microbial survival and growth models with flight experiments on Mars precursor missions with appropriate ground testing.
10) Do cultivable and non-cultivable microorganisms differ in their responses to the biocidal and inhibitory factors listed above under martian conditions?

3D. Determination of Acceptable Contamination Rates and Thresholds

There exists a notable lack of quantitative values regarding acceptable contamination rates for human exploration. Determining the threshold at which planetary protection constraints are violated in an 'open system' such as an exploration zone is required before a detailed analysis of human exploration scenarios can be performed. This KG will require input from all other sections to build a model of contamination evolution that can be used for defining the critical contamination threshold, and thus the acceptable contamination rates for exploration.

Specifically, quantitative information is urgently required regarding contamination rates and microbial contaminations in the following areas:

1) Landed equipment, habitats and laboratories in central EZ.
2) Crewed systems for mobility throughout the EZ.
3) Activities involving purposeful interactions with regolith (extraction of resources, buried nuclear power sources).

3E. Protection Mechanisms for Organisms on Mars

Although numerous biocidal and inhibitory factors operate in the subsurface, surface, and aeolian environments on Mars (see above), there are several conditions (e.g., dust loading on landers [Schuerger *et al.*, 2012], terrain shadowing of UV [Moores *et al.*, 2007], spacecraft geometries [La Duc *et al.*, 2014]) that will act to buffer or protect Earth microorganisms that are relevant to future human missions to Mars. Although the Splinter Group 3 did not develop a full and complete list of all plausible natural protection processes on Mars, the following five parameters were identified that require additional research:

1) Dust loading on spacecraft surfaces that act to enhance the shielding of Earth microorganisms from combinations of biocidal (e.g., UV irradiation, extreme desiccation) or inhibitory (e.g., low temperature and pressure).
2) Terrain and/or spacecraft shadowing may also enhance shielding of Earth microbes by decreasing UV irradiation and wind dispersal of microbial cells on spacecraft surfaces.
3) The formation, transport, and shielding efficiency of particulates released from habitats.
4) Structural architecture/site arrangement of landed elements may also yield protected niches around landed human structures that will protect Earth microorganisms from the harsh conditions on Mars (e.g., increased humidity under large structures).

5) Presence of biofilms around attached cells from Earth may offer additional protection from martian biocidal factors. Thus, characterization of the presence or absence of microbial biofilms on aggregate or individual cells on spacecraft must be performed.

3F. Degradation of Landed Materials by the Martian Environment

Compared to a wide range of environments on Earth, the martian environment is preservative from the standpoint of its extremely low humidity at all times except the deepest cold of night. However, without a substantial ozone layer compared to Earth, the ultraviolet radiation is not only more intense on Mars but extends to much shorter wavelengths (UV attenuated by CO_2 at 190 nm) (Cockell *et al.*, 2000; Patel *et al.*, 2003). In addition to the effects of direct UV, there are a host of photochemical products, especially various species of oxidants in the atmosphere and also in the global soil, which are apparently responsible for the degradation of the previously expected amounts of organics at the surface (Davila *et al.*, 2008; Yen *et al.*, 2000).

Although this extended UV and the gaseous and solid oxidants are generally sterilizing to all unprotected organisms, from microbes to megafauna, they may also be degradative to some spacecraft materials. This creates the potential to release trapped but viable organisms that otherwise would not be a potential threat to planetary protection.

Observations of Mars landers and rovers have not revealed obvious evidence of severe erosion due to photochemical effects, nor has there been obvious mechanical abrasion by saltating sand grains, with the only mechanical effects being due to interactions with rocks (e.g., MSL rover wheels) (Townsend *et al*., 2014). Thus, although this factor is potentially important, the current assessment is that conservative engineering practices seem to be effective in minimizing such damage. After more than 13 years of exposure on the martian surface, the Mars Exploration Rover (MER) Opportunity seems in excellent condition from the standpoint of exposed materials (Townsend *et al.*, 2014).

