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Final Report: Forums on Next-Generation Microbiological Requirements for Space Flight

Human Research Program
Space Human Factors & Habitability
Advanced Environmental Health Project

Principal Investigator:
Duane L. Pierson, PhD[1]

Co-Investigators:
C. Mark Ott, PhD[1]
Victoria A. Castro[2]
Todd Elliott[2]
Cherie M. Oubre, PhD[2]

[1]*NASA Johnson Space Center, Houston, TX*
[2]*Wyle Science, Technology and Engineering Group, Houston, TX*

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Acronyms

CDC	Centers for Disease Control and Prevention
CFU	colony-forming unit
CTB	cargo transfer bag
DNA	deoxyribonucleic acid
EB	Enterobacteriaceae
EPA	Environmental Protection Agency
FGB	Functional Cargo Block
HAACP	Hazard Analysis & Critical Control Points
HRP	Human Research Program
ICMSF	International Commission on Microbiological Specifications for Foods
ISS	International Space Station
JSC	Johnson Space Center
LOC/LOM	loss of crew/loss of mission
MRSA	methicillin-resistant *Staphylococcus aureus*
NEA	near-Earth asteroid
NEO	near-Earth object
PCR	polymerase chain reaction
PWD	potable water dispenser
RNA	ribonucleic acid
UTMB	University of Texas Medical Branch
VZV	varicella-zoster virus
WRS	water recovery system

Executive Summary

Microbiological requirements that mitigate risk to the crew during space flight missions are periodically updated to reflect changes in the scientific understanding of microbial pathogenicity, our knowledge of the response of microorganisms to the space flight environment, effects of space flight upon human immunity, and advances in monitoring technology. Three expert panels held from 2011 through 2013 performed a review of microbiological requirements, based on new knowledge and available technologies. These panels focused on requirements associated with potable water, space flight food, and vehicle air and surfaces specifically described in *NASA Space Flight Human System Standard (NASA-STD-3001)*. The forum on Space Flight Foods was co-sponsored by the Space Food Systems Laboratory at the Johnson Space Center.

Evaluation of the recommendations from all three forums indicated 12 common themes, such as the need for appropriate microbiological training for individuals who would be developing or operating space flight systems and guidelines for current and future microbiological monitoring technology. The reoccurrence of these themes in multiple panels reinforced the importance of the consideration of these recommendations.

These forums provided the opportunity to formally update and document our knowledge base for space flight microbiological requirements. As this type of forum has not occurred in the past 20 years, the amount of data and documentation was extensive. To streamline future efforts, smaller forums should occur at closer intervals, such as every 5 years, to both decrease the information to be reviewed and enable more rapid implementation of new technology and scientific findings.

While implementation of these recommendations was outside of the scope of this study, the co-sponsorship of a forum with the Space Food Systems Laboratory accelerated the acceptance of the recommendations toward updated food monitoring requirements. In addition, recommendations concerning potable water monitoring have already been incorporated into a draft of a new set of requirements for microbiological water quality that is being used to help design advanced microbiological monitoring hardware for space flight. Many of the recommendations in this report addressed medical operations, environmental sciences, and engineering activities. As such, this report will be forwarded for consideration to Space and Clinical Operations Division, the Biomedical Research and Environmental Sciences Division, and the Engineering Directorate for their consideration.

Introduction

Crew health is associated with the microbial environment of space vehicles, and the future of human space flight will continue to include interaction of humans and microorganisms. Risks to crew health from these microorganisms must be mitigated. Space flight programs accomplish this by adhering to standards and requirements such as those found in *NASA Space Flight Human System Standard (NASA-STD-3001), Volume 1: Crew Health* and *Volume 2: Human Factors, Habitability, and Environmental Health*. Many of these requirements and standards are in the form of limits on microbial concentrations and diversity for potable water, food, and surfaces and air, which space vehicle components must pass before, during, and/or after space flight missions. Crewmembers are also held to medical microbiology standards that define pathogen screening and immunizations.

As space exploration progresses toward missions that could include lunar habitats, Lagrangian points, near-Earth objects (NEOs), and Mars, current strategies for mitigating microbiological risks to crew health and performance may not be acceptable. For example, current microbiological monitoring protocols use classic culture-based methods for the enumeration and identification of microbes that rely on large volumes of consumables with limited shelf lives, a specialized skill set, and bulky equipment items better suited for ground-based laboratories. While more suitable alternatives are available, switching to new advanced monitoring technologies is difficult, as use of culture-based methodology dictates the units for NASA's current acceptability limits for monitoring, which are defined using colony-forming units (CFUs), representing the growth of one microorganism at a single location on a solid nutrient medium. Thus, the use of next-generation monitoring technologies, such as DNA or RNA sequencing, is restricted as its measurements cannot easily be related to CFUs.

These monitoring constraints, as well as the considerable microbiological knowledge, or "lessons learned", that have become available over the life of the International Space Station (ISS), have led to the need to engage a panel of internal and external experts to review current requirements for mitigation of microbial contamination and to provide input that will be used in requirements definition for future vehicle design and mission operations. To define how these lessons learned and technological opportunities could improve NASA's current microbiological requirements, the Human Research Program sponsored a series of three forums comprised of intramural and extramural experts in microbiology, medicine, and engineering to provide recommendations for updates to requirements for current and future space flight missions. These experts included those from academia, industry such as BioMerieux and Ecolab, and government agencies including the Centers for Disease Control and Prevention (CDC), the U.S Army, and the Environmental Protection Agency (EPA). To focus these forums, the attendees were charged to assess current requirements associated with three different sample types, specifically: (1) potable water, (2) space flight foods, and (3) vehicle air and surfaces. The forum reviewing space flight foods was held in collaboration with the NASA Johnson Space Center (JSC) Space Food Systems Laboratory.

Forum Recommendations

The experts from all three forums provided 68 recommendations. Detail and discussion of these recommendations can be found in the individual forum reports in Appendices C, D, and E. These recommendations included opinions on current vehicle design and space flight operations, integration of current and near-term monitoring technologies, practical monitoring modifications, and specific recommendations for future research and collaborations.

Evaluation of all reviews from the three forums provided organizing themes leading to 12 distinguishable categories of related recommendations for guidance to the entirety of potable water, food, and surface and air microbiology. The 12 categories are listed below with supporting rationale and the recommendation identifier denoting the forum (W = water, F = food, and SA = surface and air), and the specific recommendations. Please note that three of the recommendations from the forum discussing space flight food suggested the elimination of specific testing and these recommendations were not included in the following list.

Group 1: NASA should manage the microbiological requirements for different design reference missions (ISS, asteroid, moon, Mars) separately.

Rationale: Different missions will possess different levels of microbial risk; for example, there is less microbial risk in a bag of sterile water for ISS than there is in water recycled from solid waste on a Mars habitat. Deep space missions may require more mitigation of microbial risk and more stringent requirements than a sortie mission to ISS in a space capsule.

Specific Surface and Air Recommendation:

1. NASA should separate the microbiological requirements for the ISS from those for long-duration, deep-space missions. (SA 15)

Group 2: NASA should develop testing criteria rationale and decision-making matrices for specifications and procedures involved with quality control and risk management of food production and testing.

Rationale: Items such as testing criteria rationale and decision-making matrices are critical to quality control and risk management of food production and testing.

The food microbiology experts recommended that rationale for testing criteria and a decision-making matrix be included in the requirement document entitled Microbiological Specification and Testing Procedure for Foods Which Are Not Commercially Sterile (SD-T-0251,), which defines the sampling and microbial testing of space foods. This should include critical control points.

Specific Food Recommendation:

1. A rationale should be provided for the testing criteria in SD-T-0251, and a decision-making matrix should be developed. (F1)

Group 3: NASA should develop strategies to mitigate risks to food systems caused by human error.

Rationale: Human error is a significant cause of hazards in systems requiring human interaction and NASA reviews and mitigates the risk of human error in space vehicles.

The food microbiology experts recommended mitigating human error risks, strategies – such as for astronaut food handlers – to reduce risks in future bioregenerative food systems, including contamination of the system during operations by human error.

Specific Food Recommendations:

1. A strategy to reduce risks to future bioregenerative food systems that are caused by human error should be developed. (F24)

Group 4: NASA should continue to mitigate the presence of obligate pathogens and opportunistic pathogens, including coliforms, other Enterobacteriaceae family organisms, yeasts, and molds. Special attention should be paid to *Salmonella, Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus, Aspergillus niger, Legionella pneumophila,* and norovirus.

Rationale: During the discussions, the experts at the three forums mentioned groups of concern and specific species of organisms. These microorganisms include bacteria, fungi, and viruses. There was not an objective of creating a list of microorganisms at the forums; however, it may be appropriate to develop watch lists or panels of indicators for microbial risk. Obligate and opportunistic pathogens are always a concern and will be included as additional watch list microorganisms.

Specific Food Recommendations:

1. Enterobacteriaceae (EB) testing should be performed in lieu of coliform testing. (F4)
2. *Salmonella* testing for all beverages, including cocoa, should be maintained, but the enrichment step should be modified. (F9)
3. All dried products, including any beverages (real juice, flavored drinks, milk, cocoa, etc.) should be tested for EB. (F10)
4. All items in the food category of nuts should be tested for *Salmonella*. (F12)
5. All baked goods with water activity (Aw) of 0.6 or more should be tested for yeasts and mold. (F14)
6. All baked goods should be tested for *Salmonella*. (F15)
7. Dried fruit should be tested for EB and *Salmonella*. (F17)
8. Rehydratable foods should be tested for EB and *Salmonella*, in addition to performing post-packaging aerobic plate counts and augmented environmental testing of the processing area, such as the freeze-drying room, for *Salmonella*. (F19)

Specific Surface and Air Recommendation:

9. NASA should be most concerned with the following microorganisms with respect to surface and air microbiology: *Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus, Aspergillus niger, Legionella pneumophila*, and *Norovirus*.(SA12)

Group 5: NASA should increase efforts to reach out to external partners, consider new technologies, use current culture modeling tools, and perform more research on known issues, such as immune systems, crew allergies, and bioaerosols.

Rationale: NASA currently collaborates, uses new technology and tools, and performs a wide range of research. The experts had specific recommendations to increase these efforts.

The Decadal Survey on Biological and Physical Sciences in Space, "Recapturing a Future for Space Exploration: Life and Physical Sciences Research for a New Era", released in 2011 suggests that "mechanisms of immune system changes during space flight" are the highest-priority research topic in animal and human biology for NASA. It also suggests that "aerosol deposition in the lungs of humans and animals in reduced gravity" is a highest-priority research topic in animal and human biology for NASA in the context of lunar and Mars dust. These are important topics for NASA and for understanding the microbiology of the space environment for crew health.

It should be noted that immune dysregulation and bioaerosols were considered key research gaps by the external experts; both were conclusions that the experts came to on their own without any discussion about the Decadal Survey.

Specific Food Recommendations:

1. The Space Food Systems Laboratory should reach out to spice and tea manufacturers to discuss the microbial load of their products.(F7)
2. New retort technologies for food preparation should be considered.(F29)
3. The use of microencapsulated distribution of probiotic organisms and other technologies should be investigated to improve shelf life and quality control. (F31)
4. Research in the rotating wall vessel should be expanded to include other organisms on the CDC's list of foodborne diseases, especially *Norovirus* and spore-forming bacteria. (F32)
5. Research on bioregenerative food systems should be expanded. (F33)
6. Collaborative research with major food producers should be encouraged for mutual benefit, such as understanding their processing and testing programs. F34)

Specific Surface and Air Recommendations:

7. NASA should study the anecdotal reports of allergies that are reported by the ISS crewmembers, because these allergies have unknown and unexplained origins and could affect mission and crew. (SA1)
8. NASA should study the impact of the deep-space environment on the immune system. (SA9)
9. NASA should sponsor studies into the behavior of bioaerosols, such as spores, droplets, and micronuclei, in microgravity. (SA10)

Group 6: NASA should employ a future microbial monitoring system with the following attributes:

- Use reagents with a long shelf life (years)
- Use diverse range of sample matrices (food, water, urine, blood)
- Be equivalent to technologies approved by microbiology standards organizations
- Be verifiable in ground laboratories
- May support a panel of indicators of unsafe water, food, air, or surfaces
- Be easy to use, rapid, and simple
- Possess the ability to measure viability

Rationale: Future monitoring systems were discussed by experts at each of the forums and the possibilities included a diverse selection from lateral flow technologies (i.e., dipsticks) to more advanced DNA detection systems. Each group of experts focused on a different aspect of a future

monitoring system and this list represents the sum of all attributes to date. The Microbiology Laboratory considers these attributes to be good guidance for the development of future microbial monitoring systems.

Specific Food Recommendations:

1. New technologies to be used for the enumeration and identification of microorganisms onboard long-duration missions should require reagents without a short shelf life, and technology development also should focus on sample preparation for a diverse range of sample matrices. (F27)
2. Regardless of the choice of new technology, the hardware and process should be equivalent to those approved by AOAC International and verifiable in the ground laboratory. (F28)

Specific Surface and Air Recommendation:

3. NASA should support the development of in-flight molecular testing to enable identification of microorganisms that would serve as indicators of an unsafe vehicle and detection of antibiotic resistance to support selection and use of the limited spectrum of onboard antibiotics. (SA13)

Specific Water Recommendations:

4. The microbiological monitoring hardware should be easy to use, provide results rapidly, and use technology that is as simple as possible. (W11)
5. The microbiological monitoring hardware should include a method of measuring the viability of the microbes present. (W12)

Group 7: NASA should perform risk assessments on systems with microbial risk and immune function should be a factor in microbial risk assessment.

Rationale: Risk assessment was a topic at each of the forums and risk assessments have been performed on potable water. There were two risk experts that supported the forums. The food microbiology experts recommended a formal risk assessment for future food systems, including the bioregenerative food system.

There is uncertainty in the behavior of the human immune system, especially in deep space missions. Immune dysregulation should be a key factor in future risk assessments. The past risk assessments that have been performed focused on source and organism, without consideration of the uncertainty of immune dysregulation.

Specific Food Recommendation:

1. Formal microbial risk assessments for the food systems, including on-orbit packaged foods and future bioregenerative food systems, should be performed to fully understand the overall risk. (F25)

Specific Surface and Air Recommendation:

2. NASA should include alterations in crewmembers' immune systems as a factor in its microbial risk assessment. (SA14)

Group 8: NASA should mitigate microbial risk by educating people who interact with microbial-risk containing systems in contamination control.

Rationale: Education would be for training the crew, engineers, designers, and others who do not have a specialty in microbiology or contamination control. There have been lessons learned in the

past in which education and training would have mitigated the occurrence of a system contamination. Education would also mitigate clinical microbial risk in the human system, in regard to hygiene and infection control.

Specific Food Recommendations:

1. Food from future bioregenerative food systems should be managed by designated, trained personnel on the mission. (F22)

Specific Water Recommendations:

2. The designers, engineers, and technicians who develop and service the water system and users of the system should be educated about hygiene and infection control. (W7)
3. Crewmembers should be educated about hygiene and infection control. (W17)

Group 9: NASA should increase the use of system design strategies to mitigate risk in microbial risk containing systems using strategies such as the following: parametric measurements, redundancy, biocides, maintenance, remediation, materials selection, and human factors engineering.

Rationale: The implementation of specific system design strategies can mitigate risks to a level that would reduce the frequency of monitoring significantly, saving crew time and effort, and cargo mass. This includes parametric measurements, such as temperature and flow rate, to measure system performance; and the use of biocides, not only in hardware but in the human system (disinfectant and antiseptics). NASA currently uses system design to mitigate microbial risk and the experts agreed that specific strategies would enhance this approach.

Specific Surface and Air Recommendations:

1. NASA should investigate newly developed disinfectants and antiseptics for the disinfection of surfaces and skin. (SA6)
2. NASA should use human factors engineering to enhance environmental disinfection capabilities. (SA7)
3. NASA should increase use of antimicrobials and nonporous materials as components of subsystems and as a materials selection strategy for reducing environmental microbial levels. (SA8)

Specific Water Recommendations:

4. The water system should be designed to minimize areas within the system's components that could allow bacterial growth. (W2)
5. The water system should include the use of a residual biocide. (W3)
6. The water system should include the use of dissimilar redundancy to prevent contamination of potable water. (W4)
7. The design of the water system should include features that provide system data to users that allow them to assess the performance of the system and the potability of the water it is dispensing. (W6)
8. The water system should have a remediation capability. (W8)
9. The water system should be easy to maintain. (W10)

Group 10: NASA should increase process strategies to mitigate risks in microbial risk containing systems, including the crew, with the following processes where appropriate: vaccination, screening and decolonization, quarantine, sterilization, pasteurization, disinfection, and use of probiotics.

Rationale: The implementation of specific process strategies can mitigate risks to a level that would reduce the frequency of monitoring significantly, saving crew time and effort, and cargo mass. This is in addition to many processes that NASA already uses, such as screening crewmembers for specific microorganisms or monitoring water systems for coliform bacteria.

Specific Food Recommendations:

1. All cook or kill steps should be validated. (F5)
2. Ingredients likely to have a high microbial load, such as spices, should be tested, even if they are in small amounts in the final product. (F6)
3. Only pasteurized versions of real-juice beverages should be used in the Space Food System. (F8)
4. A 2-log kill step should be used for nuts other than peanuts. (F11)
5. The sources of frozen fruits should be tracked. (F21)
6. The components of future bioregenerative food systems should be sterile or thoroughly disinfected to minimize pathogenic risk to the food supply. (F23)
7. The Exploration program should use a reverse quarantine of the crew, their families, and the critical personnel for an extended period before flight. (F26)
8. Probiotic foods, which have been demonstrated to inhibit infection by likely pathogens, should be provided to the crew throughout crew quarantine and all mission phases. (F30)

Specific Surface and Air Recommendations:

9. NASA should screen the crew to determine if any are asymptomatic carriers of pathogenic microorganisms, especially norovirus. (SA3)
10. NASA should develop an improved method of disinfecting Nomex® cargo bags for future missions. (SA5)

Specific Water Recommendations:

11. The water system should be sterilized immediately before flight. (W1)
12. The crewmembers should be screened for relevant obligate and opportunistic pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA); the causative agents of meningitis in adults, such as *Neisseria meningitidis* and *Streptococcus pneumoniae*; and *Streptococcus pyogenes* – the causative agent of Group A streptococcal infections. (W13)
13. The crewmembers should be vaccinated against *N. meningitidis, S. pneumoniae, S. pyogenes*, and other relevant opportunistic pathogens that are not currently in the immunization schedule. (W14)
14. The crewmembers should be decolonized so that they do not carry relevant opportunistic pathogens, such as *S. aureus* or the antibiotic-resistant strain MRSA. (W15)
15. The crewmembers should be processed through a rigorous quarantine program that uses hospital-developed isolation and infection-control regimens. (W16)

Group 11: NASA should have contingency plans for risks that occur, such as infectious disease in the crew and contamination of the systems and environment.

Rationale: The external experts agreed that it was highly probable that microbial hazards would occur and that NASA should be proactive and develop contingency plans to mitigate associated risks. These plans include controlling infection or contamination in humans and in microbial risk containing systems.

Specific Surface and Air Recommendations:

1. NASA should supply a broader range of antibiotics for future crews to use than the ones currently supplied in the medical kit. (SA2)
2. NASA should look for proper ways to treat shingles rashes on orbit. (SA4) *Post meeting note: Antivirals specific for herpes zoster are currently manifested.*

Specific Water Recommendations:

3. To minimize the risks associated with contamination of the potable water system, a remediation plan should be developed and validated.(W9)
4. The crewmembers should have other medications in addition to antibiotics available to treat infectious disease and to relieve symptoms of infection. (W18)
5. The crewmembers should use a proper infectious disease response plan. (W19)

Group 12: NASA should continue to use sampling to validate control of microbial risk in systems and environment. It should consider levels of confidence, standards from quality organizations, and frequency.

Rationale: NASA uses sampling as a method of monitoring microbial risk containing systems. The experts in Food and Surface and Air forums believed the sampling procedures needed improvement. In the Surface and Air forum, the experts did not think there was enough sampling to make judgments on the microbiology of the space vehicle environment at a high level of confidence. This discussion revealed that current monitoring of surface and air is used to validate contamination control methods, such as housekeeping.

Specific Food Recommendations:

1. The sampling procedure in Section 3 of SD-T-0251 should be updated with current methodologies used in the food industry, such as those used by the International Commission on Microbiological Specifications for Foods (ICMSF). (F2)
2. The environmental sampling plan currently in use should be augmented to add analysis of air samples from the food production areas. (F3)
3. Aerobic plate count testing for post-packaged rehydratable foods should continue. (F18)

Specific Surface and Air Recommendation:

4. Routine microbiological monitoring is appropriate as validation of contamination control protocols; however, if a greater level of confidence in the microbial risk is required for future missions, an increased number of samples and frequency of sampling must be implemented. (SA11)

Specific Water Recommendation:

5. The water system should be monitored before, during, and after flight. (W5)

Conclusion

Due to the limited knowledge about microbial responses to the space flight environment on human health and performance, microbiological requirements for early NASA space flight missions were extremely stringent. As our knowledge increased, microbiological requirements were modified to minimize the need for resources during space flight missions. Along these lines, the ISS has provided unparalleled insight into the impact of long-duration human space flight on the habitable environment. This increase in experience is complemented in parallel by recent advances in technology that hold the potential to provide better information on the crew environment using smaller, safer approaches. Three expert panels performed a review of microbiological requirements associated with potable water, space flight food, and vehicle air and surfaces, based on this new knowledge and available technologies.

