U.S. NATIONAL LABORATORY

Exploring the Microbiome/Immunome and Disease on the International Space Station—Improving Human Health on Earth

Program Guide and Workshop Report

Cleveland, OH
October 25, 2016

Workshop sessions conducted by the International Space Station U.S. National Laboratory in conjunction with the 32nd annual meeting of the American Society for Gravitational and Space Research

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ABOUT THE ORGANIZER

About the ISS National Lab: *In 2005, Congress designated the U.S. portion of the ISS as the nation's newest national laboratory to optimize its use for improving quality of life on Earth, promoting collaboration among diverse users, and advancing science, technology, engineering, and mathematics (STEM) education. This unique laboratory environment is available for use by non-NASA U.S. government agencies, academic institutions, and the private sector. The ISS National Lab manages access to the permanent microgravity research environment, a powerful vantage point in low Earth orbit, and the extreme and varied conditions of space. To learn more about the ISS National Lab, visit www.issnationallab.org.*

INTRODUCTION
The Need for Microbiome/Immunome Research

The mission of the International Space Station (ISS) U.S. National Laboratory is to promote the utilization of the ISS for spaceflight research to benefit life on Earth. Microbiome research performed in microgravity onboard the ISS National Lab has the potential to further the field of human microbiome research and its impact on the human immunome and disease.

Approximately 100 trillion microbes exist in and on the human body, outnumbering human cells by about 10 to 1. These microorganisms form the human microbiome, which until recently has been largely unstudied. With the advent of gene sequencing and innovative computational tools, we now know more about the essential functions the microbiome performs: it maintains overall human health, makes nutrients digestible that would otherwise be inaccessible to the human body, provides essential vitamins and nutrients, and plays a role in protecting the body from dangerous pathogens. Microbiome research is poised to provide insight into the human immunome—the genes and proteins that comprise the immune system—and shed light on the interconnectedness of the human microbiome and human biology.

The human microbiome is specific to each human being, creating a kind of biological signature. Distinguishing and identifying microbiota in the human microbiome may help detect disease patterns and inform future analysis. Research endeavors and initiatives such as the Human Microbiome Project and the National Microbiome Initiative, both funded by the National Institutes of Health (NIH), are charged with not only studying what makes a healthy human microbiome, but also identifying differences between a healthy microbiome and one that coexists in and on a human diagnosed with a disease. Pinpointing microbiotic changes in the health of individuals, families, and communities can lead to the identification of global microbial changes that might indicate viral infection patterns or clarify the onset and progression of disease.

Microbes are living organisms so small they cannot be seen with the naked eye. There are several types of microbes, including bacteria, fungi, archaea, protists, viruses, and microscopic animals and plants. Except for microscopic plants, the human body hosts every type of microbe.

Using the Space Environment to Accelerate Microbiome/Immunome Research

The microbiome comprises not just bacteria, but other living microorganisms such as viruses and fungi that saturate the human body and the world around us. Microbial communities exist in the environments in which we live and work, and these environmental assemblages of microbes constitute their own microbiome. In any environment, the human microbiome interacts with the environmental one, allowing microbes to adapt or colonize on surfaces and in building mechanics/systems (i.e., air conditioning, plumbing, etc.). This concept of microbial contamination/transmission is especially of interest in this age of the built environment—environments created by humans and not occurring naturally. Since people in developed countries spend approximately 90% of their time indoors, the relationship between the human microbiome and the live/work environment has become more multifaceted and complex.

Microbes respond differently in microgravity, oftentimes providing research results quite different to those in ground experiments. Bringing microbiome research into the space environment has the potential to influence various aspects of human health, including the future of antibiotic resistance and disease treatment.

The National Academies of Sciences, Engineering, and Medicine recently published a report on microbiomes of the built environment, drawing attention to indoor microbiology, the human microbiome, and human health as they relate to human-made environments. The information included in the report is intended to inform the future of building design to promote health and decrease energy usage, identify areas where more research is needed, and address sustainability issues. In terms of the ISS, the report might also serve as an introduction to the complexities of microorganisms in built environments such as the ISS.