3G. Induced Environmental Conditions around Structure

The infrastructure created by a human-rated Mars base may incidentally create a Mars Special Region and thereby become a source of amplified contamination. This could be catastrophic for Planetary Protection if such a mini-Special Region were active for an extended period. Also, such an equipment-induced Special Regions could compromise any attempts at life detection, and give false positives concerning the biohazard potential of the environment.

If structure or mobile hardware elements inject thermal energy into the overburden sufficient to heat environmental ice up to or above the melting point of water, a Special Region may be created. This could occur whether by direct conduction or thermal radiation to a nearby surface. Given the reality that some terrestrial microbes will be released unavoidably from spacesuits and airlocks into the environment, such Special Regions could pose a serious long-term source of forward contamination of Mars.

This problem is one that must be subjected to analysis or to preventative measures by the engineering design of the base (e.g., thermal radiators oriented upward to minimize radiance impinging on the ground surface). The following five R&TD are suggested for future research:

1) Quantification of humidity diffusion rates into the subsurface.
2) Quantify diffusion rates and persistence of atmospheric oxidants into the soil beneath the habitat.
3) Model the thermal, moisture, and microbial transport out of habitats and other relevant surface assets into the covered terrain.
4) Measure outgassing of organic volatiles (e.g. methane, ethylene etc.) out of the habitats.
5) Vertical and horizontal subsurface transport modelling.

3H. Sensitivity of Non-Cultivable Species to Biocidal Factors

See Knowledge Gap 3C on biocidal factors. Experiments could be conducted in terrestrial laboratories by using aliquots from natural ecosystems, with assays before and after exposure to simulated martian insults (e.g., UV; oxidizing species). If there are unexpected survival rates for the non-cultivable components, there could be ramifications for planetary protection. However, the fact that these species have not been successfully cultured under favourable laboratory conditions indicates they have a strong dependence on other organisms which are more robust. Hence, these organisms may be even more susceptible to the rigors of an alien environment.

Furthermore, there are no existing requirements for the assay or control of such organisms in Planetary Protection protocols. In general, such organisms would comprise an interdependent colony of multiple organisms, whereas the contamination on spacecraft and in cleanrooms have generally been of individual heat-resistant organisms, as evidenced by the very low numbers of colony counts from swab samples of areas that are huge compared to the size of an individual organism or group of organisms.

Splinter group 3 overall priority ranking

The following assumptions are made for the definition of ranking/prioritization of KG 3A-3H above:

> '**High'** priority is defined as time critical to define requirements and design hardware.
>
> '**Medium'** priority is defined as not immediately critical to define requirements and design hardware but still required.

High priority research topics:

- What measurements and models are needed to determine atmospheric transport of mobilized mission-induced contaminants? (3A)
- How do interactions of biocidal factors affect microbial survival, growth, and evolution on Mars? (3C)
- There is a recognized lack of acceptable contamination generation rates or thresholds for: (3D)
  - Landing, equipment, and habitation sites.
  - Mobile crewed systems.
  - Subsurface contamination.
  - Extraction of resources (e.g. local water/ices and hydrated minerals).

Medium Priority research topics:

- What data and models are needed to determine the subsurface transport of mobilized mission-induced contaminants. (3B)
- What are the potential natural protection mechanisms on Mars (e.g. dust loading, biofilms, shadowing etc.)? (3E)
- How does the Mars environment (e.g. UV, oxidants, etc.) degrade landed materials and do the resultant products represent a contamination source (e.g. false positive indications of life)? (3E)
- Can the induced environmental conditions below or around landed structures create Special Regions (e.g. increased humidity, thermal perturbations)? (3G)
- Do cultivable species differ from non-cultivable species in relation to biocidal responses and transport models on Mars? (3H)

Relevance to the Moon and Asteroids as Destinations for addressing Knowledge Gaps

The following table summarizes the priority of each KG for human exploration of Mars, and associated relevance for the cases of the Moon and asteroids.