Evaluation of the recommendations from all three forums indicated 12 common themes. The reoccurrence of these themes in multiple panels reinforced the importance of the consideration of these recommendations. For example, experts from the three panels stressed the need for appropriate microbiological training for individuals who would be developing or operating space flight systems. Likewise, multiple panels also agreed that the use of microbial monitoring, especially for the identification of potentially pathogenic microorganisms, was critical in minimizing risk to the crew. Interestingly, one additional common theme was the opinions of the panels on technological advances and how they impacted future ground-based and in-flight monitoring.

While implementation of these recommendations was outside of the scope of this study, the co-sponsorship of a forum with the Space Food Systems Laboratory accelerated the acceptance of the recommendations toward updated food monitoring requirements. In addition, recommendations concerning potable water monitoring have already been incorporated into a draft of a new set of requirements for microbiological water quality that are being used to help design advanced microbiological monitoring hardware for space flight. Many of the recommendations in this report addressed medical operations, environmental sciences, and engineering activities. As such, this report will be forwarded for consideration to Space and Clinical Operations Division, the Biomedical Research and Environmental Sciences Division, and the Engineering Directorate for their consideration.

These forums provided the opportunity to formally update and document our knowledge base for space flight microbiological requirements. As this type of forum has not occurred in the past 20 years, the amount of data and documentation was extensive. To streamline future efforts, smaller forums should occur at closer intervals, such as every 5 years, to both decrease the information to be reviewed and enable implementation of new technology and scientific findings.

Appendices

Five appendices are included in this final report to provide more detail on the recommendations.

Appendix A: Recommendations Matrix: This is a table with the original recommendations from the microbiology experts as reported in the interim reports for water, food, and surface and air forums. Each recommendation has a unique identifier based on which forum and which order the recommendation appears in the interim report; for example, the first water recommendation is W1. Also noted is how the recommendation fits in the specific aims of the original project plan. The specific aims are briefly listed here:

- *Aim 1*: Remove, optimize, or add requirements for vehicle design and mission operations
- *Aim 2*: Assess next-generation monitoring technologies and technology gaps
- *Aim 3*: Describe monitoring requirements for immediate or future development
- *Research and Collaborations*: These recommendations address collaborations that may be useful or research gaps that would provide useful information. These were not a part of the specific aims, but still provide valuable consideration.

Appendix B: Recommendations Group Matrix: This is a table of the summary recommendation groups, each with a unique identifier and notation of which recommendation is included in each group.
The following appendices are the interim reports describing the proceedings of each of the forums. These interim reports were delivered to the subject matter experts to give them an opportunity to comment. Specific comments to the body of these reports are included in footnotes in these appendices where appropriate. These comments provide very useful detail about the specific recommendation and they should be reviewed before implementing a recommendation.

Appendix C: Interim Report: Recommendations from the Forum on the Next Generation Microbiological Water Requirements for Space Flight

Appendix D: Interim Report: Recommendations from the Forum on Next Generation Microbiological Food Requirements for Space Flight

Appendix E: Interim Report: Recommendations from the Forum on Next-Generation Microbiological Surface and Air Requirements for Space Flight

Appendix A – Recommendations Matrix

ID	Forum	Recommendation	Specific Aims addressed
W1	Water	The water system should be sterilized immediately before flight.	1
W2	Water	The water system should be designed to minimize areas within the system's components that could allow bacterial growth.	1
W3	Water	The water system should include the use of a residual biocide.	1
W4	Water	The water system should include the use of dissimilar redundancy to prevent contamination of potable water.	1
W5	Water	The water system should be monitored before, during, and after flight.	3
W6	Water	The design of the water system should include features that provide system data to users that allow them to assess the performance of the system and the potability of the water it is dispensing.	1, 3
W7	Water	The designers, engineers, and technicians who develop and service the water system and users of the system should be educated about hygiene and infection control.	1
W8	Water	The water system should have a remediation capability.	1
W9	Water	In the event of the need for the water system to be remediated, the crewmembers should have a remediation plan available.	1
W10	Water	The water system should be easy to maintain.	1
W11	Water	The microbiological monitoring hardware should be easy to use, provide results rapidly, and use technology that is as simple as possible.	2, 3
W12	Water	The microbiological monitoring hardware should include a method of measuring the viability of the microbes present.	2, 3
W13	Water	The crewmembers should be screened for relevant obligate and opportunistic pathogens, such as methicillin-resistant *S. aureus* (MRSA); the causative agents of meningitis in adults, such as *Neisseria meningitidis* and *S. pneumoniae*; and *S. pyogenes*, the causative agent of Group A streptococcal infections.	3
W14	Water	The crewmembers should be vaccinated against *N. meningitidis*, *S. pneumoniae*, *S. pyogenes*, and other relevant opportunistic pathogens that are not currently in the immunization schedule.	1
W15	Water	The crewmembers should be decolonized so that they do not carry relevant opportunistic pathogens such as *S. aureus* or the antibiotic-resistant strain, MRSA.	1
W16	Water	The crewmembers should be processed through a rigorous quarantine program that uses hospital-developed isolation and infection-control regimens.	1
W17	Water	Crewmembers should be educated about hygiene and infection control.	1
W18	Water	The crewmembers should have other medication in addition to antibiotics available to them to treat infectious disease and to control symptoms of infection.	1
W19	Water	The crewmembers should use a proper infectious disease response plan.	1
F1	Food	A rationale should be provided for the testing criteria in SD-T-0251, and a decision-making matrix should be developed.	1

ID	Forum	Recommendation	Specific Aims addressed
F2	Food	The sampling procedure in Section 3 of SD-T-0251 should be updated with current methodologies used in the food industry, such as those used by the International Commission on Microbiological Specifications for Foods (ICMSF).	1
F3	Food	The environmental sampling plan currently in use should be augmented to add analysis of air samples from the food production areas.	1
F4	Food	Enterobacteriaceae (EB) testing should be performed in lieu of coliform testing.	1, 3
F5	Food	All cook or kill steps should be validated.	1
F6	Food	Ingredients likely to have a high microbial load, such as spices, should be tested, even if they are in small amounts in the final product.	1, 3
F7	Food	The Space Food Systems Laboratory should reach out to spice and tea manufacturers to discuss the microbial load of their products.	R&C
F8	Food	Only pasteurized versions of real-juice beverages should be used in the Space Food System.	1
F9	Food	*Salmonella* testing for all beverages, including cocoa, should be maintained, but the enrichment step should be modified.	1, 3
F10	Food	All dried products, including any beverages (real juice, flavored drinks, milk, cocoa, etc.) should be tested for EB.	1, 3
F11	Food	A 2-log kill step should be used for nuts other than peanuts.	1
F12	Food	All items in the food category of nuts should be tested for *Salmonella*.	1, 3
F13	Food	Testing of nuts for yeast and mold should be eliminated.	1
F14	Food	All baked goods with Aw of 0.6 or more should be tested for yeasts and mold.	1, 3
F15	Food	All baked goods should be tested for *Salmonella*.	1, 3
F16	Food	Yeast and mold testing for candy should be eliminated.	1
F17	Food	Dried fruit should be tested for EB and *Salmonella*.	1, 3
F18	Food	Aerobic plate count testing for post-packaged rehydratable foods should continue.	1, 3
F19	Food	Rehydratable foods should be tested for EB and *Salmonella*, in addition to performing post-packaging aerobic plate counts and augmented environmental testing of the processing area, such as the freeze-drying room, for *Salmonella*.	1, 3
F20	Food	Testing of Staphylococcus enterotoxin in rehydratable foods should be eliminated.	1
F21	Food	The sources of frozen fruits should be tracked.	1

ID	Forum	Recommendation	Specific Aims addressed
F22	Food	Food from future bioregenerative food systems should be managed by designated, trained personnel on the mission.	1
F23	Food	The components of future bioregenerative food systems should be sterile or thoroughly disinfected to minimize pathogenic risk to the food supply.	1, 3
F24	Food	A strategy to reduce risks to future bioregenerative food systems that are caused by human error should be developed.	1
F25	Food	Formal microbial risk assessments for the food systems, including on-orbit packaged foods and future bioregenerative food systems, should be performed to fully understand the overall risk.	1
F26	Food	The Exploration program should use a reverse quarantine of the crew, their families, and the critical personnel for a couple of months before flight.	1
F27	Food	New technologies to be used for the enumeration and identification of microorganisms onboard long-duration missions should require reagents without a short shelf life, and technology development also should focus on sample preparation for a diverse range of sample matrices.	2, 3
F28	Food	Regardless of the choice of new technology, the hardware and process should be equivalent to those approved by AOAC International and verifiable in the ground laboratory.	2, 3
F29	Food	New retort technologies for food preparation should be considered.	R&C
F30	Food	Probiotic foods, which have been demonstrated to inhibit infection by likely pathogens, should be provided to the crew throughout crew quarantine and all mission phases.	1
F31	Food	The use of microencapsulated distribution of probiotic organisms and other technologies should be investigated to improve shelf life and quality control.	R&C
F32	Food	Research in the rotating wall vessel should be expanded to include other organisms on the CDC's list of foodborne diseases, especially norovirus and spore-forming bacteria.	R&C
F33	Food	Research on bioregenerative food systems should be expanded.	R&C
F34	Food	Collaborative research with major food producers should be encouraged for mutual benefit, such as understanding their processing and testing programs.	R&C
SA1	Surface & Air	NASA should study the anecdotal reports of allergies that are reported by the ISS crewmembers, because these allergies have unknown and unexplained origins and could affect mission and crew.	R&C
SA2	Surface & Air	NASA should supply a broader range of antibiotics for future crews to use than the ones currently supplied in the medical kit.	1
SA3	Surface & Air	NASA should screen the crew to determine if any are asymptomatic carriers of pathogenic microorganisms, especially norovirus.	3
SA4	Surface & Air	NASA should look for proper ways to treat shingles rashes on orbit.	R&C
SA5	Surface & Air	NASA should develop an improved method of disinfecting Nomex® cargo bags for future missions.	R&C

ID	Forum	Recommendation	Specific Aims addressed
SA6	Surface & Air	NASA should investigate newly developed disinfectants and antiseptics for the disinfection of surfaces and skin.	R&C
SA7	Surface & Air	NASA should use human factors engineering to enhance environmental disinfection capabilities.	1
SA8	Surface & Air	NASA should increase use of antimicrobials and nonporous materials as components of subsystems and as a materials selection strategy for reducing environmental microbial levels.	1
SA9	Surface & Air	NASA should study the impact of the deep-space environment on the immune system.	R&C
SA10	Surface & Air	NASA should sponsor studies into the behavior of bioaerosols, such as spores, droplets, and micronuclei in microgravity.	R&C
SA11	Surface & Air	Routine microbiological monitoring is appropriate as validation of contamination control protocols; however, if a greater level of confidence in the microbial risk is required for future missions, an increased number of samples and frequency of sampling would need to be implemented.	3
SA12	Surface & Air	NASA should be most concerned with the following microorganisms with respect to surface and air microbiology: *Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus, Aspergillus niger, Legionella pneumophila*, and norovirus.	3
SA13	Surface & Air	NASA should support the development of in-flight molecular testing to identify a panel of microorganisms that would serve as indicators of an unsafe vehicle and for detecting antibiotic resistance, to inform selection of a limited spectrum of onboard antibiotics.	2, 3
SA14	Surface & Air	NASA should include alterations in crewmembers' immune systems as a factor in its microbial risk assessment.	R&C
SA15	Surface & Air	NASA should separate the microbiological requirements for the ISS from those for long-duration, deep-space missions.	1

W = Water, F = Food, SA = Surface and Air, R&C = Research and Collaborations

Appendix B – Recommendation Group Matrix

ID	Group	Supporting Recommendations
Group 1	NASA should manage the microbiological requirements for different design reference missions (ISS, asteroid, moon, Mars) separately.	SA15
Group 2	NASA should develop testing criteria rationale and decision-making matrices for quality control and risk management	F1
Group 3	NASA should develop strategies to mitigate risks to microbial risk containing systems caused by human error.	F24
Group 4	NASA should continue to mitigate the presence of obligate pathogens and opportunistic pathogens, including coliforms, other Enterobacteriaceae family organisms, yeasts, and molds. Special attention should be paid to *Salmonella*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus terreus*, *Aspergillus niger*, *Legionella pneumophila*, and norovirus.	F4, F9, F10, F12, F14, F15, F17, F19, SA12
Group 5	NASA should increase efforts to reach out to external partners, consider new technologies, use current culture modeling tools, and expand research on known issues, such as immune systems, crew allergies, and bioaerosols.	F7, F29, F31, F32, F33, F34, SA1, SA9, SA10
Group 6	NASA should employ a future microbial monitoring system with the following attributes: • Use reagents with long shelf life (years) • Use diverse range of sample matrices (food, water, urine, blood) • Be equivalent to technologies approved by microbiology standards organizations • Be verifiable in ground laboratories • May support a panel of indicators of unsafe water, food, air, or surfaces • Be easy to use, rapid, and simple • Possess the ability to measure viability	F27, F28, SA13, W11, W12
Group 7	NASA should perform risk assessments on systems with microbial risk, and immune function should be a factor in microbial risk assessment.	F25, SA14
Group 8	NASA should mitigate microbial risk by educating people who interact with microbial risk containing systems in contamination control.	F22, W7, W17
Group 9	NASA should increase the use of system design strategies to mitigate risk in microbial risk containing systems using strategies such as the following where appropriate: parametric measurements, redundancy, biocides, maintenance, remediation, materials selection, and human factors engineering.	SA8, SA7, SA6, W6, W4, W3, W2, W10, W8
Group 10	NASA should increase process strategies to mitigate risks in microbial risk containing systems, including the crew, with the following processes where appropriate: vaccination, screening and decolonization, quarantine, sterilization/ pasteurization/ disinfection, and use of probiotics.	F5, F6, F8, F11, F21, F23, F26, F30, SA3, SA5, W1, W13, W14, W15, W16
Group 11	NASA should have contingency plans for risks that occur, such as infectious disease in the crew and contamination of the systems and environment.	SA2, SA4, W9, W18, W19,
Group 12	NASA should continue to use sampling to validate control of microbial risk in systems and environment. It should consider levels of confidence, standards from quality organizations, and frequency.	F2, F3, F18, SA11, W5

Appendix C –Interim Report: Recommendations from the Forum on the Next Generation Microbiological Water Requirements for Space Flight

1. Executive Summary

In July 2011, the Johnson Space Center (JSC) Microbiology Laboratory convened a panel of water microbiology experts (the Forum on the Next Generation of Microbiological Water Requirements for Space Flight or "Forum") to develop and recommend a new set of requirements for vehicle design and mission operations, including microbiological monitoring, to be based on "lessons learned" and new technology. Discussions at the Forum were focused on requirements contained in *NASA Standard 3001, Volume 2: Human Factors, Habitability, and Environmental Health*, and other engineering design standards and requirements for hardware and vehicle design that are derived from *Volume 2*; however, the content of many recommendations would also fall under *NASA Standard 3001, Volume 1: Crew Health*. All of the recommendations will be forwarded to appropriate individuals in the Space and Clinical Operations Division of the Human Health and Performance Directorate and in the Engineering Directorate for their consideration. Recommendations from this Forum also focused on microbial monitoring technology and associated monitoring strategies. ***This interim report only documents the discussions at the Forum and may be modified by discussions at future forums before the final report is submitted in September 2013.***

Key findings from this Forum include these:

- Preventive design and operational strategies should be stringent and should be the focus of most of NASA's resources, as they are cost-effective and can be attained with conventional technology. Hardware for microbial monitoring should be simple and must be able to measure the viability of microorganisms in a sample. Multiple monitoring technologies can be used as long as the microorganisms being identified can also be confirmed as viable.
- One key source of infectious agents will always be the crew, and appropriate preventive measures should be taken before flight.
- Evidence showing alterations in the immune function of crewmembers and in microbial virulence complicates risk assessments and creates the need for very conservative requirements.
- Water systems should be thoroughly disinfected (sterilized if possible) before flight and retain a residual biocide throughout the mission.

2. Introduction

As humans begin to explore farther into space, microorganisms will travel with them. To decrease the incidence of crew health risks associated with the provision of potable water, requirements are developed and imposed on vehicle design and operations. The effectiveness of these requirements is traditionally evaluated by microbiological monitoring, which can occur before flight, during flight, and/or after flight, depending on the architecture of the space flight mission. Current requirements associated with microbiological monitoring are predicated on a culture-based methodology in which microorganisms are grown on a semi-solid growth medium and colonies are enumerated. Subsequent identification of the organisms requires specialized labor and large equipment items, and historically, identification has been performed on Earth.

As NASA extends human exploration beyond low-Earth orbit, adequate resources may not be available to maintain current monitoring strategies. One such limitation is NASA's current culture-based monitoring methodology that relies on large volumes of consumables with limited shelf lives. The use of culture-based monitoring methodology also has led to NASA's current monitoring requirements, which are limited as they use "measurement criteria" in the form of colony-forming units (CFUs) –representing the growth of one microorganism at a single location on an agar medium – per a given volume. Thus, the use of alternative monitoring technologies, such as DNA-based technology, is restricted, as its measurements cannot easily be related to CFUs.

These limitations, combined with the extensive amount of new knowledge that has become available over the life of the International Space Station (ISS), have led to the need for a panel of internal and external experts to review current requirements for mitigation of microbial contamination and to provide recommendations for updated requirements for vehicle design and mission operations.

External expert participants in this Forum:

- Glen Mayhall, MD, Infectious Disease Department, University of Texas Medical Branch (UTMB)
- Kristina Mena, MSPH, PhD, School of Public Health, University of Texas Health Science Center
- Robert Quick, MD, Enteric Diseases Branch, Centers for Disease Control and Prevention (CDC)
- Steven C. Richards, PhD, PE, BCEE, Col (R), US Army, Supervisory Environmental Engineer, U.S. Army Institute of Public Health (*Dr. Richards was not available to participate in the forum, but he kindly provided comments to the interim report on potable water).*
- Brandolyn Thran, PhD, Army Institute of Public Health

Additional expert participants in this Forum:

- Monserrate Roman, Marshall Space Flight Center
- Karen Pickering, PhD, Exploration Life Support Water Recovery Systems, JSC
- Robert Haddon, MD, Space Medicine Division, JSC
- Victoria Castro, Microbiology Laboratory, JSC
- Mark Ott, PhD, Microbiology Laboratory, JSC
- Duane Pierson, PhD, Microbiology Laboratory, JSC

Before making the recommendations, the Forum participants engaged in substantial discussion about the microbiological aspects of space flight. Ten presentations were given to the panel, and a summary is included in Section 4. The Microbiology Requirements Framework, a communication tool, was developed to be used by the Forum and is explained in Section 5. The recommendations are in Section 6. A summary of the discussions that led to the development of the recommendations is in Section 7.

3. Project Goals

The purpose of the Forum was to develop and recommend a new set of requirements for vehicle design and mission operations, including microbiological monitoring, based on "lessons learned" and new technology. This task is a subset of the Human Research Program (HRP) direct research study "Technology and Requirements Development Microorganism Identification," which includes the development of new microbiology requirements for potable water, air and surfaces, and space flight food.

The specific aims of the study are the following:

> Aim 1 – To use historical data to assess optimal requirements for vehicle design and mission operations. This "lessons learned" approach will be incorporated into a microbial risk assessment approach to recommend removal of unnecessary requirements, optimization of current requirements, and implementation of new requirements.
>
> Aim 2 – To assess current and near-term technologies for application to next-generation monitoring requirements. This aim will also focus on defining gaps between optimal requirements and available technologies and flight resources.
>
> Aim 3 – To define practical monitoring requirements for (a) immediate development of next-generation ground operations and flight hardware and (b) long-term goals for future requirements, if different from those delivered in (a).

To address Aim 1, the panel listened to presentations about the history of the water system on the ISS, the occurrence of microbiological events, the medical occurrence of infectious disease, lessons learned, and current requirements and operations. They also participated in tours to help them understand the water systems, how they are developed, how the water is carried to the ISS, and how it is processed. This included tours of the water system lab in Building 7 and the Mockup Facility in Building 9.

To address Aim 2, the panel discussed several technologies and requirements. This was not at the level of requirements definition, which is a specific systems engineering process. Instead, the panel made recommendations for the current requirements and how they could be improved for future missions. The technologies and requirements were discussed together because, in microbiology, the requirements usually dictate the technology. For example, the measurement of CFUs is used to determine whether the system is meeting the current microbiology requirements. This dictates that the technology to be used will be heterotrophic plate counts. Several technologies were discussed, including DNA sequencing, mass spectrometry, Raman spectroscopy, flow cytometry, heterotrophic plate count, and biochemical identification methods.

Aim 3 is the definition of practical monitoring requirements. ***These recommendations are the beginning of the process to define those requirements***. This report is the first of three interim reports describing the findings of each of the forums, on potable water, air and surfaces, and food. Ultimately, the unit of measurement for microbiological requirements needs to be addressed to define the requirements. The panel unanimously agreed that viability is a critical measurement for making decisions on water quality in terms of microbiology, and currently that measurement is in CFUs. In the microbiology laboratory, heterotrophic plate count is the gold standard used to determine CFU in water.

In summary, highly advanced technologies do not seem necessary to measure the operational environments of water systems either on the ISS or on exploration missions to a near-Earth object (NEO) or Mars. The smarter method is to prevent pathogens from entering the water system by proactively removing them from the system, the vehicle, and the crew on the ground, engineering the system to

provide performance feedback with respect to critical processing points, and providing the resources to remediate and monitor the system when the crew needs them.

A reasonable amount of uncertainty exists with respect to questions about host-microbe interactions, such as whether immune dysregulation is occurring or if bacteria do become more virulent in microgravity. As data are gathered and these questions are answered, microbiological requirements will have to be re-evaluated to ensure crew health and safety.