The ISS serves as home to astronauts during their time on station and houses advanced state-of-the-art technologies, such as tools and systems for biology, human physiology, physical science, and materials research. The 357-foot long station also accommodates multipurpose facilities and Earth Science facilities, including incubators, freezers, microscopes, and centrifuges; available advanced capabilities include bone densitometry, DNA sequencing, and protein crystal growth. The built environment of the ISS is unique because of its location in microgravity and its access and exposure to harsh conditions (such as radiation exposure and extreme temperatures). It is also different from many other built environments because it is considered a semi-closed environment, meaning that it has limitations as to what enters and exits its contained environment. Few built, semi-closed environments exist, and keeping them semi-closed often proves difficult.

Because of its location and the unique methods necessary to introduce new astronauts or materials, the ISS does not share the more common challenges to maintaining a semi-closed status: ventilation source and occupancy load. More specifically, the air onboard station is recirculated using high-efficiency particulate air (HEPA) filters, restricting the introduction of “fresh” air and the number of individuals that enter or exit is extremely limited to the number of astronauts on station (full crewed at six astronauts). In addition, the experiments/payloads are often packed in clean rooms and delivered to and from station using well-maintained and impeccably clean transport modules.

The functionality of the ISS plays an integral role in the stability of the microbial communities onboard it, as the growth rate of existing organisms on the ISS may fluctuate based on various mechanical/maintenance changes (e.g., air flow, water filtration, temperature). Different microorganisms are introduced when, for example, new equipment is installed, or an astronaut enters the ISS. Additionally, astronauts and the objects they touch or interact with daily, along with food particles from the meals they consume, may yield distinct microbial differences that would be reflected in microbial data collection efforts. Each change yields a unique environment with equally distinct microbial differences and, thus, presents diverse data collection potential for microbial research.

The National Aeronautics and Space Administration (NASA) has more than ten years of data on microbial isolates collected from the ISS to be used in research focused on understanding the development of microbial communities on station. In addition, the HEPA filters used in the air filtration system will be sent back to Earth for microbial analysis. While microbial research of built environments is still relatively new, current microbial research here on Earth using these existing data collected from the ISS over time informs maintenance and safety practices of current built environments like the ISS and directs development decisions concerning the next generation of human-made habitats and space stations.

Microbiome/Immunome Research in Space to Benefit Life on Earth

Life sciences research over the past several decades has demonstrated that many physiological alterations occur in humans and animals during spaceflight. These include muscle atrophy, loss of bone density, immune dysfunction, and cardiovascular deconditioning. Some of these changes mimic the degradation our bodies experience during normal aging here on Earth. In spaceflight, however, these changes occur more rapidly, and they occur in very healthy adults. Thus, researchers are interested in studying this accelerated aging as a means of understanding disease processes. The long-term intent may be to develop pharmaceuticals or therapeutics to minimize or prevent maladies such as osteoporosis.

The Microbiome of Built Environments (MoBE): The MoBE is a relatively new area of research that focuses on the unique ecosystem created by microbial communities inside a human-made environment, such as the ISS.

Similarly, microbiome research on the ISS contributes valuable information to microbiome/immunome research efforts here on Earth by identifying existing gaps in research and informing future research efforts. Removing just one element (microgravity) from Earth-based research provides data that could never be collected on the ground; until the ISS, the evolution and lifespan of microorganisms had been restricted to Earth and standard gravity. Continually gathering information on the behaviors and mechanisms of microorganisms, genes, and proteins in low Earth orbit will help us understand how they influence human health in standard gravity. As we investigate life sciences research data from spaceflight, we can study the expression of genes that dictate the activity of cells in human bodies. Collecting additional data on gene expression patterns may help identify potential targets for new medicines and redirect future research endeavors.