Knowledge Gap	Description	Mars	Moon	Asteroids
3A	Measurements and models needed to determine atmospheric transport of contaminants	High	Not relevant	Not relevant
3B	Measurement and models needed to determine subsurface transport of contaminants	Medium	Medium	Medium
3C	Effects of biocidal factors on survival, growth and adaptation of microorganisms	High	Medium	Medium
3D	Determination of acceptable contamination rates and thresholds	High	High	Medium
3E	Protection mechanisms for organisms	Medium	Medium	Medium
3F	Degradation of landed materials by the environment	Medium	Medium	Medium
3G	Induced environmental conditions around structure	Medium	Medium	Medium
3H	Sensitivity of non-cultivable species to biocidal factors	Medium	Medium	Low

Table 4: Priority of KG identified by splinter group 3 and relevance for different target bodies.

Post-Workshop Synthesis

The prioritized KG described in the different splinter groups have been evaluated by the workshop organisers after the workshop (tabulated in Appendix III). Based on this evaluation the workshop organisers identified four high priority **Knowledge Gap Areas** (broader in scope than the specific individual KG considered in the workshop splinter groups) and potential ways to address them. In this context, high priority Knowledge Gap Areas are defined as time critical to establish quantitative planetary protection requirements and to design hardware and operations in compliance with these requirements.

High priority Knowledge Gap Areas

1. Natural transport of terrestrial biological contamination on Mars
2. Status and evolution of microbiome on robotic and human flight systems
3. Synergistic biocidal effects of the martian environment on the survival and growth of spacecraft (robotic and human) associated microbiome
4. Acceptable level for biological and organic contamination release from human support systems

Actions to close the high priority Knowledge Gap Areas

- Measurements on the surface of Mars to acquire high frequency meteorological data over at least a full martian year at multiple fixed locations for each proposed EZ to develop, test and validate contamination transport models; these measurements need to include, at a minimum, turbulent fluxes of heat and momentum, basic measurements of air temperature, pressure, humidity and wind velocity, the dust concentration and atmospheric column abundance, deposition and erosion rates of dust
- Development of microbiota and microbiome monitoring capabilities and systematic measure the microbial diversity and its evolution over time for robotic Mars spacecrafts (ground based during hardware assembly, test and launch operations) and human spacecrafts (ground based during hardware assembly, test and launch operations and in-flight e.g., on the ISS or successor vehicles)
- Ground based measurements of the synergistic biocidal effects on the microbial survival and growth of spacecraft associated microorganisms.
- Measurements to characterise the release of biological and organic contamination from human support systems (e.g., EVA suit, air locks, habitat).

Conclusions

Not all places on Mars are equal in terms of providing the right conditions for microbial growth. As a response to this fact, the concept of “Special Regions” on Mars was introduced in the COSPAR Planetary Protection Policy in 2002. Robotic missions landing on Mars and not accessing a Mars Special Region only need to have basic biological contamination control measures in place. Missions accessing a Mars Special Region, however, need to follow much more stringent biological contamination control measures. In our present state of knowledge, this recognition of differential need of protection will likely continue into the human exploration era. Enabling human missions to Mars consistent with the planetary protection goals requires a more refined partitioning of the martian surface. The KG and associated actions to address them presented in the splinter groups and as synthesized by the workshop organisers are in line with previous findings going back to the Pingree Park workshop in 2001. From the synthesis of all the splinter group reports, we concluded that Knowledge Gap Area 1 “Natural transport of terrestrial biological contamination on Mars “is the highest priority because an informed partitioning of the martian surface and the establishment of quantitative planetary protection requirements are only possible based on increased understanding of the natural transport of biological contamination on Mars, requiring new measurements at Mars.

Looking ahead, one logical next step would be to ensure the high priority actions are reflected in the Global Exploration Roadmap of the ISECG, facilitating the type of measurements necessary to address the KG through flight opportunities in the frame of robotic missions to Mars and the ISS utilisation program. In particular robotic missions to Mars would cover two aspects at once: enabling scientific measurements in preparation for human missions to Mars in line with MEPAG goal IV (prepare for human exploration) and an additional opportunity for scientific investigations in line with MEPAG goal II (understanding the processes and history of climate on Mars).