4. Summary of Presentations

Current Water Recovery Systems

Dr. Pickering gave a presentation describing the current potable water Environmental Control and Life Support System on the ISS, including water sources from visiting vehicles (including Space Shuttle orbiters, Progress vehicles, the H-II Transfer Vehicle, and the automated transfer vehicle), and water storage containers like the contingency water containers and contingency water containers for iodinated water). She presented an overview of the current U.S. Water Recovery System, including the subsystems used to recover water from humidity condensate and urine. The main points of her presentation were that (1) microbial requirements support both crew health and system operation needs, (2) multiple microbial mitigation techniques are needed for effective control, and (3) remediation techniques should be prepositioned for microbial contamination events.

ISS Medical Operations Requirements Document Microbiology Requirements and Verification

Ms. Smith from the JSC Microbiology Laboratory described the current microbiology requirements in the ISS Medical Operations Requirements Document. She described the current monitoring methodologies that are used to collect environmental data on air, surface, and potable water before, during, and/or after flight. She also described anomalies that have occurred during the ISS Program, such as elevated bacterial levels in contingency water containers and the potable water dispenser (PWD). She also discussed contingency plans that were exercised to resolve off-nominal situations and lessons learned from these experiences. Her data indicated that the microorganisms isolated from the water systems on the ISS were common environmental flora, including *Sphingomonas, Ralstonia, Cupriavidus, Methylobacterium*, and *Burkholderia*. She also noted that to date, no coliform bacteria, indicators of fecal contamination, have been found on the ISS.

Infectious Considerations in Space Flight

Dr. Haddon provided the perspective of a NASA flight surgeon in addressing infectious disease diagnosis and mitigation. He described two non-attributable space flight cases of infectious disease in addition to current infection control strategies. His main point was that infectious processes and their management during space flight missions will be for the most part similar to the processes and treatment on Earth; however, certain specific variables associated with space flight may affect the way diseases are treated. The current posture for medical emergencies on the ISS relies on the ability to return the crew to Earth for care within 18 to 24 hours, which will not be an option on exploration missions.

> *During the discussion of* Staphylococcus aureus *being present on the ISS, the panel asked if other potentially infectious normal flora such as meningococcus, pneumococcus, and Group A streptococcus were a concern. Dr. Haddon said that they are and that the program cannot guarantee that those organisms cannot get onto a space vehicle such as the ISS or an exploration spacecraft, and that the agency will have to plan on that situation.*
>
> *During the discussion of varicella-zoster virus (VZV), the causative agent of chickenpox and shingles, Dr. Haddon described how there is increased shedding of VZV in astronauts, and there is debate on whether or not a shingles case has occurred during flight. The astronauts are vaccinated against*

VZV. One of the greatest dangers of shingles during flight is not the shingles rash itself, but the chance that virus shedding from the vesicles could be transferred to spacecraft air by means of skin flakes or aerosols that do not settle in microgravity, and be aspirated by an astronaut without immunity to VZV, resulting in varicella pneumonia, a condition with a high mortality rate.

What Are the Chances?

Dr. Mena presented the results of her quantitative microbial risk assessment model of the ISS potable water system, which she developed over the past 4 years. This paradigm, composed of evaluations of crew exposure, hazard identification and characterization, and expected dose-response, is used to predict public health impact of the system. One novel finding for the ISS water system is that characterization of the microbe, rather than the quantity of microorganisms, drives the health risk. In other words, whether a pathogen is present is more important than the bacterial concentration. This result differs from the approach that many municipal water systems use in which the exposure and amount of consumption drive the risk.

Exploration Operational Concepts

Dr. Kundrot, the Deputy Program Scientist for the HRP, presented an overview of the flexible path, described by the Augustine Commission in the report *Seeking a Human Space flight Program Worthy of a Great Nation* in 2009. The flexible path includes a multi-option plan to go to Lagrange points, planetary orbits, and landings, including rendezvous with NEOs or asteroids. He also described operational concepts from the Human Exploration Framework Team for missions to NEOs and Mars. A mission to an NEO would take about 1 year, and the crew could abort the mission; however, it could take them at least 1 month to start their return to Earth. The habitat with the crew's consumables, such as food and water, would be launched almost a year before the crew arrives to begin the voyage and those items would have to be stable for up to 2 years in space. For a Mars mission, the transit time would be 6 months, and for an aborted return the crew would still have to orbit Mars to return to Earth. Consumables, such as food and water, would have to be stable for at least 30 months.

Water Recovery Systems for Exploration Missions

Dr. Pickering presented information on future exploration water systems for transit vehicles and habitats. Having closed systems will greatly reduce the mass needed for exploration missions, but those systems will be complex, and will include the recovery of water not just from urine and humidity condensate, but also shower waste, lavatory waste, oral hygiene, laundry water, and other water sources with high microbial loads.

The panel agreed that microbes will be present in the system, and that NASA should be prepared to control them. This would include controlling microbes using sterilization or aseptic techniques when possible. For transit missions, such as to an NEO, the most likely water system to be used would be a partially closed loop similar to what is currently on the ISS. For long-duration exploration missions that involve habitats on the moon or Mars, the water systems would be closed loops, meaning that 100% of the water would be reclaimed.

Human Research Program Exploration Medical Capability

Mr. Wu, a manager for the Exploration Medical Capability Element of the Human Research Program, presented an overview of Exploration Medical Capability, and the Exploration Medical Conditions List, which contains about 85 conditions derived from the ISS medical checklist, the Shuttle medical checklist, in-flight medical condition occurrences, and NASA flight surgeon subject matter expertise. He also described technology development projects, such as the reusable handheld electrolyte and lab technology for humans (rHealth) that is a microfluidic biosensor; and in-flight blood analysis technology for astronaut health monitoring that is based on flow cytometry.

Immunosuppression Expectations

Dr. Crucian, the HRP Immunology Discipline Lead and Laboratory Co-Manager, presented an overview of space flight immune dysregulation. Many immunological studies have been performed on the Shuttle and the ISS that point to an immune suppression during flight that is unrelated to launch and landing. Although the exact mechanism causing this immune dysregulation in crewmembers is unclear, the effect has been clearly identified. Data from the current "Integrated Immune" flight experiment corroborate these observations. There are technical challenges to obtaining conclusive data, which would need to be measured by an in-flight flow cytometer. Such hardware is in development and would be extremely useful, not only for immunological studies, but also for microbial identification and blood chemistry.

Microbial Response to Microgravity and Microgravity Analog Environments

Dr. Ott presented an overview of the work performed to evaluate the effects of microgravity on virulence and virulence characteristics in *Salmonella* Typhimurium and virulence characteristics in *Staphylococcus aureus*. Researchers have found, on the basis of data from murine models of infection, that the virulence of *Salmonella* is increased when it is cultured in microgravity or in space flight analog bioreactors. In these tests, *Salmonella* cultured in microgravity or bioreactors killed more mice faster than the microorganism cultured on the ground in the same conditions. Studies in *S. aureus* in rotating bioreactors have found that the microorganism displayed diminished virulence characteristics. Interestingly, the changes observed in both organisms were regulated by the same molecular mechanism, suggesting that an evolutionarily conserved mechanism of virulence can be found in multiple microorganisms that manifest it in different phenotypic ways. This hypothesis is supported by space flight analog data from other microorganisms in which virulence and gene expression changes have been noted: *Escherichia coli*, *Pseudomonas aeruginosa*, *Saccharomyces cerevisiae*, and *Candida albicans*.

Development of Microbial Monitoring Capabilities for the ISS and Beyond

Ms. Roman presented findings from a microbial monitoring workshop that she coordinated in April 2011. Workshop participants included a number of experts in environmental microbiology, infectious diseases, pathogen tracking and monitoring, and food safety sampling, and included several members of the Microbiology Laboratory team. One of the key findings was that prevention was important and this could be done by housekeeping, education of the crew, minimizing conditions that cause growth, and thorough disinfection on the ground. The technology identified included genetic, quantitative biomedical, biosensor, flow cytometry, mass spectrometry, and microscopic analyses. The participants found that currently no culture-free monitoring technologies exist. This presentation was given to the panel at the end of the Forum so as to not lead the panel to their recommendations. Regardless, the conclusions of the experts in this workshop were similar to those of the water forum panel.

5. Water Microbiology Requirements Framework

In preparation for this Forum, the Water Microbiology Requirements Framework was developed by the JSC Microbiology Laboratory to use as a communication tool to help describe space flight scenarios that could have an impact on microbial contamination of potable water. The main components are (1) the factors in the system that contributes to a high microbial load, and (2) the identification of the microbes found in the water. Together, these two factors determine the hazard of pathogens getting into the water and making the crew sick. The concept of the framework is described at a very high level and in very broad terms.

System

The influence of the water system is determined by whether it possesses minimal, few, or many factors that contribute to a high microbial load. These are defined as low, medium, and high impacts based on historical data.

This impact is affected by the complexity of a system and takes into account the number of users, user interfaces, moving parts, junction points, entry points, dead spaces, and subsystems.

The impact of a system does not equal the amount of risk in a system. Categorization of a system as one that includes many factors that contribute to a high microbe presence could simply mean that it is an advanced water system, without inferring that it is risky.

Low System Impact: Some examples of systems with low impact are drinking bags. These systems are for the most part water containers.

Medium System Impact: Some examples of systems with medium impact are systems currently on the ISS, including the Russian stored water distribution system, the Russian condensate water processor, and the U.S. Regenerative Environmental Control and Life Support System, which includes the water recovery system (WRS) and potable water dispenser (PWD). These systems are composed of tanks filled with water delivered from Earth or they are capable of humidity condensate or urine recovery coupled with thermal and chemical processing steps.

High System Impact: Currently, we are considering these to be advanced systems that have neither been designed yet nor been demonstrated in space flight.

Source

The source of the water influences what microorganisms are found in it. Sources are categorized as low, medium, or high impact on the basis of microbiological knowledge.

Low Source Impact: includes water that is sterile or contains microorganisms with minimal risk of causing disease, such as *Cupriavidus metallidurans.*

Medium Source Impact: includes water that contains microorganisms that cause disease under certain conditions, such as immunosuppression. Such organisms include *Pseudomonas aeruginosa*. Humidity condensate is a water source that fits this category.

High Source Impact: includes water that contains microorganisms that are known to cause disease in humans. Such organisms include *Salmonella enterica*.

The Water Microbiology Requirements Framework is a matrix of the system impact and the source impact on a 3 x 3 chart (Figure 1). It is subdivided into three categories; A (low-low, low-medium), B (low-high, medium-medium), and C (medium-high, high-high). We have further subdivided each section by individual boxes, for example three boxes in A (1, 2, 3), three boxes in B (4, 5, 6), and three boxes in C (7, 8, 9).

The goal is to have a method of describing the water and the system in ‘big picture’ terms to discuss mitigation plans.

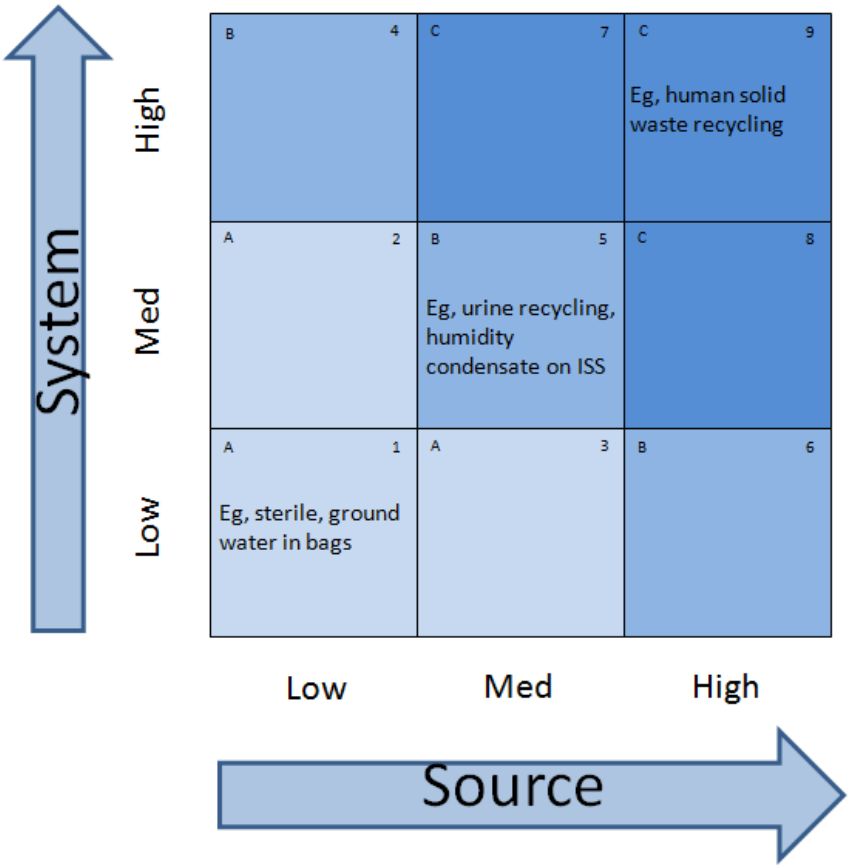

Figure 1: Water Microbiology Requirements Framework.

The mitigation plans for each of the framework scenarios range from no mitigation to something very labor-intensive and technically complicated; for example, using hardware to determine the full microbiome of the water. Mitigation plans could include preflight screenings of nasopharyngeal or stool samples, water filtration, biocide, water remediation kits, or in-flight monitoring for specific organisms. They could include water system design modifications (to minimize biofilm formation, for example). Many of these mitigation plans have arisen from "lessons learned" on the ISS. Proposed mitigation hardware is limited by mass, power, consumables, and training. Mitigation hardware most likely will include miniaturized versions of current laboratory hardware or even technology that is either on the cutting edge or nonexistent today.

A companion table to the Water Microbiology Requirements Framework is the mitigation table (Figure 2). It maps the possible mitigation plans to the boxes in the Water Microbiology Requirements Framework.

Cat.	Sub	Criteria	Examples	Strategy
A	1	Low System Low Source	Sterile or ground water in bags	No in-flight monitoring, no remediation
	2	Medium System Low Source		
	3	Low System Medium Source		
B	4	High System Low Source		
	5	Medium System Medium Source	Urine recycling, humidity condensate on ISS	Culture-based in-flight characterization and enumeration, remediation capability, pre/post flight characterization and enumeration
	6	Low System High Source		
C	7	High System Medium Source		
	8	Medium System High Source		
	9	High System High Source	Human solid waste recycling	In-flight full microbiome ID, remediation kit

Figure 2: Water Microbiology Requirements Framework mitigation table.

The Water Microbiology Requirements Framework was presented to the panel by Dr. Ott and used as a communication tool during the course of the discussion section of the Forum.

> *Dr. Thran commented that the Framework looks like a risk matrix. The panel agreed that it does; however it was careful to note that it was not a risk matrix. For example, a highly complex water system that recovers water from human solid waste and would reside in the Framework box with the highest system and source impacts is not necessarily a technology to be avoided and mitigated. Such technology would have to possess certain requirements and monitoring technologies to ensure that the water was potable.*
>
> *The panel agreed with the concept of the Framework Mitigation Plan matrix. The number of hazards does increase as the system becomes more complex and the water source becomes a more complex microbial environment.*
>
> *For example, the simplest water system for a short-duration mission to the ISS may be composed of sterile bags of water. The next level of complexity and source hazard would be a system like the PWD that dispenses water recovered from urine and humidity condensate from the air. A more advanced water system might include recovery of water from laundry and shower activities, which would*

definitely introduce fecal coliforms from clothes and the body into the system; such a system does increase the hazard. A system that recovers water from human solid waste, which is not currently in development, would have the most hazards for a water-recovery system.

6. Recommendations

During the Forum on Next Generation Microbiological Water Requirements for Space Flight, the expert panel of microbiologists made 19 recommendations pertaining to NASA standards and requirements. The Forum met the specific aims of this task by using historical data to assess optimal requirements, assessing current and near-term technologies, and recommending practical monitoring requirements.

Recommendations to Standards and Requirements

Of the 19 recommendations to microbiology requirements made by the panel, 12 pertained to water systems operations and monitoring requirements and standards in *NASA Standard 3001, Volume 2, Human Factors, Habitability, and Environmental Health.* These recommendations would also be applicable to other engineering design standards and requirements for hardware and vehicle design that are derived from *Volume 2*. These recommendations will be delivered by this report to the Human Research Program and the Office of the Chief Medical Officer.

During the discussion of the water systems, the panel found that the crew health aspect was tightly intertwined with environmental health and could not be separated from it. Seven recommendations were made to crew health requirements and standards in *NASA Standard 3001, Volume 1, Crew Health*. These recommendations made by the expert panel of microbiologists are somewhat out of the scope of this forum, but because the discussion did revolve so much around crew health, these recommendations are presented here for the benefit of interested stakeholders, including the Space Medicine Division.

The panel's recommendations were not prioritized; however, they were categorized as either prevention or control. The most effective means of maintaining a safe water system is prevention; for example, to reduce as much as possible the exposure of water systems of vehicles and habitats to potential contamination by microbes. Prevention will save NASA a large amount of time and effort in reducing bacterial counts during the mission. Regarding control, it was said many times throughout the course of the Forum that "microbes win," meaning that because microorganisms are so prevalent in the environment, they will eventually become a problem unless effort and resources are expended to control them after they are established either in the water system or in the crew.

<u>Prevention Recommendations Relating to Human Factors, Habitability, and Environmental Health</u>

(W1) The water system should be sterilized immediately before flight.

Rationale: During the installation of the PWD of the ISS WRS, the hardware was packed and sat dormant for several months. During checkout, the system was found to have elevated levels of bacteria present. If the system had been sterilized, the bacteria levels would have been zero. Such methods would avoid the costs and effort of remediating the system.[1]

[1]*Comment from Dr. Richards: "In developing sanitation requirements for our field water bottling systems, we employed HAACP procedures, and among other things, use a steam generator to run 120ºC steam through the system for 30 minutes prior to commencing operations each morning. Probably not a practical procedure for space flight, but if sufficient heat and water were available, it could be considered for a remediation capability. For our field water systems in general, we have our soldiers super-chlorinate their field water storage and distribution equipment with a 100 mg/L chlorine solution for 60 minutes prior to putting it into use (quarterly or semiannually). It's not sterilization, but pretty strong disinfection/sanitation procedures."*

(W2) The water system should be designed to minimize areas within the system's components that could allow bacterial growth.

Rationale: In the design of the PWD, there is a line from the water tank with a point-of-use filter that is a considerable distance from the dispensing needle. Bacteria in amounts that have exceeded microbial limitations have accumulated in the hose between the filter and the dispensing needle. In addition to this, the presence of bacteria causes the formation of biofilms, which can make chemical disinfection of the system difficult. A point-of-use filter should be immediately upstream of a dispenser and tubing, and hoses should be resistant to the development of biofilms. Other concerns include secondary contamination of the dispensing needle, which is open to the vehicle environment and could be contaminated during the rehydration of food.[2,3]

[2]Comment from Dr. Mayhall: "…concern I have is about Legionella, because, outside of an infected host, it mainly reproduces in free living amoebas. In water systems it reproduces in amoebas and periodically re-enters amoebas when threatened by disinfectants. The amoebas are resistant to disinfectants that are used to kill Legionella in water. If water becomes contaminated with Legionella, it could be very problematic to remove the Legionella from the water system."

[3]Comment from Dr. Richards: "Move the dispensing needle as close to the storage container as possible (or vice-versa), reducing the length of the connecting tube to a minimum. Ensure that it is used frequently so the water does not remain stagnant in the line for long periods. May consider using silver-impregnated tubing to reduce biofilm and/or if a filter is placed at the outlet, consider silver impregnated filter material so bacterial growth on/in the filter is inhibited. I recall that NASA used silver-impregnated carbon blocks several years ago to inhibit bacterial growth in their carbon filters, so the idea is not new to NASA. Maintaining a biocide residual in the stored water and throughout the system would reduce biofilm growth, growth on the filter, and growth on the dispensing needle (unless the filter is a carbon filter that removes the biocide)."

(W3) The water system should include the use of a residual biocide.

Rationale: The current U.S. water system on the ISS uses iodine as a biocide, and the Russian system uses silver ions. The panel agreed that using a biocide was a wise decision. It should be noted that bacteria can develop resistance to either of these biocides. Pseudomonads, microorganisms commonly found in the U.S. water system, can grow in the presence of iodine; other microbes can grow in the presence of silver ions.[4]

[4]Comment from Dr. Richards: "I receive anecdotal evidence periodically from a vendor friend that low concentrations of silver ions in water are healthful for many ailments, and silver has a bactericidal effect, as mentioned in the report. I would be interested to know if the Russians have documented any noticeable effects from using it as their residual disinfectant. At concentrations less than the EPA secondary standard of 100 g/L, it has reportedly reduced the nosocomial infection rate at a Texas hospital for several years, nearly eliminated MRSA in men's locker rooms at Arizona State University, and eliminated the need to clean the ice machines that it feeds at the same university. I was involved in a cursory evaluation of a silver ion generator system that was installed to feed silver ions into the water line of a burn unit at a military hospital several years ago, and the results (again anecdotal) indicated reduced infection and improved healing of the burn victims in that unit. I don't know why it wasn't pursued further… you might consider adding a UV radiation source either in the storage or delivery system to operate constantly or periodically depending on need and power availability, to work in conjunction with the biocide residual. West Point did some research a few years ago demonstrating a synergistic bactericidal effect between silver and UV radiation. It didn't matter which occurred first, either one potentiated the other. UV should be effective since the turbidity of the water, I presume, is very low, and transmittance should be good."

(W4) The water system should include the use of dissimilar redundancy to prevent contamination of potable water.