> *Understanding the relationship of the human microbiome and immunome in a semi-closed environment such as the ISS may bring forth breakthroughs in drug development, microbial health, and the prevention of disease spread.*

Workshop Goals

To explore the potential benefits of microgravity research for advancing current terrestrial studies into the human microbiome, the ISS National Lab brought together leading researchers and industry experts to discuss the current state of microbiome research and opportunities for microgravity-enabled knowledge advancement. The *Exploring Microbiome/Immunome and Disease on the International Space Station* workshop participants were tasked with defining current challenges in research and development to assess the interaction of microbiota, immune function, ecosystem health, and human disease. Participants also identified focus areas for research in microgravity to address current challenges in microbiome/immunome research and opportunities for collaborations between government agencies as well as public-private partnerships. Consideration was given to collaborative funding opportunities, as well as science, technology, engineering, and mathematics (STEM) education initiatives related to the microbiome and microgravity.

The workshop brought together thought leaders from the microbiome/immunome research community, including experts from government, academia, and private industry—some meeting face-to-face for the first time. Experts in the fields of space exploration, astronaut health, and ISS National Lab management expanded on microbiome research potential in space and in a semi-closed built environment. The ultimate intent of the workshop was to define how diverse organizations can come together to further microbiome/immunome research and how best to utilize the ISS National Lab in that endeavor.

The workshop focused on the following topics:

1. **The National Microbiome Initiative and interagency research on the ISS**
2. **The microbiology of the built environment: On Earth and in space**
3. **The microbiology of the human environment**
4. **What is next for microbiome research in space**

Conclusion

The following sections contain information gathered during the workshop, as well as background on the history and current state of microbiome/immunome research. This information highlights preliminary research questions, challenges faced in the field, and thoughts on the advantages of conducting microbiome/immunome research in microgravity. The enclosed summations are intended as a starting point on a path toward optimal use of the ISS National Lab to enable future microbiome/immunome research.

WORKSHOP AGENDA

Event held October 25, 2016

*Presentations available at **www.issnationallab.org/workshops/microbiome***

- **Welcome/Introductions**
  Debbie Wells, ISS National Lab
- **Workshop Objectives for the ISS National Lab**
  Michael Roberts, Ph.D., ISS National Lab
- **Lessons Learned from the Environmental "Omics" of ISS**
  Kasthuri Venkateswaran, Ph.D., NASA-JPL
- **Microbiology and Human Spaceflight Applications**
  Mark Ott, Ph.D., NASA-JSC
- **The National Microbiome Initiative and Agriculture Research on the ISS**
  Elizabeth Stulberg, Ph.D., USDA, NASA Journey to Mars and ISS Science
- **Harnessing Microbes for Good Health**
  Susan Erdman, DVM
- **NASA's Journey to Mars and ISS Science**
  Julie Robinson, Ph.D., NASA-JSC
- **Concurrent Break-Out Sessions**
  Four roundtable discussions involving all participants
- **Consolidation of Requirements and Group Concurrence**

WORKSHOP PARTICIPANTS

Richard Arnold, Ph.D.
Director, Aerospace Research Directorate
Naval Aerospace Medical Research Unit – Dayton

Anne Cheever, Ph.D.
Associate and Lead Scientist
Booz Allen Hamilton – DARPA

Linda Chrisey, Ph.D.
Program Office, Naval Biosciences and Biocentric Technology
Department of Defense, Office of Naval Research

Karim Dabbagh, Ph.D.
Chief Scientific Officer
Second Genome, Inc.