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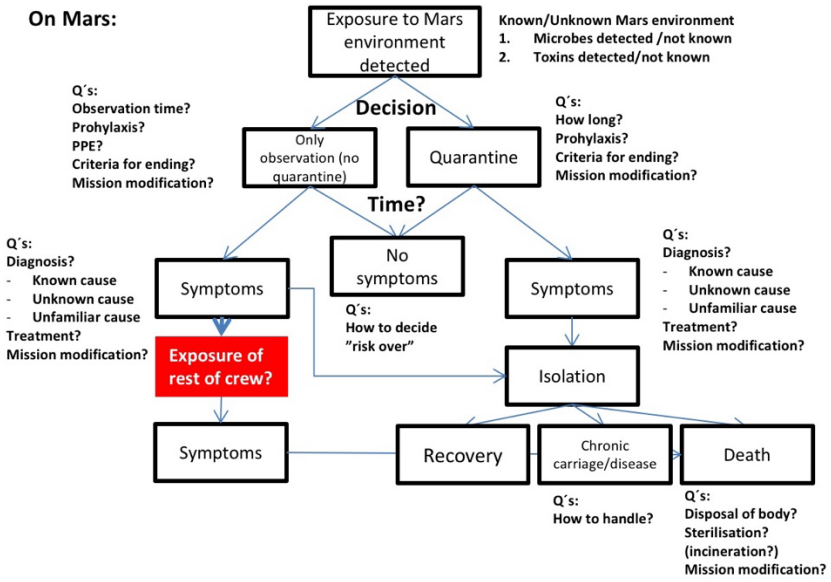
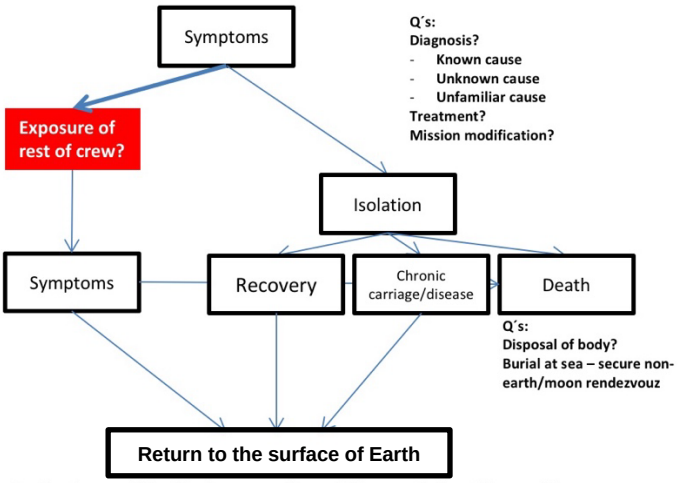
Appendix II : Decision tree from Group 1 describing the thought process for dealing with astronaut illness/suspected infection

The Splinter Group 1 on Microbial and Human Health Monitoring used the illustration below as a tool in the discussion to refine and prioritize knowledge gaps. It is captured here as an artefact of the workshop, which may be useful as an aid to future discussion.



Return flight: in case of infection or suspected infection – origin can be Earth OR Mars

- Isolate and treat sick member until recovered and noninfectious
- Consider non-recovery scenario (death/long-term incapacitation)



Return to Surface of Earth: Irrespective of if crew is healthy or ill or recovered - > quarantined or Isolated in pre-assigned biosecure facility

- time and procedures for different scenarios to be determined

Appendix III : Summary tables of identified KG priorities and destinations for all splinter groups

During the post-workshop analysis, two summary tables were compiled: First, Table III-1, which shows the KG prioritization and criticality for all splinter groups, together with potential targets destinations for addressing the knowledge gaps. However, since each of the splinter groups addressed their set tasks from different perspectives and with different foci, the targeting activity was done with different levels of rigor and fidelity, so some of the table entries are incomplete.