Rationale: The current U.S. water system on the ISS uses dissimilar redundancy to treat the water. This includes filtration, residual biocide, and heat, which is a byproduct of the catalytic oxidizer. Water systems more complex than a sterile bag of water should contain barriers to contamination that are appropriate for the hazards of the water source and system.[5]

[5]Comment from Dr. Richards: "In our deployment operations we employ field water systems which are more akin to your space systems than our fixed installation systems, and we use a "multiple-barrier approach" to ensure the provision of safe drinking water (as do other water purveyors and EPA). The classic barrier names vary in description with the organization and application, but generally consist of preventing contamination from entering or passing through the several stages of drinking water management systems. Typically they include some form of the following:

1. Source water selection and protection
2. Use of certified water treatment equipment (which may also include multiple barriers) and employ certified operators who operate and maintain the equipment in a sanitary manner
3. Use of effective water disinfection techniques
4. Protect treated water quality through distribution system operation and maintenance
5. Perform operational and water quality monitoring and provide medical oversight

These barriers to contaminants ensure to the extent possible that the product water is safe to drink. Weakness in any barrier weakens the overall system, but weakness in one or more barriers can be compensated for by strengthening others. An example is that in the deployed environment we sometimes don't have much choice as to the quality of the water source (barrier 1), and our ability to provide proper medical oversight (barrier 5) is usually strained by the lack of appropriately trained public health personnel. To compensate for those weaknesses, we strongly recommend (used to require) reverse osmosis treatment systems operated by highly trained and certified operators (barrier 2), effective disinfection of 2 mg/L free chlorine after 30 minutes contact time (barrier 3), and maintenance of a comparatively strong free chlorine residual during distribution (1 mg/L) (barrier 4)…"

(W5) The water system should be monitored before, during, and after flight.

Rationale: Currently ISS water is monitored and characterized on a regular schedule, every 30 to 90 days, depending on the water system. It is possible that in-flight monitoring of exploration water systems would not have to be as rigidly scheduled as monitoring of ISS systems, because the systems on the ISS have been well characterized after up to 10 years of operation. Monitoring may be performed in response to a microbial event in the water system, detected by other means, such as system performance feedback. Preflight screening could be performed on the ground as it is now. Certification of sterile bags of water could preclude preflight or in-flight testing as long as the water bags were intact. Post flight screening of water systems is a necessity to measure the performance of the water system; however, the sample could be extracted from the system before a water landing, which is the anticipated landing operation in exploration missions. Such a sample could be considered a late in-flight sample.

(W6) The design of the water system should include features that provide system data to users that allow them to assess the performance of the system and the potability of the water it is dispensing.

Rationale: The water system should provide data that will allow crews to make judgments on the performance of the system and potability of the water. For example, pressure measurements across a water filter could supply information about its integrity. Measurements of temperature in the oxidizing chamber provide information about the effectiveness of this component of the water system. The system should provide sufficient data to allow a user to know when conditions exist that could allow the microbial content of water to exceed limits for the potability quality of the water. This is analogous to the monitoring component of the Hazard Analysis and Critical Control Points (HACCP) strategy used in food and pharmaceutical safety. HACCP was developed by NASA and Pillsbury for use in the manufacture of space foods. HACCP consists of 7 principles: conducting a hazard analyses, determining critical points, establishing critical limits, establishing monitoring procedures, establishing corrective actions, establishing verification procedures, and establishing record-keeping procedures.[6]

[6]*Comment from Dr. Richards: "Inline continuous water quality parameter monitoring is becoming more common and viable. Continuous turbidity monitors or particle counters and total dissolved solids (salinity) monitors are common. Different instruments could be used to provide continuous or periodic feedback as to the "health of the system," their selection being depending on the ratio of recycled water to fresh water (and I guess duration of the*

mission). As mentioned in the discussions, pressure drop across filters is also a good indicator of filter function. We have a small system that fits inside a 1- or 2-inch line and reports 8 water quality characteristics every few seconds wirelessly, programmable to report to Twitter, as an example (but probably not useful in space-but could report to a data collection device as desired). HAACP is a great strategy to help determine what can and needs to be monitored."

(W7) The designers, engineers, and technicians who develop and service the water system and users of the system should be educated about hygiene and infection control.

> *Rationale: The panel suggested that the program would benefit if all individuals involved in the assembly, testing, and checkout and use of water systems were educated about techniques needed to maintain asepsis in water systems.*[7]

[7]Comment from Dr. Richards: "Our program at the Army Institute of Public Health works closely with Army, Navy, and Marine Corps Research, Development, Testing, and Engineering communities in water-related projects to provide medical oversight and input into their products. We are engineers and scientists who understand both medical and engineering aspects of the design and production of water treatment systems and devices. We participate in most of the teams, committees, and workgroups they have for water system development. They seem to appreciate our participation"

Control Recommendations Relating to Human Factors, Habitability, and Environmental Health

(W8) The water system should have a remediation capability.

> *Rationale: In the current U.S. WRS on the ISS, the PWD was remediated after elevated microbial counts were found. This remediation required that a bag of iodine solution be transported to the ISS. The system was flushed and the problem was alleviated. Developers of exploration water systems should expect contamination to occur and should design features into the system that will remediate or aid in the remediation of contamination. One of the prevailing quotations from the Forum was "Microbes win," meaning that bacteria, viruses, fungi, and protozoa are a part of our environment and they will find a niche and establish themselves there. Exploration crews should be equipped with the tools they need to remediate microbial fouling of the water system. This includes regenerative sterilization technology by electrochemical means or ultraviolet (UV) irradiation.*[8]

[8]Comment from Dr. Richards: "Having the ability to super-iodate, super chlorinate or otherwise be able to "shock" the system is a good idea. Disinfection efficacy is a result of a combination of time and concentration (CT). If you are using chlorine or iodine, you can increase the CT by either raising the concentration or the time. EPA in its Surface Water Treatment Rule provided required CTs to achieve desired log-reduction of microorganisms in water systems for chlorine. As mentioned previously, we use 100 mg/L free chlorine for 60 minutes, or a CT of 6,000 mg/L/min to superchlorinate. We and the American Water Works Association also allow systems to use 10 mg/L free chlorine for 24 hours to achieve sanitation. Having the ability to vary either or both the dose of disinfectant and the time provides options to remediate with the same equipment you are providing the residual with."

(W9) In the event of the need for the water system to be remediated, the crewmembers should have a remediation plan available.

> *Rationale: On exploration missions, crew resources will be constrained and resupply will not be available. There should be a plan for corrective action just as there is one for the response to a fire in the vehicle. A remediation plan should be multi-level, with the response tailored for the microbial situation. For example, if engineering data show that the water system has not performed properly, a balanced response plan should exist in which investigative tools, such as a coliform kit or microbial capture device, should be available and used only as needed.*[9]

[9]Comment from Dr. Richards: "Depending on the availability of space and weight for the spacecraft, the crew could take individual water purification devices for each member, or a single small unit water purifier as individual or group emergency backup for the water system. These units range from disinfection-only to various kinds of filters, and combinations of both… We performed two studies for the Army Studies Program Office, evaluating as many

individual and small unit water purifiers as we could lay our hands on, a few years ago, and have those reports and a website available to see the results of our studies, if that would be of interest to the forum or to NASA in general. ”

(W10) The water system should be easy to maintain.

Rationale: The water system should be designed so that crewmembers could perform autonomous maintenance during flight such as replacing filters and hoses to remediate the system if needed.[10, 11]

[10]Comment from Dr. Richards: “Replaceable or removable and cleanable system components would make this easier.”

[11]Comment from Dr. Mena: “…a strategy would ideally anticipate (and prevent) influences [such as human error] by the flight crew.”

(W11) The microbiological monitoring hardware should be easy to use, provide results rapidly, and use technology that is as simple as possible.

Rationale: Currently water is monitored for microbes in 2 ways that are analogous to ground monitoring. The number of microbes is monitored using the microbial capture device, which consists of a water filter impregnated with growth medium, on which bacteria form viable colonies that metabolize a chemical that facilitates manual enumeration of the colonies.. The water is also tested for coliforms, an indicator of fecal contamination, and is measured by a color change in the medium. In general, these 2 technologies are easy and “low tech.” Dr. Quick was invited to attend the Forum specifically because of his expertise in remote locations, such as South Africa, Southeast Asia, and South America, where easy, fast, and low-tech monitoring hardware is desired. The potable water science community is performing research to develop a dipstick-like device that will detect the presence of E. coli *has begun. The Microbiology Laboratory is also monitoring advancements in water testing methodology and technology.*[12]

[12]Comment from Dr. Richards: “An electronic coliform analyzer system that has been under development for several years (funded via an Army Small Business Innovation Research program) that tracks the growth rate of organisms filtered from water in a nutrient broth and can identify coliform and *E. coli* bacteria is currently being tested by EPA as an alternative test procedure (ATP). It can reportedly verify that water is coliform/ *E. coli* free in 8 hours instead of the current 18 or 24 hours required by the presence/absence methods we use. We are watching closely to see if it passes the EPA tests and is awarded EPA’s ATP status.”

(W12) The microbiological monitoring hardware should include a method of measuring the viability of the microbes present.

Rationale: Viability is a key component of the knowledge about microbes in the water. Dr. Ott presented data pertaining to the metagenomics of water samples from the ISS, which listed a number of key microbes that have been found in the water using classic methods, including culture, and 16S rDNA sequence identification. Some of these microorganisms would cause concern if they were viable, and the genetic methods used, which are extremely powerful for identifying the complete microbiome of the water system, cannot determine whether the DNA measured comes from a viable cell or from free DNA in the water from a lysed or otherwise nonviable organism. A paper was discussed (La Duc, et al. 2004 Habitation 10:39-48) in which Salmonella typhi was found in Kennedy Space Center supplied water, but was not culturable. It was most likely from dead cells killed in the water treatment process and not of concern. Dead microorganisms are not as critical a concern to the crew as viable microorganisms are. There are technologies in development that measure viability other than simply culturing on a plate.[13, 14]

[13]Comment from Dr. Richards: “I believe culturing microorganisms is still the best way to determine viable organisms. HPC combined with presence/absence total coliforms and *E. coli* provide information on the general microbial state of the system as well as potential fecal or other human contamination. They also are methods that meet the bacterial standards in NASA-STD-3001, VOLUME 2. The coliform analyzer mentioned above obviously measures only viable organisms.”

[14]Comments from Dr. Mena: A) "…the presence/absence of viable organisms is not definitively addressed through a culture test. Many microbes can enter a viable but nonculturable (VBNC) state, so some organisms may be missed. In addition, the culturing of heterotrophic plate count (HPC) bacteria provides useful information on water quality changes over time, but since most members of this group of bacteria are not pathogens, a positive culture is not indicative of a health risk. The ultimate purpose of water monitoring for space flight is to prevent adverse effects among the flight crew. Ideally, the routine testing of water would indicate a water quality problem before flight crew are impacted, but this is not typically possible due to the delay before results are generated – particularly with culture analyses. The discussions throughout the Forum concluded that the identification of viable microorganisms would lead to the development of appropriate corrective measures. In addition, a specific identification of the pathogen compromising the water supply would aid in either treating an ill crewmember or anticipating potential health problems. Moreover, knowing the unique characteristics of a particular microbe may also provide clues to the source of the problem so that an appropriate mitigation strategy can be implemented."

B) "Although culturing organisms was the overwhelming favorite of the Forum panel, the usefulness of the monitoring results generated from this method is not straightforward. Viability is not definitively assessed, and the culture medium employed may not detect/identify a particular microbe. Although enumeration can be recorded, the usefulness of this information is questioned since it is the virulence of the pathogen – rather than the number of pathogens – that impacts health risk. The number of organisms in the water supply is important, however, when considering a nonculture method. Those methods are typically not as sensitive as culturing and therefore require a higher number of organisms present to be detected. An advantage of non-culture methods is that results are obtained much quicker. Also, specific microorganisms can be targeted in the analysis, which would provide the information needed by microbiologists, engineers, and flight surgeons to take appropriate remedial action. Some of the non-culture methods discussed included those involving polymerase chain reaction (PCR) and flow cytometry. Monitoring water quality parameters with instruments could indicate changes in water quality to trigger microbial monitoring."

C) "While viability is an important consideration, particularly when assessing health risks, it may be difficult to quickly and definitively obtain that information for a positive sample. From a risk assessment perspective, knowing which microorganism is present in the water supply is a priority since survivability and infectivity vary greatly among waterborne microorganisms, and have shown to directly impact health risk. An integrated culture-molecular approach may be an option as long as the combination minimizes the disadvantages of each methodology. Future components of a microbial monitoring regimen in the space environment may be "lab-on-a-chip" systems, which are microfluidic devices applicable for the detection of waterborne microbes at a fraction of the cost of conventional methods. Researchers at The University of Arizona are designing these miniaturized flow cytometer systems for the detection of bacteria, enteric viruses, and protozoa using engineered molecular beacons. The molecular probes target unique microbial nucleotides, producing a fluorescent signal. This novel approach integrates handheld optical detection systems for fast, quantitative signal measurement in the field. The advantages of microfluidic systems reduce reagent volumes, system cost, size and power requirements, while addressing the need for rapid, real-time environmental monitoring, and disease diagnostics. These "lab-on-a-chip" systems offer advantages that make them good candidates for a water monitoring tool during longer missions. Their effectiveness in a microgravity environment needs to be assessed."

D) "…monitoring needs to be designed to generate the information necessary for an appropriate response to a contamination event."

Prevention Recommendations Relating to Crew Health

(W13) The crewmembers should be screened for relevant obligate and opportunistic pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA); the causative agents of meningitis in adults, such as *Neisseria meningitidis* and *Streptococcus pneumoniae,* and *Streptococcus pyogenes,* the causative agent of Group A streptococcal infections.

Rationale: In terms of crew health, the greatest risk of infectious disease will come from the organisms the crewmembers already carry in their bodies, such as MRSA. Dr. Haddon noted that it has been shown that crewmembers have gone to the ISS without the non-resistant S. aureus *and returned with it. Currently the crew is screened for MRSA, syphilis, HIV,* Helicobacter pylori, *tuberculosis, and hepatitis A, B, and C. Other disqualifying infections include malaria, Lyme disease,*

mission-impacting herpes simplex I and II, intestinal parasites, herpes zoster, and post-herpetic neuralgia, but additional screening that would include the organisms identified above should be done to determine if any candidate crewmembers carry other dormant infections that may become active in long-duration exploration missions, when radiation levels are elevated and crewmembers' immune systems are suppressed.[15]

[15]Comment from Dr. Mayhall: "I have a great concern about *Norovirus* with respect to long-term space exploration. The virus is highly infectious with only a few viral particles need to cause infection. It is a nonenveloped virus and very difficult to kill with disinfectants requiring sodium hypochlorite (5000 ppm) for inactivation. Although the route of transmission is basically fecal-oral, it may also be airborne with viral particles inhaled and then swallowed. If *Norovirus* ended up on a space craft, it might be very difficult to eradicate."

(W14) The crewmembers should be vaccinated against *N. meningitidis*, *S. pneumoniae*, *S. pyogenes*, and other relevant opportunistic pathogens that are not currently in the immunization schedule.

Rationale: Currently crewmembers are immunized for a number of infectious diseases, including diphtheria and tetanus (DT), polio, hepatitis A, hepatitis B, varicella, and measles, mumps, and rubella, according to the Medical Requirements Document MR089S. For exploration missions this immunization schedule should be complemented with immunizations against obligate and opportunistic pathogens that the crewmembers may carry with them and subsequently release during immune system dysregulation.

(W15) The crewmembers should be decolonized so that they do not carry relevant opportunistic pathogens such as *S. aureus* or the antibiotic-resistant strain, MRSA.

Rationale: It is known that once a microorganism is carried to a space vehicle environment by a crewmember, that microorganism will become a part of that environment. For example, colonization of crewmembers by S. aureus *has occurred on the ISS. Exploration crewmembers who are positive for* S. aureus *or other opportunistic pathogens should be decolonized before flight. However, a decolonized crewmember of an exploration-class mission that docks at the ISS may be exposed to microbes that are already on the ISS.*[16]

[16]Comment from Dr. Thran: "…if you do that, what are unintended consequences? Does that make that crewmember more susceptible to something else?"

(W16) The crewmembers should be processed through a rigorous quarantine program that uses hospital-developed isolation and infection-control regimens.

Rationale: Once exploration crewmembers are screened, immunized, and decolonized of obligate or opportunistic pathogens, such as S. aureus, they should be quarantined in a rigorous infection control protocol so that they are not exposed to additional obligate and opportunistic pathogens during public outreach, celebratory events, or nominal pre-launch activities.

(W17) Crewmembers should be educated about hygiene and infection control.

Rationale: Many infectious diseases can be controlled by behavior and situational awareness, such as the proper method of controlling a sneeze. Many misconceptions are prevalent about microbiology in general, such as the difference between commensal microbes and pathogens. An adequate education program would provide operational knowledge to prevent infection while dispelling misconceptions.

Control Recommendations Relating to Crew Health

(W18) The crewmembers should have other medication in addition to antibiotics available to them to treat infectious disease and to control symptoms of infection.

Rationale: Currently the ISS medical kit contains a host of antibiotic, antiviral, and antifungal medications, including antiseptic swabs and wipes. Other medications to treat effects of waterborne illness should include anti-emetics and bismuth subsalicylate.

(W19) The crewmembers should use a proper infectious disease response plan.

Rationale: Along with the proper medicines, the crewmembers should use a proper response plan to treat infectious disease, especially in regard to antibiotic medicines. Antibiotics should be used sparingly and under the proper control strategy to avoid the development of antibiotic resistance, or the overgrowth of commensal organisms such as Clostridium difficile, *which can cause life-threatening, debilitating diarrhea. The plan should also address the control and isolation of infectious biological fluids such as mucus, sputum, blood, and diarrhea.*

7. Discussion

Dr. Ott led the discussion sessions of the Forum, starting with an explanation of the Water Microbiology Requirements Framework as described in Section 5. Considering the many factors affecting water systems and sources, in terms of current knowledge and expectations on mission design, immune dysregulation, and virulence changes, the panel discussed Risk and Mitigation, Strategy, Technology, and Research Gaps. Dr. Ott began with a discussion attempting to define the endpoint of the "risk" that the panel would be further discussing, as different members of the panel may see different endpoints for the risk. Space flight programs are often concerned with the risk of loss of a crew or the loss of a mission (LOC/LOM). Another perspective might be from a clinician who may be more interested in the manifestation of disease. A water systems engineer may look at the risk of having microbial concentrations elevated over acceptability limits, increasing the risk of biofouling in the system. Each of these levels of risk has different impacts, with LOC/LOM having the greatest. From a hierarchical standpoint, a poorly defined level of uncertainty exists between high microbial levels, the presence of medically significant organisms, a disease state, and LOM/LOC.

Dr. Thran explained that the Army defines risk as a function of probability of exposure and severity. They have a risk matrix with probability of exposure and severity as axes and the risk is defined in terms of mission capability, which is similar to what NASA does. The Army further delineates the disease in terms of its effects, such as whether it is debilitating or lethal and how different people react to it.

Dr. Quick explained that the CDC does not focus on risk, but on prevention and control. The CDC's work is based on evidence, and there is not a lot of evidence for microbiology occurrences in the space program due to the small number of astronauts, a very small occurrence of illness, and an absence of data. He thought that prevention of a microbial occurrence on a space vehicle "is almost the entire ballgame. Do everything you can before the launch and after launch do what you can with your limited resources with something quick and easy."

Dr. Mena said that in her risk work with the food industry and municipal water utilities, they focus on probability of infection, which has less risk than disease and more risk than the presence of a medically significant microorganism, with the purpose of circumventing disease.

Dr. Mayhall added that biofilms are a considerable hazard that will need to be addressed specifically because of *Legionella*, which can form biofilms or establish themselves in biofilms formed by other organisms. Dr. Quick suggested that a lot of research could be done in biofilms. Dr. Ott commented that biofilms are under consideration. This led to a discussion about the hazards of biofilms in terms of the endotoxins produced: the level of endotoxins that make people sick from drinking water versus using the

water in injectable fluids. There is some concern about chemical agents in a recycled water system, such as endotoxins, antibiotics, or toxic chemicals, being concentrated by the system itself over the course of several years. Currently, it appears that the water system is removing endotoxins and antibiotics, but more should be known about how they are removed and what happens to them. Currently, the PWD and Russian systems contain endotoxin concentrations that are lower than the limits for injectable fluids, according to measurements in the Microbiology Laboratory. Dr. Pierson suggested that humans can take in huge amounts of endotoxin during ingestion, especially during the ingestion of food, when the number of heterotrophic plate count microorganisms can be up to 20,000 per gram of food. Injectable fluids, however, have much lower limits.

During the discussion of biofilms and endotoxin, Dr. Ott made note that the system requirements and the crew health requirements are intertwined. If the system allows the growth of biofilms that shed bacteria and endotoxin, it will affect the health of the crew. Many times throughout the course of the Forum, the panel made recommendations to crew health requirements as well as water system requirements.

Mr. Canga, the manager of the Advanced Environmental Health research portfolio and former risk manager in the Space Shuttle Program, suggested that the agency understand the causal chains that lead up to a risk. What causes the risk? What has caused illness from the water or elevated microbial counts in the past?

Dr. Thran suggested that NASA understand which level of risk it is addressing, and whether it is LOC/LOM, disease, presence of medically significant microorganisms, or elevated microbial counts. She said we need to make sure that we know what a risk is (the probability of something happening) versus what a hazard is (something that leads up to a risk).

Dr. Ott led the panel through a number of key discussion questions:

- If potable water is defined as water that can be safely used during a mission, what are the particular risk concerns that could occur from consumption, hygiene, eye wash, or wound cleaning?

