MICROBIAL MONITORING CHALLENGES AND NEEDS FOR MARS APPLICATIONS. M. C. Roman¹, C. M. Ott², V. A. Castro³, M. N. Birmele⁴, M. S. Roberts⁴, K. J. Venkateswaran⁵ and D. L. Jan⁵

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Introduction: The monitoring of microorganisms will be an important part of a mission to Mars. Microbial monitoring equipment will be needed to look for the presence of microorganisms on the planet, to confirm that planetary protection measures are working, to monitor the health of plants, bioreactors and humans living in a habitat and to monitor the performance of the life support systems that will keep them alive during their stay on Mars. Coordinating the different microbial monitoring needs during the early days of mission planning, can provide NASA with equipment that could meet more than one need while also providing complementary analysis options, which can enhance the research capabilities. The early coordination between the different NASA groups that will need microbial monitoring equipment on the surface of Mars, could also make the mission more affordable, as development of the needed equipment could be potentially cost shared.

Challenges and Needs: Humans have been exploring space for more than 50 years. For all those years, microorganisms have accompanied both manned and un-manned spacecraft and cargo. Microorganisms that are commonly found on Earth easily adapt to space environments or mutate to survive in very harsh conditions. Their presence in spacecraft and cargo has caused problems in areas like crew health, life support systems performance, and degradation of material in the cabin. Other sources of the microbial burden in a spacecraft include experimental animals, plants, supplies, food, and water. Humans that had contact with the spacecraft before and during the mission are the chief contributors (about 95% of recovered organisms) to the microbial populations in the cabin.. The sterilization of the spacecraft that will host humans in long duration missions would be a difficult and costly operation that will not provide a long-term solution to the microbial colonization of the vessels. As soon as a human is exposed to the spacecraft, microorganisms will start populating the new environment.

During early NASA missions the microbial population of spacecraft, cargo (including water/food) and crew was carefully monitored before and after all flights using ground analysis. To minimize the risk of contaminating Earth with microorganisms from the lunar surface, even the crew was quarantined after missions. The vehicle was disinfected before the launch, but microbial control in the cabin during the mission was minimal. When the Space Shuttle program started to take humans to space, the inside of the vehicle was carefully disinfected before each flight and the microbial population of the crew was once again investigated before and after the mission. Microbial control inside the Space Shuttle was improved, but areas like air filtration needed to be improved. Apollo and Space Shuttle missions were short duration missions, compared to the current missions to the International Space Station (ISS). Microbial monitoring of the short duration missions was accomplished by returning samples to the ground; no in-flight monitoring capabilities were required. As mission duration increases, disinfection of the crew cabin is more challenging because the spacecraft is not returned to the ground, like the ISS. The risk from the microbial load to hardware and crew also increases. Microbial monitoring aboard the ISS is limited to microbial enumerations. For microbial identification, samples have to be returned to the ground for analysis.

To ensure that pathogenic microorganisms are minimized to the extent possible in spaceflight, preventive measures are taken. For instance in the International Space Station, there are HEPA (or equivalent) filters in the air regeneration system to remove particulates and microorganisms, relative humidity is reduced, and materials are carefully selected. In addition, in-flight monitoring of the air, surfaces, and water are conducted, as well as routine housekeeping and inspections. Due to the potential for health impacts on the crew and for degradation of air, water supplies, and equipment, it is important to address fundamental issues surrounding the sources and risks of pathogenic microorganisms in spacecraft.

Mitigation of the microbial population risk to the crew, vehicle and life support systems involves several different strategies that will include controlling the microbial load (in numbers and diversity) and monitoring. Controlling the microbial population load includes careful disinfection of all surfaces and controlling the crew temperature and humidity to prevent condensation. Active and aggressive control of the microbial population is a very important risk mitigation tool for a long duration mission. To meet planetary protection obligations integrated system technologies to protect human life from alien microorganisms (should they exist) and to shield engineering systems from biocorrosion are required. In addition, assurance of compliance with evolving standards for planetary protection (both forward and backward contamination) relating to the human exploration of Mars is essential. Finally, a sound technical basis is needed to determine whether the inadvertent shedding of bio-contaminants from human explorers can be minimized to such a degree that the search for life can continue in an unobstructed, meaningful manner. Then there is the increased need to grow food for long duration humanassociated missions due to limited resupply options. Monitoring plants and crops, especially the wet rooting environment, for microbes is required to ensure a safe and palatable food source. To accomplish that goal, a real time integrated system is required to identify and characterize human associated pathogens that may be introduced into a plant growth system and become a potential cause of food-borne illness. Previous microbiological analyses of edible crops grown in a variety of plant growth chambers have shown that the numbers on some crops exceeds the current microbiological standards. A tool is needed to evaluate the effectiveness of post-harvest sanitizing methods to reduce microbial numbers including potential pathogens and monitor critical control points in the cultivation and food preparation processes.

Automated microbial monitoring can be used as part of a comprehensive risk mitigation strategy in long duration missions. It can provide the crew with a way of regularly characterizing the microbial population inside the cabin. During nominal conditions, a baseline database can be acquired and stored. When/if conditions deteriorate, the crew will be able to monitor the effectiveness of remediation activities or watch for the off-nominal condition to stabilize. Off-the-shelf automated microbial monitoring systems that provide semi-quantification and identification of the microbial population are currently available. This kind of analysis can provide the crew with a way to know what kind of microorganisms they are dealing with and help expedite the remediation process tailored to the species responsible for the problem. Although it appears that no technology from those currently available represents a "catch-all solution" to the needs of long term

spaceflights, several technologies offer significant advantages over the current practice.

At least some of the technologies available, when optimized for the special needs and conditions of spacecraft, such as challenges like; sample collection and preparation, partial gravity, cross contamination, disinfection of the systems between samples, calibration, expendables, biohazard waste generation, library of unknown organisms, can represent robust and costeffective means to assess the health of the crew and the environment. A key deliverable would be an integrated microbial monitoring system, validated in a terrestrial Mars analog environment and ready for deployment on a human mission. Such a system is essential for missions to comply with requirements to avoid harmful contamination and thereby facilitate human exploration. The proposed integrated microbial monitoring system will bolster confidence in, and lend support to, planetary protection efforts, hardware reliability, and sustained crew health. By forewarning human explorers of any significant fluctuations in microbial burden, the system allows the crew to take immediate actions to significantly diminish any threat to crew health, or deterioration of the habitation module resulting from bio-corrosion. In addition, the advancement of enumeration and identification capabilities would provide the ability to provide a daily assessment of systems and increase risk mitigation respectively.

In an effort to reformulate the Mars Exploration Program, NASA will need to evaluate microbial monitoring challenges and mitigation strategies. The high risk potential for microbial contamination and biofilm formation is a safety, crew health, planetary protection, food source, and hardware hazard with costly consequences. The first line of defense during long duration missions would be to develop microbial control methods and then focus on microbial monitoring as a mitigation strategy. Current microbial monitoring practices in spacecraft rely on relatively slow, manual, highly consumable processes which are then reliant upon earth analysis. The utilization of currently available hardware with advanced analytical options for spaceflight environmental monitoring will be crucial for furthering human exploration to Mars.