TABLE III-1: Overview of All Splinter Group Findings	Priority/Criticality			Possible Locations?				
GROUP 1: Microbial & Human Health Monitoring	Time/Mission			**Mars**	**Moon**	**asteroids**	**Earth**	**ISS**
1A. Microbial monitoring of the environment	H				M?	M?	H	
1B. Microbial monitoring of humans	H						H	H
1C. Mitigation of microbial growth in spacecraft systems	H				M/H?	M/H?	H	H?
1D. Operational guidelines for PP and crew health	L							
GROUP 2: Technology & Operations for Contamination Control	Time	Mission						
2A. Bioburden/transport/operations during short vs. long stays	M	M					M,M	
2B. Microbial/organic releases from humans and support systems	H	H					H,H	H
2C. Protocols for decontamination & verification procedures	M	H					M,H	H
2D. Design of quarantine facilities/methodologies at different mission phases	L	L					L,L	
2E. Martian environmental conditions variation over time with respect to growth of Earth microorganisms	L	H		L,H				
2F. Research needed to make ISRU & PP goals compatible	M	M		M,M			M,M	
2G. Acceptable contamination level from wastes left behind, including constraints on vented materials	L	L					L,L	
FORMER 2H. DELETED								
2I. Approaches to achieve 'Break the chain" requirements	L	L					L,L	
2J. Global distribution/depth of subsurface ice and evidence of extant life	H	H		H,H				
2K. Evolution of planetary protection requirements/goals from robotic precursor through to human missions & exploration zones	H	M		H,M			H,M	
GROUP 3: Natural Transport of Contamination on Mars	Time/Mission							
3A. Measurements/models needed to determine atmospheric transport of contaminants	H			H	X	X		
3B. Measurements/models for subsurface transport of contaminants	M			M	M	M		
3C. Effect of biocidal factors on survival factors on growth and adaptation of microorganisms	H			H	M	M		
3D. Determination of acceptable contamination rates & thresholds	H			H	H	M	H	
3E. Protection mechanisms for organisms on Mars	M			M	M	M		
3F. Degradation of landed materials by martian environment	M			M	M	M		
3G. Induced environmental conditions around structures	M			M	M	M		
3H. Sensitivity of non-culturable species to biocidal factors	M			M	M	L	M	

Second, using these overall data, an additional table was compiled listing only those items identified with High rankings (Table III-2). This overall ranking is the basis of the identification of the four highest priority knowledge gap

areas presented in the synthesis text, and is included here both as a record of the process, and to support future discussions.

TABLE III-2 High Priority Splinter Group Findings	Priority/Criticality			Possible Locations?				
GROUP 1: Microbial & Human Health Monitoring	Time/Mission			Mars	Moon	asteroids	Earth	ISS
1A. Microbial monitoring of the environment	H				M?	M?	H	
1B. Microbial monitoring of humans	H						H	H
1C. Mitigation of microbial growth in spacecraft systems	H				M/H?	M/H?	H	H?
GROUP 2: Technology & Operations for Contamination Control	Time	Mission						
2B. Microbial/organic releases from humans and support systems	H	H					H,H	H
2C. Protocols for decontamination & verification procedures	M	H					M,H	H
2E. Martian environmental conditions variation over time with respect to growth of Earth microorganisms	L	H		L,H				
2J. Global distribution/depth of subsurface ice and evidence of extant life	H	H		H,H				
2K. Evolution of planetary protection requirements/goals from robotic precursor through to human missions & exploration zones	H	M		H,M			H,M	
GROUP 3: Natural Transport of Contamination on Mars	Time/Mission							
3A. Measurements/models needed to determine atmospheric transport of contaminants	H			H	X	X		
3C. Effect of biocidal factors on survival factors on growth and adaptation of microorganisms	H			H	M	M		
3D. Determination of acceptable contamination rates & thresholds	H			H	H	M	H	