  The panel agreed that potable water can be used safely for consumption and hygiene. It is even safe to be used for an eye wash if the eyes were already healthy. This depends on the nature of the need for the eyewash, such as washing out minor debris versus washing out of a tissue-damaging caustic chemical. As long as soap is added, potable water can be used safely for minor wound cleaning. If the wound is a severe burn, potable water should not be used because of the potential for growth of bacteria in burned tissue.

- Which obligate pathogens are the greatest threats given current and expected space flight water systems?

  The panel did not identify particular organisms during this discussion. They suggested that the particularly hazardous microorganisms, such as MRSA, Salmonella, *etc., be screened and decolonized out of the system and crew before launch during preventive measures. Dr. Mena added that according to her research, because the water sources on the space vehicle are few, if a pathogen is present, the odds of it infecting a crewmember are very high; thus she believes that identification of those pathogens is important.*

  There are indicator organisms, such as E. coli *and* Enterococcus, *that are not necessarily pathogens, but instead indicators that the water has been contaminated with fecal material. The panel agreed that if the pathogens are prevented from getting on board, then there is not as much risk.*

  Dr. Quick added that if a gastroenterological event does occur in flight, more effort should be spent on containing the diarrhea, treating the person, and fixing the system than identifying the causative microorganism.

- Which opportunistic pathogens are the greatest threats given current and expected space flight water systems?

  Pseudomonas aeruginosa *was discussed as a particular opportunistic pathogen that occurs in the water on the ISS. The hazard would depend on the amount of* Pseudomonas *present in the system. There is a dose-response aspect to infection by microorganisms, and it is species-dependent. Drinking water with* Pseudomonas *is not a threat to healthy people. The unknown in this case is the change in host-microbe interactions in space flight. Suppression of the immunity of crewmembers or an increase in the virulence of the microorganism would increase the level of uncertainty.*

- Is preflight monitoring required for all space flight water systems?

  The panel agreed that prevention is the best method for mitigation and that preflight monitoring is required for prevention.

- When is postflight monitoring required?

  The panel agreed that it is important to have postflight data to verify that the quality of water is the same after the mission and to make sure the quality does not degrade. Water landings may make this technically difficult, but an in-flight sample taken just before landing could meet science requirements.

- For each condition described in the framework, when is monitoring required? Specifically:
  - When is enumeration required?

    If it is a sterile bag of water, Dr. Mayhall said that it is important to monitor to make sure it is sterile; however, characterization is not necessary. In the hospital environment, equipment is sterilized and is indicated "sterile" as long as the packing is intact. The panel agreed that in simple systems, sterility can be indicated and be verified by engineering methods. A sealed bag of water would have to be compromised or opened for microorganisms to contaminate it. In that case, enumeration by culture is not necessary. However, an open system such as the PWD is a different case because water and microbes from the environment are constantly entering the system.

  - Are enumeration analogues (for example, DNA copy number) adequate?

    Dr. Quick prefers culturing for enumeration and molecular techniques for characterization. For screening water, culturing for viability is best. The panel agreed that a measurement of viability is essential for the detection of microorganisms in the water.

  - When is characterization required?

    Dr. Quick said that he has investigated many outbreaks in which they did not characterize the microbe from the source, but they found it in the patients. A lot of time and effort can be spent on characterization without a high yield. Dr. Ott pointed back to Dr. Haddon's presentation when he said that as a flight surgeon, he does not necessarily identify the microbe causing the illness; he is concerned with stopping its effect on the patient.

  - What information should be required for characterization?
    - Viability?

      Viability is essential for detection of microorganisms, and the current technology to measure this is culture-based.

    - Genus-species identification?

Species-level identification would be nice to have, but difficult to perform in spacecraft. Many of the current technologies to identify microorganisms to the species level are resource and time expensive. Genetic and protein-based methods do not necessarily measure viability, and without knowing whether the microorganism is viable, it is impossible to determine whether it is a threat. For example, there is a paper (La Duc, et al. 2004 Habitation *10:39-48) that reports on evaluation of the microbial diversity of water at Kennedy Space Center using molecular cloning techniques and reports that* Salmonella *Typhi was found. Such information is very interesting from a microbiology perspective, but the organism was not culturable, and its viability was undetectable. It is currently not practical for crewmembers to perform advanced molecular procedures to assess organism viability on ISS or on a deep space mission.*

- Virulence factors?

  The measurement of virulence factors in flight is not necessary.

- Characteristics of microbial response to countermeasures (for example, antibiotic resistance)?

  Antibiotic resistance is good information when studying an outbreak, to advise on treatment with antibiotics. A clinician would still treat the illness presumptively with an antibiotic and if it did not work, try something else. The crew may not have the luxury of trying a different antibiotic. The practical strategy of trying the first best treatment and not worrying about the identity of the specific organism recalls what Dr. Haddon said about stopping the bug, not necessarily identifying it.

- For each condition described in the framework, what other mitigation strategies (such as remediation, or fault tolerance and redundancy) should we use?

Many other types of mitigation strategies are available. Without going through each condition in the framework, the list included these:

- Filters

  Dr. Mayhall said that UTMB uses water filtration to remove Legionella *from the water. Those filters do not last very long and must be changed out frequently. Replacement of filters would cost upmass, volume, and effort. Dr. Dan Gazda of the Water and Food Analytical Laboratory made the point that spacecraft water systems use a much higher quality of water than municipal water and will not have the same amount of bacteria and particulates. Dr. Pickering said the water system engineers currently use large Pall filters, but the small Millipore filtration disc filters should work, so that they could be changed out more often, with a smaller, lighter filter.*

- Regenerative Disinfectant

  Dr. Pickering said that a regenerative electrochemical disinfectant is in development that will be able to generate sodium hypochlorite or hydrogen peroxide.

- Redundancy

  For 3-day missions to the ISS, several layers of redundant microbial mitigation steps on a water system are not necessary. For those missions, the water source may be sterile bags of water. On short missions, if a PWD-like device is used that dispenses water from a holding tank redundancy is not needed beyond the primary filter component of the system. In other words, for 3-day missions, a biocide and oxygen catalyst would not be necessary; however, water quality should be verified before flight.

  Levels of redundancy would be triggered by introduction of microbially rich sources, such as urine and fecal particles from laundry and bathing, or even humidity condensate.

- Should system remediation (such as disinfection shock) count as a level of redundancy?

  Remediation should not be considered a level of redundancy. The panel did agree that some form of remediation must be available. In the case of a short mission using individual water bags, the redundancy is inherent as the crewmember would just use a different bag of water.

  The panel reinforced the idea that systems with medium system impacts should have some redundancy. Currently, dissimilar redundancy exists in the WRS, meaning that one antimicrobial control is chemical (biocide), another is physical (heat), and the last is mechanical (filter).

- Should medical treatment (*e.g., antibiotics*) count as a level of redundancy?

  No. The topic of antibiotics led to a lot of discussion by the panel. Microorganisms will not develop antibiotic resistance in the space vehicle unless they are pressured by antibiotics in the system. The microbes will only bring whatever antibiotic resistance they already possess. If the crew uses the antibiotic medicines without the proper dosage and length of treatment, then indeed microbes can develop antibiotic resistance. This led to the recommendation that there be education about antibiotics and an infection control plan for regimented control of antibiotic use. Another consideration when using antibiotics is whether or not the antibiotics are removed by the water system when it is recycled. There was concern about antibiotics, hormones, endotoxins, and estrogenic compounds accumulating in the system as the water is recycled many times. Dr. Pickering and Ms. Roman are both working on those issues and they both thought that the current system and a biological system in development do take out the antibiotics. The final issue with antibiotics is that they can kill off the beneficial, commensal organisms in the human gut so that the normally benign microbe Clostridium difficile *can overgrow and cause life-threatening diarrhea. This is a known effect of antibiotic usage that could affect the mission and crew.*

  *With respect to other medical treatments, the panel strongly agreed that it is critical to have crewmembers screened, decolonized, and put through a robust quarantine process before flight, and that the water system should be sterilized, and the vehicle should be sanitized as practically as it can be. The human pathogens that the panel was most concerned about would come from other humans (*E. coli, *MRSA, etc.). Screening, decolonizing, and quarantining will be key to controlling pathogens in the crew and in the water system.*

- Could we use engineering measurements (such as pressure drop across filters, temperature readings during pasteurization, biocide level) of the water system to determine potability of the water in a manner similar to HACCP?

  The panel agreed that using engineering measurements was a good strategy. Engineering measurements would allow crews to avoid some of the routine monitoring that is currently used on the ISS water. This would save time, effort, and consumables for when the crew really needed to use them.

  When the panel was discussing the lifespan of the system, Dr. Pickering made the point that engineering a water system to perform for 30 months (the duration expected for an asteroid mission) is easier to do than to engineer a long-lifespan system like the one on the ISS, which is designed to last for 15 years. It was an interesting juxtaposition with the usual point that deep-space missions will be more hazardous due to lack of resupply and long return times. Those missions will not last as long as the entire lifespan of the ISS.

  Dr. Jan, the Advanced Environmental Monitoring and Control Manager from the Jet Propulsion Laboratory, thought we should develop a water system remediation plan, and the panel agreed. This plan would be similar to a fire response plan. First, the proper materials are used in the

design of the system. Second, something triggers the crew to investigate the water system, instead of routine monitoring. Third, the response to remediate the system occurs while a backup supply of water is available. Finally, the crew verifies that the system is once again potable. Dr. Quick added that this strategy would help the crew save its diagnostic tools for more critical issues.

There was some discussion about how to know when the water system is contaminated: the crew decides that the water tastes or smells contaminated, or they even realize that the water is making them sick. The panel thought that the trigger should come before the crew gets sick. Dr. Thran suggested that it should involve some indicator that is easy to use and does not require culture, like a dipstick.

Dr. Quick said that a dipstick indicator of water quality is the Holy Grail of water quality investigators in third-world countries. Technology is in development, but it is not ready. A low-tech, low-effort way of determining water quality is the way to go, especially in distant locations with few resources such as on exploration missions.

Dr. Ott led the panel through a discussion of monitoring technologies, which involve two types of microbiological methods: classical and advanced.

Classical microbiological methods include heterotrophic plate count, which is a count of the colonies formed on a culture medium by single microorganisms in a sample, and characterization through growth and biochemical responses. Biochemical profiles are measured by well-researched methods based on patterns of nutrient utilization by different microorganisms. Classical methods rely on viability, are simple and low-tech, and possess the sensitivity to detect a single microorganism.

Advanced microbiological methods include molecular analyses used to identify microbes and their virulence factors by DNA sequencing. Flow cytometry is another advanced method, which uses dyes and antibodies that bind to specific organisms and is already a technology of interest to the space flight medical community. Protein analysis by mass spectrometry can generate organism-specific spectra. The spectra are compared against signature spectra of known organisms, and thus mass spectrometry can identify organisms in an unknown sample. This technology is of interest to other environmental research groups, such as the Water and Food Analytical Laboratory at JSC, which has mass spectrometers. It is possible that mass spectrometry tests can be combined to provide results to disciplines of chemistry and microbiology. Other advanced methods are being developed in the research community. These methods typically use a lot of consumables and require advanced training on very large pieces of hardware. The key drawback with most advanced methods is that they do not directly measure viability.

Ms. Castro discussed a system that utilized an enrichment step before DNA identification. Enrichment is the amplification of viable microorganisms in a culture medium before DNA extraction. Two issues were brought up regarding molecular methods at this point: (1) Different microorganisms require different DNA extraction methods to disrupt the cell membranes, especially if the microbe is a fungus, and (2) If, during the use of a molecular method, the product of a PCR reaction contaminates the interior of the spacecraft (a nonhazardous event), the subsequent sampling for that organism will always come up positive. The latter is a technical risk common in terrestrial laboratories.

Dr. Quick emphasized that in his experience in remote locations, simple, low-tech, and easy is the preferred approach. His research community is seeking a simple, small test for bacterial contamination of water, like a dipstick, but technical obstacles exist. Their goals are for the technology to be compact, simple, and inexpensive, and to require little to no power.

Dr. Gazda of the Water and Food Analytical Laboratory at JSC thought that a combined environmental monitor to look at chemical and microbiological contamination is a good approach, but such technology may be far off because the chemical compounds of interest, especially on the microbiology side, are not currently known. Dr. Gazda has experience in developing low-tech, straight-forward colorimetric chemical assays for use on the ISS.

With respect to culture plates for future systems, preliminary data show that media plates may last for up to 2 years. Dehydrated media may be a feasible option. The Food Laboratory at JSC is currently developing packaging material for long-duration storage of food, and that research may be applicable to the preservation of culture plates used in classical microbiology methods.

Dr. Ott presented some preliminary data from ISS water systems that were analyzed using a newly developed molecular method called pyrosequencing, which is an advanced sequencing method that can analyze the DNA from all the microorganisms in a sample, even ones that cannot be cultured. This type of study is called metagenomics. He presented a list of microorganisms from the PWD that had been identified at the genus level and asked the panel how useful the information was if they had to make a microbiological judgment about the water. The panel agreed that as this method does not measure viability, the data only beg for more data. They were not able to make a judgment about the water because there was no viability measurement, and there was no specificity beyond the genus identification. For example, the PWD hot water contained the genus *Escherichia*, but they could not tell if it was *Escherichia coli*, which is a fecal coliform indicator. Also, this PWD hot water has no viable bacteria in it, according to heterotrophic plate count. So the water has a questionable microbe in it, but it is not viable; thus, the genetic information alone is not enough to evoke a remediation response, but the viability measure assures that there is most likely no threat. The pyrosequencing method most likely detected a piece of *Escherichia* DNA in the PWD hot water, not a viable organism. The panel agreed that viability is a key measurement in microbial monitoring. Dead pathogens in the water are not a hazard.

Ms. Roman agreed that measurement of viability and the number of bacteria is essential for engineering as well, and the molecular data are interesting, but useful only if they follow a trend; for example, if one of the microorganisms is becoming dominant.

Dr. Mayhall was not concerned with any of the microorganisms in the list, as long as the crew was healthy, but he brought up the caveat that the immune system is dysregulated in microgravity and in a radiation environment. Dr. Quick suggested looking for a good cohort resembling crewmembers on a deep-space mission, possibly kidney transplant patients.

Dr. Thran told how a similar study in the Army using advanced molecular methods caused much concern among her leadership because of the ubiquitous presence of microorganisms in Afghanistan samples. She explained that it was caused by a misperception of microbiology by lay people, and that scientists fail at communicating the existence of normal flora that are not medically significant, and that it is important to educate people so that they know that unless microorganisms are medically significant and present in dose-response concentrations, they are of little concern.

Dr. Mayhall added that what crewmembers carry in their bodies as normal flora is more hazardous than the list of microorganisms discovered by pyrosequencing that were in the ISS water. The panel agreed that it is the microorganisms that crewmembers carry or that are in the system that will be of concern, and if their presence is prevented on the ground by proactive measures, then the likelihood of having a microbiological hazard in space is low.

To help summarize the types of technology needed and associated targets, Dr. Ott brought a cup of water from an unknown source and asked the question, “What would a crewmember need to know to judge the health aspect of the water?” The panel asked questions such as:

- Where did the water come from?
- Was the cup clean?
- Has the water been used more than once?
- What is the temperature or turbidity?
- Is the system that generated it certified?
- What is the amount of biocide in it?

Suggested Research Studies

During the course of the discussion, the panel made a number of suggestions regarding studies to close knowledge gaps:

- NASA should find a cohort among the general population who have immunosuppression similar to that expected to be experienced by crewmembers of exploration missions, and should investigate the efficacy of preventive measures and treatment regimens for infectious diseases among this population of immunosuppressed individuals. Kidney transplant patients might provide such a cohort. Such a study could provide information about dose response to various levels of exposure of certain microorganisms that could be found in the water system. Most of the microorganisms currently found in the ISS water system are considered harmless, but that consideration is a function of the dose response in normal healthy persons without immunosuppression. Such an assumption may not be possible with mounting evidence of immune dysregulation in space flight.
- NASA should understand the impact that biofilms and subsequent endotoxin generation will have on the water system and on the health of long-duration crews. How much endotoxin can make crewmembers sick through ingestion, injection, and inhalation? Does endotoxin become more potent in microgravity?
- NASA water system engineers, chemists, and microbiologists should understand the interaction of compounds such as antibiotics and estrogenic compounds in a recycling water system.

Appendix D – Interim Report: Recommendations from the Forum on Next Generation Microbiological Food Requirements for Space Flight

1. Executive Summary

In a collaboration between the International Space Station (ISS) Program and the Human Research Program (HRP), the Johnson Space Center (JSC) Space Food Systems Laboratory and the JSC Microbiology Laboratory cosponsored a forum in April 2012 during which food microbiology experts from other federal agencies, academia, and industry were invited to provide opinions on the current microbiological specifications for space food products in *Microbiological Specification and Testing Procedure for Foods Which Are Not Commercially Sterile* (SD-T-0251), and to discuss next-generation food microbiology requirements for future exploration missions. ***This interim report only documents the recommendations, considerations, and concerns provided by the subject matter experts during the Forum and may be modified by future discussions with key stakeholders before the final report is submitted in September 2013.***

A final report will be delivered by the HRP to the JSC Chief Medical Officer. The Chief Medical Officer will distribute the report to stakeholders who have the requisite expertise to assess the feasibility of the recommendations.

Several recommendations concerning laboratory testing were well received and deemed operationally feasible by stakeholders; implementation of these recommendations in laboratory operations is under way.

Key recommendations from this Forum include these:

- The document *Microbiological Specification and Testing Procedure for Foods Which Are Not Commercially Sterile* (SD-T-0251) should be updated to ensure that, where feasible, food testing and sampling are aligned with current food industry practices.
- In the investigation of probiotics for the space program, both microencapsulated probiotics and shelf-stable foods containing probiotics should be explored.
- The bioregenerative food system will introduce new safety risks. A strategy that minimizes risk both before and during flight should be developed.
- Future technologies on space exploration vehicles should include
  - Microbial identification or monitoring systems that obviate the need for reagents with a short shelf life, and
  - Sample preparation procedures that address the expected diversity of types of microbiological samples, including clinical and environmental samples.

2. Introduction

Microorganisms are a significant component of the human environment, and no matter how far exploration missions extend that environment into space, microorganisms will be present. Microbial risks associated with the space food system are mitigated through the Hazard Analysis Critical Control Point (HACCP) system, which was developed by NASA, Pillsbury, and the U.S. Army. The HACCP plan for verifying risk mitigation includes microbial testing of food products. Periodic review of microbial testing requirements is necessary so that those requirements comply with changes in technology development and government regulations in the food industry. As human space exploration extends beyond Earth orbit, next-generation food requirements may need to address a bioregenerative food system, provide mission operations guidelines, and include new technologies for onboard microbial testing, food processing, and probiotic use.

The Space Food Systems Laboratory and the Microbiology Laboratory collaborated to cosponsor a panel of internal and external experts who have an extensive knowledge base of food industry testing standards and lessons learned in the Space Shuttle and ISS Programs. The Forum on Next Generation Microbiological Food Requirements for Space Flight was designed to develop recommendations pertaining to requirements currently used to monitor food quality and recommendations pertaining to issues expected to arise during future exploration missions. This interim report is a deliverable of the HRP task "Next-Generation Microbial Requirements," which includes the development of new microbiology requirements for potable water, air, surfaces, and space flight food.

External expert participants in this Forum:

- Stan Bailey, PhD, Director of Scientific Affairs, BioMerieux
- Arun Bhunia, BVSc, PhD, Professor, Purdue University
- Robert Buchanan, PhD, Professor, University of Maryland
- Mike Doyle, PhD, Regents Professor, University of Georgia
- John Hanlin, PhD, Vice-President of Public Health, Ecolab, Inc.
- Kristina Mena, PhD, Associate Professor, The University of Texas Health Science Center at Houston
- Elsa Murano, PhD, Professor and President Emerita, Texas A&M University
- Sam Nugen, PhD, Assistant Professor, University of Massachusetts
- Andy Senecal, PhD, U.S. Army Natick Soldier Research Development & Engineering Center
- Robert Tauxe, PhD, Deputy Director, Division of Foodborne, Waterborne and Environmental Diseases, Centers for Disease Control

Additional expert participants in this Forum:

- Bekki Bruce, Microbiology Laboratory, JSC
- Victoria Castro, Microbiology Laboratory, JSC
- Maya Cooper, Space Food Systems Laboratory, JSC
- Grace Douglas, PhD, Space Food Systems Laboratory, JSC
- Todd Elliott, Microbiology Laboratory, JSC
- Robert Haddon, MD, Space and Clinical Operations Division, JSC
- Vickie Kloeris, Space Food Systems Laboratory, JSC
- Tatyana Modlin, MD, Microbiology Laboratory, JSC
- Mark Ott, PhD, Microbiology Laboratory, JSC
- Cherie Oubre, PhD, Microbiology Laboratory, JSC
- Michele Perchonok, PhD, Space Food Systems Laboratory, JSC
- Duane Pierson, PhD, Microbiology Laboratory, JSC
- Melanie Smith, Microbiology Laboratory, JSC

Report Sections

Section 3: Through the HRP, the Microbiology Laboratory has been developing recommendations for microbial requirements for potable water, food, air, and surfaces in the directed study – "Next-Generation Microbial Requirements." The goals and specific aims of this task are detailed in this section.

Section 4: Seven presentations were given to the panel, and this section is a summary of those presentations.

Section 5: Before the Forum was conducted, a list of questions was developed to stimulate discussion among the visiting experts. These questions focused on key topics in current requirements as well as future exploration requirements.

Section 6: Two types of information are captured here. Key considerations were noted by the forum development team after the Forum as points to contemplate before levying recommendations as requirements on a project or program. In addition, the panel made several suggestions for the laboratories to take actions that had been in development previously or were in development at the time the Forum was held, and the status of these actions is described.

Section 7: There were a number of considerations noted by the Forum development team after the Forum as points to contemplate before levying recommendations as requirements on a project or program.