Karen Dannemiller, Ph.D.
Assistant Professor, Environmental Health Sciences
The Ohio State University

Maria Dominguez-Bello, Ph.D.
Associate Professor, Department of Medicine
NYU School of Medicine

Susan E. Erdman, Ph.D.
Assistant Director and Chief of Clinical Resources, Division of Comparative Medicine
Massachusetts Institute of Technology

David Fothergill, Ph.D.
Scientific Director
Naval Submarine Medical Research Laboratory

George Fox, Ph.D.
Professor, Department of Biology and Biochemistry
University of Houston

John Horack, Ph.D.
Professor and Neil Armstrong Chair
The Ohio State University

Jonathan Galazka, Ph.D.
Scientist
NASA / AMES Research Center

Amy Jenkins, Ph.D.
Senior Scientist
Schafer Corporation / DARPA SETA

Fathi Karouia, Ph.D.
Researcher
NASA / AMES Research Center

Alexander Khoruts, Ph.D.
Professor of Medicine, Division of Gastroenterology, Hepatology and Nutrition
University of Minnesota

Andrew Lee, Ph.D.
Systems Engineer and Technical Advisor
Booz Allen Hamilton / DARPA

Jennifer Martiny, Ph.D.
Professor, Ecology and Evolutionary Biology
University of California, Irvine

Aaron Mills, Ph.D.
Professor, Environmental Sciences
University of Virginia

David A. Mills, Ph.D.
Professor and Peter J. Shields Endowed Chair
University of California, Davis

Andrew Moeller, Ph.D.
Miller Research Fellow
University of California, Berkeley

Ganesh B.M. Mohan, Ph.D.
CalTech-JPL Postdoctoral Scholar
NASA / Jet Propulsion Laboratory

Karen Mumy, Ph.D.
Deputy Director, Environmental Health Effects Research Directorate
Naval Aerospace Medical Research Unit

Mark Ott, Ph.D.
Senior Microbiologist
NASA / Johnson Space Center

Cheri Oubre, Ph.D.
HRP MicroHost Discipline Scientist
KBR Wyle / AMES Research Center

Julie Robinson, Ph.D.
Chief Scientist, International Space Station
NASA / Johnson Space Center

Kevin Sato, Ph.D.
Chief Scientist / Senior Project Scientist
KBR Wyle / AMES Research Center

Nitin Singh, Ph.D.
Postdoctoral Fellow
NASA / Jet Propulsion Laboratory

Michael Strong, Ph.D.
Associate Professor, Center for Genes, Environment and Health
National Jewish Health

Elizabeth Stulberg, Ph.D.
Agricultural Science Fellow
U.S. Department of Agriculture

Poorani Subramanian, Ph.D.
Senior Scientist
CosmosID, Inc.

Elizabeth Taylor
Space Biology Project Manager
NASA / AMES Research Center

David Tomko, Ph.D.
Space Biology Program Scientist
NASA Headquarters

Fred Turek, Ph.D.
Center Director, Center for Sleep and Circadian Biology
Northwestern University

Paul Turner, Ph.D.
Professor of Ecology and Evolutionary Biology
Yale University

Sayaka Umemura, Ph.D.
Deputy Director, JAXA Houston Office
JAXA

Camilla Urbaniak, Ph.D.
Postdoctoral Fellow
NASA / Jet Propulsion Laboratory

Kasthuri Venkateswaran, Ph.D.
Senior Research Scientist
NASA / Jet Propulsion Laboratory

Martha Vitaterna, Ph.D.
Research Associate Professor
Northwestern University

Alexander Voorhies, Ph.D.
Postdoctoral Fellow
J. Craig Venter Institute

Renee Wegrzyn, Ph.D.
Program Manager
DARPA

ISS National Lab Representatives

Cathy Abidin
Portfolio Project Manager

Randy Giles, Ph.D.
Chief Scientist

Marc Giulianotti, Ph.D.
Senior Associate Program Scientist

Michael Roberts, Ph.D.
Deputy Chief Scientist

Liz Warren, Ph.D.
Associate Program Scientist

Debbie Wells
Program Manager

Chasity Wilson
Events Specialist

BREAK-OUT SESSION CONCEPTS

An average of 100 trillion microbes live in and on the human body, making the human microbiome a significant source of information regarding human health. The genetic diversity of the human microbiome and the crucial role it plays in combating disease—along with the impact it has on the genes and proteins that comprise the immune system (i.e., the "immunome")—continue to garner attention from the research community.