3. Human Research Program Task Goals

The purpose of this Forum was to gather recommendations from a panel of food microbiology experts on current microbiological specifications and next-generation microbiology requirements for space food products. This task is a subset of the HRP directed research study "Next-Generation Microbial Requirements," which includes the development of new microbiology requirements for potable water, air and surfaces, and space flight food. This interim report is a deliverable specific to this HRP task.

The following are the specific aims of the study:

> Aim 1 – To use historical data to assess optimal requirements for vehicle design and mission operations. This "lessons learned" approach will be incorporated into a microbial risk assessment approach to recommend removal of unnecessary requirements, optimization of current requirements, and implementation of new requirements.
>
> Aim 2 – To assess current and near-term technologies for application toward next-generation monitoring requirements. This aim will also focus on defining gaps between optimal requirements and available technologies and flight resources.
>
> Aim 3 – To define practical monitoring requirements for (a) immediate development of next-generation ground operations and flight hardware and (b) long-term goals for future requirements, if different from those delivered in (a).

To address Aim 1 during the Forum, the panel listened to presentations about the history of the space food system on the ISS, the occurrence of microbiological events, the medical occurrence of infectious disease, lessons learned, and current requirements and operations. Panel members also participated in tours to help the panel understand the space food system, how foods are developed, how they are processed on the ground, and how they are transported to the ISS. Tours of the Space Food Systems Laboratory in Building 17, the Microbiology Laboratory in Building 37, and the Mockup Facility in Building 9 were included.

To address Aim 2, the panel discussed several technologies and requirements. This was not at the level of requirements definition, which is a specific systems engineering process. Instead, the panel made high-level recommendations pertaining to requirements and technologies. In microbiology, the requirement usually dictates the technology. For example, the current microbiology requirements use the measurement of colony-forming units (CFUs) to determine whether the system is meeting the requirement. This dictates that the technology to be used will be heterotrophic plate counts. A spectral-based technology, such as mass spectrometry or Raman spectroscopy, would not use the measurement of CFU and would require different measurements and requirements.

Aim 3 is the definition of practical requirements. *These recommendations are the beginning of the process to define those requirements*. This report is the second of three interim reports describing the findings of each of the forums on potable water, air and surfaces, and food. The *Interim Report: Forum on the Next-Generation Microbiological Water Requirements for Space Flight* was released in October 2011.

The recommendations presented here are of two distinct types: recommendations related to the microbial specifications of the food testing documented in *Microbiological Specification and Testing Procedure for Foods Which Are Not Commercially Sterile* (SD-T-0251), and recommendations related to a broader range of operational and technical aspects of the immediate future of the space food system on the ISS and a more distant future with the exploration missions to asteroids and Mars. The future missions will include not only the packaged food system but also a bioregenerative food system.

4. Summary of Presentations

Overview of Medical and Microbiological Aspects of Space Flight: Robert Haddon, MD

Dr. Haddon provided the perspective of a NASA flight surgeon in addressing infectious disease diagnosis and mitigation. Crewmembers are screened for many microorganisms before flight, including HIV, *Helicobacter pylori*, and organisms causing syphilis; hepatitis A, B, and C; tuberculosis; and methicillin-resistant *Staphylococcus aureus* (MRSA). In-flight treatment includes antibiotics, fluid replacement, supplemental oxygen, wound care supplies, and waste disposal equipment. Postflight infection management consists of surveillance of MRSA, environmental samples, and efficacy of pharmaceuticals; immune system assays; definitive care; physical rehabilitation; and social reintegration. Dr. Haddon's key message was that space flight infections will be with familiar microorganisms, but with variations related to effects of the space environment, such as altered immune response and changes in virulence.

Environmental Microbiology of Space Flight: C. Mark Ott, PhD

Dr. Ott discussed various aspects of the environmental impact of microorganisms on space vehicles. A microbial risk assessment is necessary to develop the appropriate requirements that protect the crew without wasting resources. Preflight activities include clinical and environmental monitoring, food sampling, and biosafety review of payloads. In-flight monitoring consists of sampling air, surfaces, and potable water of the space vehicle. Dr. Ott described specific cases of microorganisms being found in food samples during standard testing using the specifications found in SD-T-0251. He discussed the alterations in host immune response and microbial virulence. His key points were that the lessons learned from the ISS and the Space Shuttle Program were an excellent view of "space normal," the data reflected detection of normal environmental flora and the presence of opportunistic pathogens, and prevention is the most cost-effective countermeasure; however, contamination cannot be avoided. The lack of information about microorganisms and their impact during space flight missions remains fairly substantial, resulting in a conservative approach to requirements

Space Food System Overview: Vickie Kloeris, MS

Ms. Kloeris provided examples of the different types of food prepared in the Space Food Systems Laboratory, including powdered drinks, rehydratable foods, thermostabilized foods, intermediate-moisture foods, and natural-form foods. She also described how the food is prepared, processed, and packaged in the laboratory. She explained how the crewmembers unpack the food and prepare it for consumption, what their dining conditions are, and which types of utensils they use.

Biomedical Aspects of Early Interplanetary Expeditions: John Charles, PhD

Dr. Charles described research on the human response to microgravity, including fluid shifts, changes in behavior, and musculoskeletal losses. In addition to the ISS, low-Earth orbit may soon be the site of other destinations, such as space stations designed by other nations or commercial entities. The Augustine report described other destinations, such as lunar orbit and surface, near-Earth objects, Lagrange points (areas of stable gravitation between the sun, Earth, and moon), and Mars. A Mars mission will consist of at least a 6-month transit to Mars and an 18-month stay on the surface before a 6-month transit to return to Earth; the entire mission will last 30 months. Most long-duration flights have been only 4-7 months long. The Mars mission will have to be mostly autonomous communications one way can take 3 to 22 minutes, depending on the positions of Earth and Mars in their orbits. The projected rates of significant illness for a Mars mission are about one person per mission requiring an emergency room capability, and one person per three missions requiring intensive care unit capability. Some of Dr. Charles's key points were these: human space flight requires research to understand the effects of space on humans, space exposure is necessary for doing such research and it cannot be duplicated, and research on the ISS is needed to reduce the risks to the crews.

Future Food Systems: Michele Perchonok, PhD, Grace Douglas, PhD, and Maya Cooper

Drs. Perchonok and Douglas and Ms. Cooper explained the future space food system, how probiotics may be introduced, and how a bioregenerative food system may be used.

The Advanced Food Technology project is developing a space food system that is safe, nutritious, and acceptable, and effectively balances space vehicle resources such as mass, power, and time. The ultimate goal is to produce a food system that supports all aspects of a Mars mission. Some potential technology needs are surface materials that minimize bacterial growth, rapid microbial testing technology that does not require an enrichment step (growing the bacteria), and safe food cleaners/sanitizers that minimize resources, such as water, mass, and volume.

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Research results suggest that probiotics can antagonize pathogens, protect against infection, prevent and treat antibiotic-associated diarrhea, reduce cancer biomarkers in the colon, reduce cold and flu-like symptoms, alleviate gastrointestinal issues, and prevent urinary tract infections. Introduction of probiotics will cause current flight-approved microbial limits to be exceeded, and a method of introducing them should be developed.

Three paths are possible for future food system development: all bioregenerative and bulk ingredients only, packaged foods only, or a combination of the two. Bioregenerative systems have a cycle of water and waste that will have an impact on the final microbial population of the biomass produced. Currently, the project is expecting the technology for the bioregenerative system to be hydroponic plant growth chambers. An important point to note is that the microbial loads in the flesh of the plants are as prevalent as the surface contamination. Similar to risks found on Earth, food processing that occurs in a Mars habitat will have risks such as improper holding of hot or cold food, poor personal hygiene, cross-contamination, inadequate cooking, and unsafe food sources.

Life on ISS: E. Michael Fincke, Colonel, USAF

Colonel Fincke explained the realities of human space flight and life on board the ISS, including the space food used on the Space Shuttle and the ISS. He said that one of the main things he missed while he was on the ISS was fresh, crunchy vegetables like those found in salads. During his time on the ISS, he noted that the Russian cosmonauts grew mizuna, a Japanese mustard green, in a terrarium for several generations; however, they were not allowed to eat it.

Funding Opportunities: Michele Perchonok, PhD

Dr. Perchonok described a number of NASA research funding opportunities, such as the Small Business Innovative Research Program and Small Business Technology Transfer Program, the NASA Solicitation and Proposal Integrated Review and Evaluation System, and the NASA Human Health and Performance Center.

5. Discussion Questions

Before the Forum convened, a list of questions was developed to stimulate discussion among the visiting experts. These questions focused on key topics in current requirements and future exploration requirements.

Food Microbiology Specifications and Requirements

1. The current specification for total aerobic microbial count is no more than 20,000 CFU/g. This has proven to be difficult to meet for certain ingredients such as tea and spices, which are added in a small amount to the final food product.
    a. Should ingredients with a high microbial load be tested before they are added to a product, or should the acceptability limit apply only to the final product that the crew will consume?
    b. If such ingredients are tested, what should the pass/fail criteria be?
    c. Do industry processes and specifications exist for small-quantity ingredient testing?
2. After review of the consultant's report, do you have any significant disagreements? Should additional tests be added to SD-T-0252 *Microbiological Specification and Testing Procedure for Commercially Sterile Foods* or to SD-T-0251 *Microbiological Specification and Testing Procedure for Foods Which Are Not Commercially Sterile*?
    a. Suppose that, because of outbreaks of foodborne illness (such as *Salmonella* in peanut butter), the FDA suggests additional testing to food producers nationwide. How should NASA manage testing procedures in response to external influences such as the FDA?
    b. Are there microorganisms that are not currently in the testing specifications that may be of concern in our freeze-dried, irradiated, thermostabilized, or natural-form foods in the space flight environment?

Future Food Systems

3. What additional microbiological risks may be present in future food systems during missions of longer duration with the possibility of a partially bioregenerative food system?

4. What additional risk mitigation strategies do you suggest for future food systems?

5. Risk vs. cost trade: Given that exploration missions can last up to 2.5 years, how conservative do you think NASA should be about microbial risks, especially considering that the probability of

illness is not the same for astronauts as it is for the general population? Astronauts are generally a healthier population, but their immunity may be altered in space.

Technology Gaps and Development

6. What developing technologies would improve testing of products? Such testing would include rapid testing with no enrichment step to test fresh produce grown in transit or on a surface mission.

7. How would you introduce, test, and extend the viability of probiotics in shelf-stable foods?

6. Recommendations

At the Forum, the expert panel of food microbiologists made a total of 34 recommendations:

- 21 recommendations pertained to current food microbiology requirements (SD-T-0251). These recommendations were either overarching for the whole set of requirements or pertained to one of four specific categories of food.
- 13 recommendations pertained to next-generation food microbiology requirements for future exploration missions.

As the requirements for food safety, food spoilage, and contamination during a mission will vary depending on the mission architecture and food provisioning systems, the panel recommended that requirements for current ISS missions and future exploration missions be developed separately.

Recommendations Pertaining to Current Food Microbiology Requirements

The primary NASA document delineating current microbiological requirements for space flight food is *Microbiological Specification and Testing Procedure for Foods Which Are Not Commercially Sterile* (SD-T-0251). These requirements are used to verify the HACCP plan for the space food system.

Before the Forum was held, an external food microbiology consultant was retained to provide initial recommendations pertaining to SD-T-0251. These recommendations were provided to the panel to stimulate discussion during the Forum. Also, the panel considered a number of discussion questions concerning food microbiology issues related to specifications, which can be found in Section 5.

There are several key considerations related to proposing recommendations as requirements. Please see Section 7 for those key considerations. Several of its suggestions for actions were already being acted upon, and those are also listed in Section 7.

After engaging in the aforementioned discussions of current microbiological food requirements and testing, the panel made the following recommendations.

Overarching Recommendations Pertaining to SD-T-0251

Seven overarching recommendations were made for changes and additions to *Microbiological Specification and Testing Procedure for Foods Which Are Not Commercially Sterile* (SD-T-0251), involving testing rationale, trends in the food industry, and outreach to food companies.

(F1) A rationale should be provided for the testing criteria in SD-T-0251, and a decision-making matrix should be developed.

The panel recommended that more information be provided with the testing specifications to minimize time and effort in future reevaluation of the requirements. Types of information that should be considered include the criteria for selection of indicator organisms, the rationale behind the need for testing of each food item or group, and the public health concern being addressed for each test. The panel also suggested that a decision-making matrix would be beneficial to explain the rationale. This matrix would describe the risk to be mitigated, including the source of the raw commodity, steps for food preparation, and the rationale behind selection of the indicator organisms.

(F2) The sampling procedure in Section 3 of SD-T-0251 should be updated with current methodologies used in the food industry, such as those used by the International Commission on Microbiological Specifications for Foods (ICMSF).

The sampling plan used should have a confidence level of 95% that the test organism is present at a defined level. The Space Food Systems Laboratory should have a sampling plan for each of the specifications and should have performance characteristics for the sampling plan. Sampling plans have affected court cases in the food industry in recent years. For example, studies have shown that if three 100-g samples were spiked for contamination, the probability of detection was only 30 percent. If thirty 10-g samples were analyzed, the probability of detection was above 95 percent.

(F3) The environmental sampling plan currently in use should be augmented to add analysis of air samples from the food production areas.

Environmental monitoring provides greater sensitivity in the isolation of potential contaminants than post-process food monitoring alone. The Space Food Systems Laboratory does environmental surface testing and should include air samples. The panel agreed that it is important to verify the air quality at least once a month. Please see Section 7 for further consideration of environmental testing.

(F4) *Enterobacteriaceae* (EB) testing should be performed in lieu of coliform testing.

Historical testing of food and water relied on the detection of coliform bacteria as indicator organisms of potential fecal contamination. The entire food industry is converting to EB testing because the Enterobacteriaceae family of bacteria includes coliform organisms in addition to clinically relevant bacteria responsible for many cases of foodborne illness, such as Salmonella and Shigella. The testing protocol and acceptability limits for EB testing will need to be determined. The panel discussed performing EB testing several times during the analysis of specific food categories, including dry powder beverages, dried fruits, and rehydratable foods.

(F5) All cook or kill steps should be validated.

The cook or kill steps are specific critical control points where the cooking temperature kills the microorganisms in the food product. Validation testing includes challenging the cook step with a known amount of a known microorganism and verifying that the microorganism has been killed. The panel noted that in the future the Food and Drug Administration (FDA) will be requiring all manufacturers of cooked food to validate the cook steps for their products to ensure that they will kill any pathogens present. This approach might decrease the inherent risk associated with random

sample monitoring. Please see Section 7 for a discussion of the impact of validation studies on the Space Food Systems Laboratory.

(F6) Ingredients likely to have a high microbial load, such as spices, should be tested, even if they are in small amounts in the final product.

The panel suggested that it is important to start with the cleanest ingredients possible regardless of the amount used. Specific examples of food contamination were discussed including botulism from spices in oil and Salmonella *contamination in broccoli powder used to flavor chips. In general, the panel agreed that allowing the use of contaminated spices was not good practice.*[1]

[1]Comment from Dr. Doyle: "Untreated spices can indeed have high microbial loads but treatment with a high dose of irradiation or ethylene oxide which are acceptable commercial practices, can mitigate the risk of pathogen contamination. Rather than not using spices as ingredients, I would suggest ensuring (by using a highly reputable supplier) the spices have received the proper treatment and verify this by microbial testing. It is imperative that a reputable spice supplier, such as McCormick Company, be used as many foreign suppliers have poor track records."

(F7) The Space Food Systems Laboratory should reach out to spice and tea manufacturers to discuss the microbial load of their products.

Currently, the Space Food Systems Laboratory procures spices directly from the grocery store because the current scale of food production is small. Several panel members were familiar with how microbial testing is performed in the spice production industry and suggested that the Space Food Systems Laboratory could discuss this with the manufacturers in order to procure spices with a reduced microbial load directly from them. Having product requirements such as steam-sterilization or irradiation may be necessary to ensure the microbial safety of spices.

The results of testing teas are often "too numerous to count" and positive for Gram negatives such as Enterobacteriaceae family, Enterobacter cloacae, Klebsiella oxytoca, Klebsiella pneumoniae, *and* Enterobacter *aerogenes. The risk is reduced by using the hot water supply on the ISS. However, it is not guaranteed that crewmembers make tea with hot water. They may actually be making it with cold water. The Centers for Disease Control and Prevention (CDC) has been watching for outbreaks of infection among the users of "sun tea," which is the brewing of teas in tepid water, but has never seen such an outbreak. On the ISS, the water temperature can vary if there is heavy usage of the hot water during the day, such as during breakfast time. There is no guarantee that the hot water on the ISS is a kill step. Also, the tea is often consumed over the course of several hours. It may be a good idea to irradiate tea at the Texas A&M electron beam facility, though the panel understood that irradiation of small lots of tea is expensive. Please see Section 7 for more on outreach to food production companies.*

<u>Recommendations Pertaining to Food Categories in SD-T-0251</u>

The panel made 14 recommendations about the categories of food products produced in the Space Food Systems Laboratory, including beverages, natural-form products, intermediate-moisture foods, and rehydratable foods.

Beverages (all powdered products)

(F8) Only pasteurized versions of real-juice beverages should be used in the Space Food System.

As most juices used for space flight are flavored drinks, this would apply to only two current items. Using pasteurized real juices would preclude specific testing for microorganisms, including enterohemorrhagic Escherichia coli *and* Cryptosporidium. *The panel suggested that a*

pasteurization requirement could be documented in the purchasing specifications of real-juice beverages.

(F9) *Salmonella* testing for all beverages, including cocoa, should be maintained, but the enrichment step should be modified.

Several technical considerations pertain to the testing of Salmonella *based on food sample chemistry. The enrichment or growth step in this category must mitigate acid injury to* Salmonella *in fruit juices. The enrichment step that the FDA's Bacteriological Analytical Manual describes should be used for that food sample product.*

(F10) All dried products, including any beverages (real juice, flavored drinks, milk, cocoa, etc.) should be tested for EB.

This recommendation is related to the food industry conversion from coliform testing to EB testing.

Natural-Form Products

(F11) A 2-log kill step should be used for nuts other than peanuts.

A 2-log kill step is a cooking step that reduces the number of microbes by two orders of magnitude, for example from 1000 to 10. The panel suggested that a requirement for a 4- to 5-log kill step for nuts other than peanuts was not feasible because there were not that many bacteria to start with. They suggested that a 2-log kill step was more realistic and will mitigate 99.99% of the risk.[2]

[2]Comment from Dr. Doyle: "The 2-log kill step should be for *Salmonella* and *E. coli* O157:H7 which are the principal pathogens of concern for nut contamination."

(F12) All items in the food category of nuts should be tested for Salmonella.

Salmonella *outbreaks involving peanuts and pistachios have occurred in the past*. Salmonella *is a significant risk in nuts.*

(F13) Testing of nuts for yeast and mold should be eliminated.

Testing for yeasts and molds is more for a long-term food spoilage issue than a short-term food safety issue. Yeast and mold growth is not an issue if the water activity (A_w) is very low. The panel added that if the concern is for Aspergillus flavus*, then one should test for aflatoxin instead.*[3]

[3]Comment from Dr. Hanlin: "The water activity level threshold for microbial growth is Aw <0.6."

(F14) All baked goods with A_w of 0.6 or more should be tested for yeasts and mold.

If a yeast or mold is in a baked good with an A_w of 0.7-0.8, it will grow; however, it may take the growth of the contaminant as long as 2 years to become a significant food safety risk. If A_w is less than 0.6, yeast and mold testing can be eliminated.[4]

[4]Comment from Dr. Doyle: "If preservatives such as propionic acid, benzoic acid and/or sorbic acid are added to bakery products at appropriate levels, mold contamination can be controlled. These food additives which are GRAS in bakery products can be used safely in bakery products having a water activity greater than 0.6; however, challenge studies using spoilage molds are needed to validate that the preservative system is effective."

(F15) All baked goods should be tested for *Salmonella*.

About 1 percent of flour samples in the food industry test positive for Salmonella.

(F16) Yeast and mold testing for candy should be eliminated.

The panel did not think it was necessary to test candy for yeast and mold.[5]

[5]Comment from Dr. Doyle: "This is an appropriate recommendation if the water activity is below 0.6 or if a suitable preservation system is used for candies above 0.6. Soft, cream-filled chocolates typically contain benzoate to prevent the growth and gas production of yeasts. Hence, the qualifications noted above should be included as criteria for eliminating yeast and mold testing of candies."

Intermediate-Moisture Foods

(F17) Dried fruit should be tested for EB and *Salmonella*.

Dried fruit is an intermediate-moisture product and needs an indicator in addition to Salmonella. *EB testing makes aerobic plate count testing unnecessary for this specific food category. Yeast and mold testing is unnecessary.*[6]

[6]Comment from Dr. Hanlin: "One organism of concern that is associated with fruit is *Cyclospora*.

Rehydratable Foods

(F18) Aerobic plate count testing for post-packaged rehydratable foods should continue.

During the packaging process, the risk of contamination with Salmonella *is minimal, and pathogen-specific testing for these products is unproductive. The odds of detecting pathogen recontamination are low. Instead, contamination should be determined by monitoring aerobic plate counts in addition to augmenting environmental testing of the processing area using air sampling.*[7]

(F19) Rehydratable foods should be tested for Enterobacteriaceae and *Salmonella*, <u>in addition to</u> performing post-packaging aerobic plate counts <u>and</u> augmented environmental testing of the processing area, such as the freeze-drying room, for *Salmonella*.

The panel agreed that these four testing criteria were required for rehydratable foods.[7]

[7]Comment from Dr. Doyle: "I suggest also testing for APC the product-contact surfaces of processing equipment used to prepare these foods, especially near the end of product runs."

(F20) Testing of *Staphylococcus* enterotoxin in rehydratable foods should be eliminated.

Staphylococcus *enterotoxin testing is for human handling and fermentation failures. The food in space food systems is not heavily handled by humans, nor is there any fermented foods.*[8]

(F21) The sources of frozen fruits should be tracked.

The panel suggested that tracking should be considered for the sources of frozen fruits like strawberries, which come into the Space Food Systems Laboratory frozen from unknown sources. Many fruits come into the U.S. from other countries and such products have caused food safety issues in the past.[9]

[8]Comment from Dr. Doyle: "Many frozen fruits do not receive a treatment that would effectively kill human pathogens. *Norovirus* and hepatitis A virus outbreaks have been associated with frozen raspberries or strawberries. Frozen fruit should receive a treatment such as thermal processing to ensure pathogen inactivation."

Recommendations Pertaining to Next-Generation Food Microbiology Requirements

Of the total of 34 recommendations by the panel, 13 recommendations were related to future exploration missions. To gain greater understanding of key issues and stimulate conversation, discussion questions were posed to the expert panel. Those discussion questions can be found in Section 5. During this discussion, the panel made the following recommendations.

(F22) Food from future bioregenerative food systems should be managed by designated, trained personnel on the mission.

Similar to the function of designated medical and science officers, lead food preparation specialists should manage the food safety requirements. Although all personnel could prepare food, one specialist should be trained to inform and guide the crew in safe food preparation and storage. These food service specialists should have a biology or food science background or training and should be responsible for the bioregenerative food system itself. The optimal situation would be that the specialist prepares all the food, but practical considerations may make this option unlikely.

(F23) The components of future bioregenerative food systems should be sterile or thoroughly disinfected to minimize pathogenic risk to the food supply.

The entire bioregenerative food system is at risk if any human or plant pathogens, such as Salmonella *or fungal spores, get into the system. The panel discussed a couple of methods to thoroughly disinfect seeds and still maintain their viability. The water should be very clean and potable. The use of gnotobiotic (germ-free) plants was discussed; however, practical considerations make this option less likely. The vehicle or habitat is a likely source of plant contamination. Food processing also would be a key potential source of contamination.*

(F24) A strategy to reduce risks to future bioregenerative food systems that are caused by human error should be developed.

Even if future bioregenerative food systems are delivered sterile, human manipulation of the system will most likely introduce microorganisms into the system.[9]

[9]Comment from Dr. Hanlin: "There should be consideration of hand hygiene and food preparation or consumption, as hands have touched many surfaces and will or may touch food and mouth during preparation, eating, and drinking."

(F25) Formal microbial risk assessments for the food systems, including on-orbit packaged foods and future bioregenerative food systems, should be performed to fully understand the overall risk.

A formal microbial risk assessment would clarify the level of acceptable risk and have an impact on sampling methods and frequency. The degree of conservatism of current and future monitoring requirements should be based on actual risk to the crew rather than risk to the general public. The panel recommended Sky Chef, a commercial supplier of food to airlines, as a source of information for quantitative risk assessments.

(F26) The Exploration program should use a reverse quarantine of the crew, their families, and the critical personnel for a couple of months before flight.

A reverse quarantine is the isolation of relatively healthy people from a population with disease. The purpose of this is to isolate the critical people and allow time for both the transmission of and recovery from any short-term illnesses, such as upper respiratory infections. This is similar to what occurs in the first months of an Antarctic mission. Usually, in the first few weeks of isolation, people spread any low-grade infectious diseases they are carrying and it propagates through the population until the infections run their course.

(F27) New technologies to be used for the enumeration and identification of microorganisms onboard long-duration missions should require reagents without a short shelf life, and technology development also should focus on sample preparation for a diverse range of sample matrices.

Recent advances in food-monitoring technology have focused on high-throughput screening with multi-pathogen and multi-analyte testing without a pre-enrichment (growth) step. Many technologies are in development, and it is uncertain which will be dominant in 5 or more years. For example, spectral-based technologies, such as Raman or Fourier-transform infrared spectroscopy, do not require the use of biological probes with short shelf lives. Other technologies that are being developed include the use of microfluidic chips to concentrate samples by means of dielectrophoresis to avoid an enrichment step. Consideration of the sampling itself is important, so that a confidence level can be projected from the sampling size. For example, in a 2-µl sample, there will need to be 3000 organisms per milliliter to have a 90% probability that there will be an organism in the small sample. Also, the instrument used for enumeration and identification will likely be needed for clinical and environmental monitoring as well, so it should also be able to test for microorganisms in blood, urine, and water.

(F28) Regardless of the choice of new technology, the hardware and process should be equivalent to those approved by AOAC International, and verifiable in the ground laboratory.

AOAC International is a scientific quality association that promotes food quality assurance, and this organization reviews current methods and technologies used in food microbiology. The technology used should be recognized by AOAC International.

(F29) New retort technologies for food preparation should be considered.

New retort technologies for food preparation are being used in the food industry, such as the Shaka system used by Hormel. These new technologies are being used to improve food quality and transform normally refrigerated products into shelf-stable products.

(F30) Probiotic foods, which have been demonstrated to inhibit infection by likely pathogens, should be provided to the crew throughout crew quarantine and all mission phases.

Probiotic foods may be used to positively influence the microbial environment or microbiome of the crew before flight. Probiotic strains of microorganisms with direct and known mechanisms of preventing likely pathogenic infections would be useful to reduce the risk of disease. [10]

[10]Comment from Dr. Hanlin: "Probiotic microorganisms can survive dehydration, and can be delivered as capsules or in a food product and are effective in protecting against food-borne pathogenic microorganisms."

(F31) The use of microencapsulated distribution of probiotic organisms and other technologies should be investigated to improve shelf life and quality control.

Microencapsulation could provide an efficient way to introduce probiotics to the crew and avoid the need to develop probiotics in shelf-stable foods. However, addition of probiotics directly to the food would ensure their consumption.

(F32) Research in the rotating wall vessel should be expanded to include other organisms on the CDC's list of foodborne diseases, especially *Norovirus* and spore-forming bacteria.

Many studies have been conducted on the behavior of Salmonella *and* Staphylococcus aureus *in low-shear modeled microgravity. The CDC's list of foodborne illnesses includes those caused by Norovirus,* Cryptosporidium*, and* Campylobacter*, and these microorganisms are good candidates for future research. The panel also recommended studying the behavior of spore-formers in microgravity because these organisms may have a significant effect on food over a 5-year shelf life.*

(F33) Research on bioregenerative food systems should be expanded.

The bioregenerative food system will be a complex system used to grow plants for human consumption. Human and plant pathogens will need to be eradicated from the system, and a contingency plan will need to be developed to restart the system after a catastrophic failure due to infection by microorganisms. The biggest potential microbiological issues would be caused by the proliferation of Listeria, Burkholderia, *or* Salmonella.

(F34) Collaborative research with major food producers should be encouraged for mutual benefit, such as understanding their processing and testing programs.

Collaborative research and interaction with food producers provides insight into their facilities, processes, and safety protocols. It would be good to have a program that would be prerequisite to HACCP, such as an ingredient approval program. Please see additional considerations in Section 7.

In summary, the Forum was a highly successful joint venture between the Space Food Systems Laboratory and the Microbiology Laboratory, acting under the auspices of the ISS Program and the Human Research Program to gather recommendations on the microbial aspects of food from a panel of external food microbiology experts from academia, industry, and government. They provided recommendations for both the specific requirements of current food products for the ISS and broader next-generation requirements for exploration missions.

A reasonable amount of uncertainty exists in the question of host-microbe interactions during space missions, such as whether immune dysregulation is occurring and whether bacteria do become more virulent. As data are gathered and these questions are answered, microbiological requirements will have to be reevaluated to ensure crew health and safety.

7. Key Considerations from Discussion Session

Two types of information are captured here. Key considerations were noted by the forum development team after the Forum as points to contemplate before levying recommendations as requirements on a project or program. In addition, the panel made several suggestions for the laboratories to take actions that had been in development previously or were in development at the time the Forum was held, and the status of these actions is described.

<u>Validation studies of cook or kill steps</u>

The validation of cook or kill steps requires adding a known pathogen to a food product and verifying that the temperatures used to cook the food also kill the pathogen. The panel believed that the validation of kill steps with the entire product is better than testing small amounts of spices, such as parsley, that are added to the final product later. The microbiological testing of the spice analyzes a very small fraction of the product, and the odds of finding a pathogen are very small. Dr. Bailey likened this concept to "trying to find a needle in a haystack." A validated kill step or cook step mitigates the risk of not finding pathogens that are present.

The panel discussed validation studies several times during the Forum and they clearly recommended the validation of cook or kill steps; however, the practicality of the approach for the extensive number of food items produced by NASA would need to be investigated. The Space Food Systems Laboratory is familiar with validation studies, and they pointed out during the course of the Forum that those studies are very expensive, and they require the testing to be performed in a facility different from the food production area because of the controlled introduction of pathogens into the food. This would necessitate the use of an outside food microbiology testing service to perform these validation studies. A requirement to perform validation studies on food products from the Space Food Systems Laboratory would add a significant cost and effort to the program, and the addition of those studies should require a feasibility assessment. The Space Food Systems Laboratory already maintains higher standards than the food industry. Such validation testing may be more critical for exploration-class missions that require foods to have a very long shelf life, such as 5 years.

References in SD-T-0251

The panel suggested that the reference materials in the SD-T-0251 should be updated. The Microbiology Laboratory has been aware that many of those references need to be updated. They have been working to update the references and will have those changes ready for the next revision of the SD-T-0251.

Augmented environmental testing of food production areas

During discussions of environmental testing, including the testing that the Space Food Systems Laboratory currently performs, the panel emphasized the importance of performing additional environmental testing, including air sampling, to enhance the detection of food pathogens instead of depending on "searching for a needle in a haystack" as suggested by Dr. Bailey. If food pathogens are in the food production area, the likelihood that they will be detected will be greater if additional environmental testing is performed than if food product sampling alone is used. Also, the environment would be the source from which pathogens could be reintroduced into the food after a cook or kill step. Additional environmental testing was considered a testing method to enhance other types of testing, not to substitute for them.

Definition of "zero bacteria"

The panel suggested that the number of bacteria in a microbial culture that has no detectable bacteria should be described as "below the lowest limit of detection" instead of zero. As microbiological monitoring is based on a probabilistic approach, Dr. Buchanan commented that the requirement would be more accurate if it referred to the sensitivity level of the monitoring technique. The Microbiology Laboratory reports this way for reasons of simplicity while operating in a system focused on engineering disciplines.

Outreach to food production companies

The panel suggested that NASA should initiate outreach to food production companies to look for areas of mutual benefit. This was a recurrent theme during the Forum. It was brought up during discussions on spices, teas, and research gaps. In the past, the Space Food Systems Laboratory has reached out to major food producers like Kraft. Although employees of such companies show much interest and excitement at first, NASA has not been able to make relationships cost-effective for the food companies because of the small purchase volume required for space food. Early in the space program, the knowledge that a company could advertise the fact that their food was used in space was enough to overcome the lack of financial incentive to work with NASA food systems; that is no longer the case in today's business environment. The Space Food Systems Laboratory currently works successfully with grocery suppliers and small food companies.[11]

[11]Comment from Dr. Doyle: "There needs to be a scientific basis to support this statement. I am aware of many small food companies that are not well-grounded in food safety principles. There are many other major food companies besides Kraft that have outstanding food safety programs. General Mills and ConAgra are two examples. More effort should be taken to align with some reputable major food companies."

Use of pasteurized liquid eggs

The panel suggested that pasteurized liquid eggs should be used for the freeze-dried egg products because they thought the scrambled egg food product was derived from shell eggs. The panel believed that using shell eggs was not worth the risk of foodborne illness when the pasteurized version carries very low risk. The Space Food Systems Laboratory verified that they currently use pasteurized liquid eggs.

Appendix E – Interim Report: Recommendations the Forum on Next-Generation Microbiological Surface and Air Requirements for Space Flight

1. Executive Summary

In February 2013, the Johnson Space Center (JSC) Microbiology Laboratory convened a panel of experts in surface and air microbiology (Forum on Next-Generation Microbiological Surface and Air Requirements for Space Flight, or "the Forum") to develop and recommend a new set of requirements for vehicle design and mission operations, including microbiological monitoring, to be based on "lessons learned" and new technology. Discussions at the Forum were focused on requirements contained in *NASA Standard 3001, Volume 2: Human Factors, Habitability, and Environmental Health*, and other engineering design standards and requirements for hardware and vehicle design that are derived from Volume 2; however, the content of many recommendations would also fall under *NASA Standard 3001, Volume 1: Crew Health*. All of the recommendations will be forwarded to appropriate individuals in the Space and Clinical Operations Division of the Human Health and Performance Directorate and in the Engineering Directorate for their consideration. Recommendations from this Forum also focused on microbial monitoring technology and associated monitoring strategies. This interim report only documents the recommendations at the Forum and may be modified by future discussions before the final report is submitted in December 2013.

Key findings from this Forum include these:

- In determining routes for contamination of environmental air and surfaces, the crew must be considered a primary source for medically significant microorganisms.
- Routine microbiological monitoring of air and surfaces is valuable as a method to verify contamination control plans.
- Current methods of disinfecting cargo bags may not be adequate, and technologies with greater stringencies, such as preflight sterilization, should be investigated. The investigation should include solutions from the disciplines of human factors and engineering, and evaluation of new disinfectants for in-flight use.
- Because the risk assessment of infectious disease depends on immune system function and the behavior of aerosols in microgravity, the uncertainty in these areas should be investigated.
- Immune system status during deep space missions is expected to differ from immune status on International Space Station (ISS) missions, so risk assessments for the two types of missions should be developed separately.

2. Introduction

The future of human space flight will continue to include interaction of humans with microorganisms that are already part of their environment. Risks to crew health from these microorganisms must be mitigated, and space flight programs mitigate crew health risks by adhering to standards and requirements such as those found in *NASA Standard 3001, Volume 1: Crew Health* and *NASA Standard 3001, Volume 2: Human Factors, Habitability, and Environmental Health*.

Many of these requirements and standards are in the form of acceptability limits, which space vehicle components must pass before, during, and/or after space flight missions. Current microbiological monitoring protocols for air and surface samples use a classic culture-based methodology for enumerating microbes. Identification at the species level requires a specialized skill set and bulky equipment items, and is performed in ground-based laboratories.

As space exploration progresses toward missions that are planned to include lunar habitats, Lagrangian points, near-Earth objects (NEOs), and Mars, current monitoring strategies may not be feasible because of limited or no resupply, delayed crew return, and delayed communications. Current culture-based monitoring methodology relies on large volumes of consumables with limited shelf lives. The use of culture-based methodology dictates NASA's current acceptability limits, which are defined by "measurement criteria" in the form of colony-forming units (CFUs) – representing the growth of one microorganism at a single location on an agar medium – in a given volume. Thus, the use of next-generation monitoring technologies, such as DNA or RNA sequencing, is restricted, as its measurements cannot easily be related to CFUs.[1]

[1]Comment from Dr. Morse: "The use of these technologies for quantitation of microbial burden is problematic as it says nothing about the viability of what is being measured. Residual nucleic acid could remain after disinfection and cleaning. The formation of a discrete colony on culture indicates that the microorganism is viable and potentially capable of infecting an individual."

These constraints, as well as the considerable microbiological knowledge that has become available over the life of the ISS, have led to the need to engage a panel of internal and external experts to review current requirements for mitigation of microbial contamination and to provide recommendations for updated requirements for vehicle design and mission operations.

External expert participants in this Forum:

- John Hanlin, Vice-President, Food Safety and Public Health, RD&E, Ecolab, Inc.
- Glen Mayhall, MD, Professor Director, Infection Control and Hospital Epidemiology, Department of Internal Medicine, Division of Infectious Diseases, University of Texas Medical Branch
- Kristina Mena, MSPH, PhD, Associate Professor and Program Head, Division of Epidemiology, Human Genetics and Environmental Sciences, School of Public Health, University of Texas Health Science Center
- Leonard Mermel, DO, ScM, AM (Hon), FSHEA, FIDSA, FACP, Medical Director, Department of Epidemiology & Infection Control, Rhode Island Hospital, Warren Alpert Medical School of Brown University
- Stephen A. Morse, MSPH, PhD, Associate Director for Environmental Microbiology, Centers for Disease Control and Prevention (CDC)
- Brandolyn Thran, PhD, Microbial Risk Analysis, Army Institute of Public Health
- Stephen Vesper, PhD, Microbiological and Chemical Exposure Assessment Research Division, National Exposure Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency

Additional expert participants in this forum:

- Doug Botkin, PhD, Microbiology Laboratory, JSC
- Bekki Bruce, Microbiology Laboratory, JSC
- Victoria Castro, Microbiology Laboratory, JSC
- Todd Elliott, Microbiology Laboratory, JSC
- Robert Haddon, MD, Space and Clinical Operations Division, JSC
- Mark Ott, PhD, Microbiology Laboratory, JSC
- Cherie Oubre, PhD, Microbiology Laboratory, JSC
- Duane Pierson, PhD, Microbiology Laboratory, JSC
- Monserrate Roman, Marshall Space Flight Center
- Melanie Smith, Microbiology Laboratory, JSC

3. Project Goals

The purpose of the Forum was to develop and recommend a new set of requirements for vehicle design and mission operations, including microbiological monitoring, that are based on "lessons learned" and new technology. This task is a subset of the Human Research Program (HRP) direct research study "Next Generation Microbiology Requirements," which includes the development of new microbiology requirements for potable water, food, and surfaces and air.

The specific aims of the study are the following:

> Aim 1 – To use historical data to assess optimal requirements for vehicle design and mission operations. This "lessons learned" approach will be incorporated into a microbial risk assessment approach to recommend removal of unnecessary requirements, optimization of current requirements, and implementation of new requirements.
>
> Aim 2 – To assess current and near-term technologies for application to next-generation monitoring requirements. This aim will also focus on defining gaps between optimal requirements and available technologies and flight resources.
>
> Aim 3 – To define (a) practical monitoring requirements for immediate development of next-generation ground operations and flight hardware and (b) long-term goals for future requirements, if different from those delivered in (a).

To address Aim 1 during the Forum, the panel listened to presentations about the history of microbiological events on the ISS, the medical occurrence of infectious disease, lessons learned, and current requirements and operations. They also participated in tours to help them understand the surface and air environment in space vehicles, including tours of the Space Vehicle Mockup Facility in Building 9.

To address Aim 2, the panel discussed several technologies and requirements, but did not perform requirements definition, which is a specific systems engineering process. Instead, the panel gave recommendations for the current requirements and how they could be improved for future missions.

Aim 3 is the definition of practical monitoring requirements. ***These recommendations are the beginning of the process to define those requirements***. This report is the third of three interim reports describing the findings of each of the forums on potable water, food, and surfaces and air.

In summary, it is important to prevent pathogens from entering the space vehicle by way of the crew or cargo, and to have the capability to disinfect surfaces and air when they become contaminated. Next-generation enumeration and identification technologies are being developed, and they will prove useful

for microbiological monitoring of surfaces and air. There is considerable uncertainty about immune dysregulation in space and the impacts of altered immunity on other aspects of microbiology.

4. Summary of Presentations

Exploration Operational Concepts

Dr. Kundrot, the Acting Program Scientist for the HRP, presented an overview of the flexible path described by the Augustine Commission in the report *Seeking a Human Space flight Program Worthy of a Great Nation* in 2009. The flexible path includes a multi-option plan to go to Lagrangian points, planetary orbits, and landings, including rendezvous with NEOs or asteroids. Dr. Kundrot also described operational concepts for design reference missions to NEOs and Mars. A mission to an NEO would take about 1 year. For a Mars mission, the transit time would be 6 months, and 500 days would be spent on the surface. Microbiology procedures for these missions would have to be highly autonomous.

Space Flight Medical Microbiology

Dr. Haddon of the Space and Clinical Operations Division provided the perspective of a NASA flight surgeon in addressing infectious disease diagnosis and mitigation. He described two non-attributable space flight cases of infectious disease in addition to current infection-control strategies and some potential risks, such as foodborne illness during quarantine. Infectious diseases and their management during space flight missions will be the same diseases and treatment as on Earth; however, certain specific variables associated with space flight may affect the way diseases are treated. Dr. Haddon also briefed the panel on immune changes in space, viral reactivation, and pathogen virulence changes, such as those found in *Salmonella*. He described the infectious disease control strategies currently used for the ISS, including screening the crew for specific pathogens and the use of antibiotics and antiseptics.

ISS Environmental Microbiology and Requirements

Dr. Botkin of the JSC Microbiology Laboratory presented an overview of the environmental monitoring of ISS surfaces, air, and potable water. The current microbiology requirements are contained in documents, such as the ISS Medical Operations Requirements Document, JSC SSP 50260. Dr. Botkin discussed how the samples are collected from space vehicles during flight, how they are analyzed in space and after return to the JSC Microbiology Laboratory. He also discussed collection of preflight samples and processing in the JSC Microbiology Laboratory. This presentation included a survey of the species of bacteria and fungi recovered from the ISS since 1998. Dr. Botkin's key point was that microbes found in space flight are similar to those found in normal terrestrial environments, except for true pathogens, which are generally excluded from the ISS. Opportunistic pathogens, such as *Staphylococcus aureus*, have also been found and are closely monitored.

Microbial Contamination on ISS: Mitigation, Events, and Lessons Learned

Ms. Bruce of the JSC Microbiology Laboratory described a number of microbial anomalies encountered during the life of the ISS. They included failure of a fire detector due to fungal growth and discoloration of a wall in the Russian Functional Cargo Block (FGB) caused by a wet environment. During checkout of the potable water dispenser (PWD), routine monitoring uncovered elevated bacterial counts. This led to a shock treatment of the PWD with an iodine disinfectant solution. Subsequent testing showed that bacterial counts were below limits for potable water. Ms. Bruce also provided a list of lessons learned that included the following: 1) crews trained in infection control can recognize early signs of environmental anomalies, 2) scientists and engineers should work hand-in-hand from the design phase and throughout operational stages, and 3) a rigorous housekeeping schedule can maintain microorganisms at safe levels.

The Surface, Water, and Air Biocharacterization Flight Experiment

Ms. Castro of the JSC Microbiology Laboratory presented information about the Surface, Water, and Air Biocharacterization Flight Experiment that characterized the microorganisms present on the ISS. This experiment used molecular methods to detect microorganisms, allergens, and microbial toxins. Samples were collected from surfaces, air, and water, and were analyzed by denaturing gel gradient electrophoresis to detect bacteria. Real-time polymerase chain reaction (PCR) was used to detect viruses, such as varicella-zoster virus and Epstein-Barr virus in surface and air samples. Deep sequencing molecular methods were used to identify fungal species from dust in the vacuum bags and HEPA filters from ISS.

Air Revitalization System of ISS

Dr. Graf of the JSC Crew and Thermal Systems Division discussed air and thermal systems and the chemical composition of the atmosphere from an engineering perspective. Due to the loss and enrichment of various elements in the ISS atmosphere, small environmental niches exist in the ISS, such as behind the Express racks, that might be of interest to microbiologists.

Cargo Bag Disinfection Process and Development

Ms. Smith of the JSC Microbiology Laboratory described the process by which the cargo transfer bags (CTBs) are disinfected before flight. Currently, they are cleaned to generally clean requirements, but sometimes exceed microbial limitations. The CTBs are cleaned with a HEPA vacuum, sanitized with a 6% hydrogen peroxide solution, and wrapped in pink polyethylene after they are dry. After this cleaning, about 4% of the CTBs exceed bacterial limits of 500 CFU/100 cm^2. About 50% of CTBs launched on an automated transfer vehicle from Kourou exceeded fungal acceptability limits after they had passed testing before shipment to French Guiana.

5. Recommendations

The expert panel of microbiologists made 15 recommendations pertaining to NASA standards and requirements during the Forum on Next-Generation Microbiological Surface and Air Requirements for Space Flight. The Forum met the specific aims of this task by using historical data to assess optimal requirements, assessing current and near-term technologies, and recommending practical monitoring requirements.

Recommendations to Standards and Requirements

The panel made 15 recommendations pertaining to microbiology requirements, in the following categories:

Medical – 4
Disinfection – 4
Research Gaps – 2
Microbiological Monitoring – 3
Project Management – 2

Medical

(SA1) NASA should study the anecdotal reports of allergies that are reported by the ISS crewmembers, because these allergies have unknown and unexplained origins and could affect mission and crew.

Rationale: Some crewmembers experience rashes, sneezing, and runny nose when they arrive at the ISS, and the symptoms persist over the course of months until they return to the ground, when the

symptoms disappear. Crewmembers pass down medicines, such as Claritin, antifungal agents, and topical and oral steroids, to new crewmembers for treatment.

The cause of these symptoms is currently unknown. Greater knowledge of the incident rate and locations where symptoms begin would provide insight into potential routes of contamination, if any.

Rashes, especially when they lead to scratching, can breach skin integrity and increase the risk of infection. Such allergies on an exploration mission could lead to other crew health issues over the course of years.

The panel had many suggestions as to the cause of these allergies, such as air quality, materials, cutaneous fungal infection, or human dander allergies. They considered this a very important issue to resolve.[2]

[2]Comment from Dr. Vesper: "…these changes are telling us there is something turning a healthy person ill." Please note: All comments from Dr. Vesper are purely his own opinions and do not represent EPA policy or opinion. These statements are for the confidential use of NASA and its contractors.

(SA2) NASA should supply a broader range of antibiotics for future crews to use than the ones currently supplied in the medical kit.

Rationale: Resistant microorganisms are being encountered increasingly by clinicians because antibiotics are overused. The chances of resistant microorganisms being transferred to space vehicles increase with time, as they become more common in the general environment. For example, Escherichia coli *that produce extended-spectrum beta-lactamase (ESBL) are being found in clinical infections. These microorganisms are a product of the overuse of antibiotics in livestock food production—they are in the food, and they are in the gastrointestinal (GI) tracts of the general population. Discussions of antibiotics to be used on future missions should address multi-drug resistance in microorganisms.*

(SA3) NASA should screen the crew to determine if any are asymptomatic carriers of pathogenic microorganisms, especially norovirus.

Rationale: Crewmembers will be the biggest source of risk to themselves because they will be the carriers of harmful pathogens in the space environment. The panel was especially concerned about asymptomatic carriers of norovirus.

Norovirus *is the most common cause of fatal gastroenteritis and causes vomiting and diarrhea, which form aerosolized droplets that can transmit the virus to others. People carry* norovirus *asymptomatically for several weeks after infection if they are normal and for up to a year if they are immunosuppressed.* Norovirus *can infect healthy people with as few as 10-20 virus particles. There is currently no vaccine or antiviral treatment for* norovirus. *The panel did not believe that NASA is prepared to handle norovirus in space flight, judging by what it knew of quantities and types of intravenous fluids and disinfectants available in flight. Bleach, which is currently incompatible with environmental systems on the ISS, is the required disinfectant for* norovirus *cases.*

The panel believed that screening for asymptomatic carriers of norovirus *should be carried out during the quarantine period before space flight. Assays, such as real-time PCR, are available that can detect* norovirus.[3]

[3]Comment from Dr. Hanlin: "NASA should leverage knowledge with the Centers of Disease Control scientist specializing in *norovirus*, Aron Hall, PhD."

(SA4) NASA should look for proper ways to treat shingles rashes on orbit.

Rationale: Varicella-zoster virus (VZV) lies latent in nerve tissue after chickenpox and can reactivate later in life, causing a painful, blistering rash known as shingles. Data show that crewmembers have higher levels of viral reactivation during space flight than they do before or after flight. VZV DNA has been found in environmental samples on the ISS, but it is not known whether this DNA is from viral particles in saliva droplets or from skin flakes from shingles rashes of crewmembers on the ISS.

The mitigation strategy for shingles in space flight is administration of the shingles vaccine to crewmembers, which should prevent shingles rashes. There has been a study on the aerosolization of VZV in shingles rashes that showed that occlusive dressings, such as those made of hydrocolloid, prevent aerosolization of VZV DNA more effectively than conventional gauze dressing. In the case of a crewmember developing a shingles rash, it would be appropriate to use such methods in wound dressing to prevent the aerosolization of VZV in space flight, instead of using standard gauze to dress such rashes.

Disinfection

(SA5) NASA should develop an improved method of disinfecting Nomex® cargo bags for future missions.

Rationale: Cargo bags are currently disinfected by scrubbing them with a 6% hydrogen peroxide solution, which has not resolved the issue of cargo bags exceeding bacterial and fungal acceptability limits. The panel agreed that the best way to disinfect cargo bags is to use gas sterilization with hydrogen peroxide or peracetic acid. These processes should be evaluated and validated. Once the bags are disinfected, preferably at the launch site, they could be validated by parametric methods (such as gas concentration, humidity level, temperature, exposure time), as an alternative to monitoring by enumerating CFUs.

It is not clear why the cargo bags continue to have microbial counts that exceed current limits. The panel suggested that a short-term study using scanning electron microscopy would most likely reveal that microorganisms are deep within the pores of the Nomex® material and sheltered from the current disinfection method. A simple visualization of the location of the microorganism would answer the question.

Future sampling of porous surfaces, especially the cargo bags, should use a vacuuming process instead of swabbing. This will increase recovery of microorganisms from the cargo bag material. Swabbing techniques are sufficient for nonporous surfaces, but not for porous ones. More microorganisms may be present on the bags than the number currently being recovered using swabbing techniques.

Procedures for handling the cargo bags after disinfection should also be defined to mitigate an increase in microbial counts. These procedures should include the use of disposable gloves and humidity control of the cargo bags to preclude proliferation of microorganisms remaining on the bags.[4]

[4]Comment from Dr. Hanlin: "Current material and design of bags make it very difficult to clean and disinfect with wet chemistry. Two additional methods to consider are: 1) irradiating the cargo bags sealed in pink poly bags so they are sterile until they need to be used and 2) impregnating a biocide into the cargo bag material at the time of manufacture."

(SA6) NASA should investigate newly developed disinfectants and antiseptics for the disinfection of surfaces and skin.

Rationale: It was reported that crewmembers routinely use skin care wipes to clean hands before food preparation. These wipes do not contain a disinfectant, alcohol, or soap. The panel believed that the use of skin care wipes and benzalkonium chloride (BZK) was insufficient for either food preparation or medical purposes. If a norovirus *outbreak occurred, BZK would be insufficient for disinfection. Humans possess antibiotic-resistant bacteria in their GI tract from eating livestock. Livestock are exposed to antibiotics, leading to the rise of antibiotic-resistant bacteria in food. The risk of transmission of antibiotic-resistant bacteria through the fecal-oral route increases without a good method of hand disinfection before food preparation. The spread of antibiotic-resistant bacteria could be facilitated by the known space flight effect of immunosuppression.*

In a clinical setting, chlorhexidine gluconate is used for disinfection of surfaces and skin. Additional antiseptics and disinfectants are available, such as newer quaternary ammonium compounds. These new compounds would need to be assayed for compatibility with environmental systems in space flight.[5]

[5]Comment from Dr. Hanlin: "There is emerging data that some quaternary ammonium disinfectants are ineffective on human norovirus. Tung, G., Macinga, D., Arbogast, J., & Jaykus, L. A. (2013). Efficacy of Commonly Used Disinfectants for Inactivation of Human Noroviruses and Their Surrogates. Journal of Food Protection®, 76(7), 1210-1217."

(SA7) NASA should use human factors engineering to enhance environmental disinfection capabilities.

Rationale: A human factors approach to handling certain aspects of disinfection could improve hygiene and reduce the spread of microorganisms on surfaces. Examples of how this approach works are new methods of changing crew's personal toilet accessories, using washable keyboards, and improving sanitation during food preparation. This approach has improved infection control in hospitals.[6]

[6]Comment from Dr. Vesper: "I think research is needed develop methods to improve hygiene tools. This might include, sweat removal during exercise, inactivation of skin microbes to some acceptable level, improved toilet facilities, perhaps self-cleaning."

(SA8) NASA should increase use of antimicrobials and nonporous materials as components of subsystems and as a materials selection strategy for reducing environmental microbial levels.

Rationale: Microbiology issues associated with the cargo bags and other cloth and porous materials could be solved with selection of appropriate materials early in the design of the living quarters of the space vehicle. Cargo bags made of Nomex® have a type of surface that promotes excursions of microbial levels above microbiological limits. It may be prudent to find a nonporous analog to Nomex for cargo bags. On the ISS in the area where the crew eats meals, there are storage bins made of cloth that is difficult to clean, especially now after several years of habitation.

Other surfaces, such as those found in the bathroom and food preparation areas, would benefit from being self-disinfecting surfaces as well. Materials are readily available that are imbued with disinfecting chemicals like silver and copper. Others have super-hydrophobic surfaces or microscopic pattern arrangement, such as NeverWet or Sharklet, respectively. NASA will need to know more about how these surfaces work to make sure they are adaptable to space vehicles. The use of self-disinfecting surfaces would decrease the need for cleaning supplies and housekeeping activities, and thus reduce upmass, waste, and crew time needed for housekeeping.

Research Gaps

(SA9) NASA should study the impact of the deep-space environment on the immune system.

Rationale: There is a lot of uncertainty about the condition of the immune system of crewmembers, especially during a deep-space mission to a near-Earth asteroid (NEA) or Mars. The Decadal Survey on Biological and Physical Sciences in Space in 2011 suggests that "mechanisms of immune system changes during space flight" are the highest-priority research topic in animal and human biology for NASA.

Much of risk mitigation relies on understanding the status of the immune system of an individual. None of the space flight analogs that are currently used to simulate the effects of space flight can simulate immune status.[7]

Causes of immunosuppression are unknown, and environmental materials could make the situation worse. The current philosophy in microbial monitoring is that high levels of nonpathogenic bacteria and fungi may predict high levels of pathogenic bacteria and fungi. If the immune system is severely compromised to a level that has not currently been documented, then the nonpathogenic bacteria and fungi themselves, growing in environmental niches in the spacecraft, may reach numbers that allow them to act as opportunistic pathogens.

During the course of discussion of hypersensitivity testing in space flight, there was mention of a medical experiment on STS-71, during the return of Atlantis from the first Mir docking in 1995. This experiment included an on-orbit skin test that led to some dramatic effects in one of the test subjects. The panel was very interested in this experience, wanted more details, and thought this account should be investigated to better understand immunity in flight.

[7]Comment from Dr. Hanlin: "The uncertainty about the immune system will impact food safety as well."

(SA10) NASA should sponsor studies into the behavior of bioaerosols, such as spores, droplets, and micronuclei in microgravity.

Rationale: No information is available about the behavior of bioaerosols in microgravity. The Decadal Survey on Biological and Physical Sciences in Space in 2011 suggests that "aerosol deposition in the lungs of humans and animals in reduced gravity" is a highest-priority research topic in animal and human biology for NASA; however, this statement was made in the context of lunar and Mars dust. Aerosol particle studies have been funded by NASA's Human Research Program (HRP) recently.

The panel was concerned with an off-nominal event of mold growing in the Functional Cargo Block (FGB) of the Russian Segment of the ISS and how the spores of this mold might be dispersed throughout the vehicle. Bioaerosol dispersion is also a concern for bacteria and viruses. Sneezing caused by upper respiratory infections can spread Staphylococcus aureus*, if it is present in the nose. Virus particles from reactivated herpesviruses, such as herpes simplex virus I and VZV, can be transmitted in saliva. In fact, VZV DNA has been found in environmental samples from the ISS.*

Future research objectives should include understanding the aerobiology of space flight, such as droplet and aerosol dispersal and clearing by HEPA filters; the effects of sneezing, coughing, and talking on bioaerosol generation and dispersal; and effects after inhalation of bioaerosols into the alveolar space. New imaging techniques, such as Schlieren imaging, can capture air flow currents and may be helpful in this kind of research.

Microbiological Monitoring

(SA11) Routine microbiological monitoring is appropriate as validation of contamination control protocols; however, if a greater level of confidence in the microbial risk is required for future missions, an increased number of samples and frequency of sampling would need to be implemented.

Rationale: The panel believed that in-flight microbial monitoring of vehicle surfaces and air is an important tool for environmental microbiology of space flight on the ISS, especially for the verification of contamination control protocols, such as for housekeeping, and for the detection and characterization of microbial anomalies, such as the mold in the Russian Segment FGB.

The panel believed that the current frequency of sampling and number of samples are not great enough to ascertain the conditions of the environment of the ISS at the high level of confidence required to use the monitoring strategy as a preventive measure. Monitoring as a preventive measure will be more important for exploration missions.

The panel mentioned a study performed by the Department of Homeland Security, "Interagency Restoration Biological Demonstration," which was conducted to understand recovery from a biological incident in a major metropolitan area. It took 6 million samples to determine if the city was clean after the mock contamination. Thus, to gain 90% confidence that the ISS is free of potentially harmful microorganisms, the sampling frequency and sample number would need to increase dramatically.

Also, the air sample volumes currently used might not be large enough. NIOSH uses a two-stage air sampler that separates the airborne particles into three size fractions and collects samples for days or weeks for molecular analysis. This may be useful in bioaerosol studies. Long-duration air samples in space flight will be much more useful than surface sampling.

In the near future, monitoring will most likely be performed by molecular means, such as PCR. Such methods are already being used in biodefense in public and postal service areas, and it is only a matter of time until such methods are compatible with space flight.[8]

[8]Comments from Dr. Vesper: A) "Another item that might be useful for finding "wet" materials is an infrared camera. There may actually be one on the ISS for other reasons. This may be useful in detecting areas where molds might grow." B) "The problem is that the monitoring results come too long after the fact. This may ok for missions that are close to earth but for long missions, up to 3 years, you can't wait for the problem to become serious. It must be stopped before it becomes dangerous." C) "For flight operations, I think that long-term air monitoring (as descried above) is superior to surface swabbing. In flight, I do not think surface sampling is useful. The one exception might be in the monitoring of "bathroom" facilities and devices and possibly food preparation areas. However, appropriate hygiene should be adequate, even in these areas. However, it sounds like appropriate hygiene may not always be utilized. Still, the results of these swab samples in designated areas would need to be available in a real time basis to have any prevention impact and remediation initiation. These real time results would require new analytical methods beyond culturing."

(SA12) NASA should be most concerned with the following microorganisms with respect to surface and air microbiology: *Aspergillus fumigatus, Aspergillus flavus, Aspergillus terreus, Aspergillus niger, Legionella pneumophila,* and *norovirus*.

Rationale: The Aspergillus *species are fungi most common in pathogenic infections in immunocompromised patients, and in the development of harmful mycotoxins, such as aflatoxin*. Legionella pneumophila *is a pathogenic bacterium responsible for Legionnaires disease*. Norovirus *is the most common cause of viral gastroenteritis.*

The panel believed that these were the most important microorganisms to prevent from entering the spacecraft environment, in addition to other opportunistic pathogens commonly associated with the human microbiome, such as Staphylococcus aureus *and* Escherichia coli.

NASA could use an indicator strategy to compile a panel of microorganisms, including those listed above, that would serve as indicators of an unsafe vehicle, instead of monitoring and identifying every microorganism found. This is a strategy used by the Environmental Protection Agency and the CDC to monitor recreational and potable waters. The Microbiology Laboratory uses a fecal coliform test as an indicator of safe potable water on the ISS. Performing a full microbiome analysis using pyrosequencing on dust samples from HEPA filters would help develop this indicator panel.

(SA13) NASA should support the development of in-flight molecular testing to identify a panel of microorganisms that would serve as indicators of an unsafe vehicle and for detecting antibiotic resistance, to inform selection of a limited spectrum of onboard antibiotics.

Rationale: Molecular identification of microorganisms and detection of antibiotic resistance using PCR is state of the art. Many of these methods are already being used in biodefense and environmental monitoring. Microarray technologies are now available that can perform hundreds of PCR reactions at one time at isothermal temperatures. This technology could be coupled with an indicator microorganism strategy noted above. The limitation of using molecular methods for identification is that most of these methods do not assess viability; however, future developments, such as detecting mRNA, may be able to infer viability status.

In addition to identification, the detection of proteins that lead to resistance to antibiotics could help the crew medical officer select which treatment is best. The capability to synthesize antibiotics on board from stable precursors would mitigate shelf-life issues with exploration missions.[9]

[9]Comment from Dr. Vesper: "I believe that instrumentation will be available for the autonomous molecular monitoring of any microbe of interest. These systems are already becoming available for point-of-use diagnoses… I would especially watch the developments in real-time nucleic acid sequence-based amplification (NASBA). One nice thing about NASBA is that one might potentially determine the viability of the microbe. There are also some very interesting isothermal technologies that are being developed. I would be thinking of moving away from culturing in the not too distant future."

Project Management

(SA14) NASA should include alterations in crewmembers' immune systems as a factor in its microbial risk assessment.

Rationale: In risk assessment, the identity of the microorganism has more impact on the calculated risk than the concentration of microbes in a volume. The possibility of greater susceptibility to infectious disease has tremendous implications for risk and would likewise have a large impact on the countermeasures necessary to mitigate that risk. The Microbiology Laboratory should pursue collaboration in NASA research efforts to better understand the immune system.[10]

[10]Comment from Dr. Vesper: "I think the most critical question is the immune function of humans in very long missions. Dr. Haddon's comment about a 3-year mission around the moon or at least a 3-year mission on the ISS, might answer this fundamental question. (If it is not possible to do a long-term human exposure, then an animal model might be considered.) The degree and type of immune dysfunction might make one susceptible to more and more microbes. For example, *Legionella pneumophila* might be more potent before some of the fungi become a problem. However, many microbes can become pathogens at some critical state of immune decline."

(SA15) NASA should separate the microbiological requirements for the ISS from those for long-duration deep-space missions.

Rationale: There are obvious differences in the mission scenarios for ISS missions and exploration missions to NEAs, Lagrangian points, or Mars. Those differences include return time to Earth, the ease of resupply, and mission duration. The panel agreed that the sets of microbial requirements for short-term missions that are readily resupplied and that the crew can easily return from, and long-term missions that cannot be resupplied and would be difficult to abort, must be separate.

Another confounding factor is the unknown effect of long-duration deep-space missions on the immune system. There is no clear understanding of how immunocompromised the crews will become.

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Duane L. Pierson; C. Mark Ott; Victoria A. Castro; Todd Elliott, Cherie M. Oubre

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Microbiological requirements that mitigate risk to the crew during space flight missions are periodically updated to reflect changes in the scientific understanding of microbial pathogenicity, our knowledge of the response of microorganisms to the space flight environment, effects of space flight upon human immunity, and advances in monitoring technology. Three expert panels held from 2011 through 2013 performed a review of microbiological requirements, based on new knowledge and available technologies. These panels focused on requirements associated with potable water, space flight food, and vehicle air and surfaces specifically described in NASA Space Flight Human System Standard (NASA-STD-3001). Evaluation of the recommendations from all three forums indicated 12 common themes, such as the need for appropriate microbiological training for individuals who would be developing or operating space flight systems and guidelines for current and future microbiological monitoring technology. The reoccurrence of these themes in multiple panels reinforced the importance of the consideration of these recommendations. These forums provided the opportunity to formally update and document our knowledge base for space flight microbiological requirements. Many of the recommendations in this report addressed medical operations, environmental sciences, and engineering activities.

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