The workshop *Exploring the Microbiome/Immunome and Disease on the International Space Station—Improving Human Health on Earth* sought to identify the critical needs and roadblocks of the microbiome research community and assess how public-private partnerships involving the ISS National Lab might address common roadblocks. **Complementing an agenda of thought-leader presentations and roundtable discussions, four targeted breakout sessions focused on:**

I: Interagency Research
II: The Built Environment
III: Humans and Microgravity
IV: What's Next?

Break-out session participants were charged with the task of assessing trends in microbiome research, highlighting collaborative research opportunities, examining the impact of a semi-closed environment on the human microbiome/immunome, and gauging the influence of environmental changes on microbial fitness. In addition, the sessions were designed to guide participants in the exploration of the relatively new field of microbiome research as it relates to space and the relationship between disease and the human microbiome on Earth.
Key findings from the four break-out sessions are presented in a condensed form in the following sections. The results of these breakout-session discussions provide a consensus on the process of promoting interagency research and are meant to be a starting point for the development of future microbiome/immunome research on the ISS.

BREAK-OUT SESSION GROUP I

The National Microbiome Initiative and Interagency Research on the ISS National Lab

Group I participants called for increased efforts to promote the unique research opportunities that the ISS National Lab offers to the scientific community, specifically in the field of microbiome/immunome research. Consideration was given to types of research that might require or benefit from the ISS environment. Group I participants concluded that increased promotion of interagency research as it relates to education and the study of the human microbiome would play a part in advancing microbiome research in space.

Group I Participants

Anne Cheever
Linda Chrisey
Ganesh B.M. Mohan
Cherie Oubre
Kevin Sato
Elizabeth Stulberg*
Elizabeth Taylor
Sayaka Umemura
Debbie Wells**

**Discussion Lead*
***ISS National Lab representative*

Group I Key Findings

Steps to advance microbiome research in space:

- Increased promotion of interagency research and education opportunities
- Increased promotion of the unique research opportunities available on the ISS National Lab
- Inclusion of nontraditional attendees at National Microbiome Initiative conferences and meetings and increased dissemination of information

Areas of microbiome research that could benefit from a microgravity environment:

- Disease modeling
- Accelerated antibiotic resistance
- Protein structure
- Filtration and CO_2 mitigation
- Food production systems
- Cryptic pathways for bacterial production of products
- Systems biology projects

Group I Abbreviated Discussion Minutes

Within the last several years, the profound relevance of the human microbiome to human health has been identified, and this realization has redirected research efforts across various research areas.

Environmental ecology, human biology, microbiology, and the study of built environments are just a few of the research fields embarking on substantial microbiome research. There are large-scale studies in development and several currently in progress. Particularly of note is the National Microbiome Initiative (NMI), which was implemented by the White House Office of Science and Technology to advance microbiome research with the express purpose of improving various aspects of human life and planetary health.

Group I participants noted that it is common policy for conferences and meetings on specific subjects like the NMI to be invitation-only. While the intent of Invitation-only events often reflects the desire to make meaningful scientific advances by bringing together subject matter experts, these events may also be missed collaborative opportunities. The inclusion of stakeholders and nontraditional attendees may improve partnerships and collaborative agreements in microbiome research. Nontraditional attendees may also contribute innovative ideas and proposals that may not have received consideration from more selective groups. In addition, participants promoted increased and robust distribution of NMI information by attendees to stakeholders and other interested parties. Information sharing and dissemination through presentations, online content, and open discussion were just a few identified methods of information sharing proposed by Group I participants.

Topics of discussion during this session also included increasing education and communication efforts concerning the promotion of novel experiments currently being conducted on the ISS. There are untapped microbiome/immunome STEM education opportunities between agencies with complimentary educational missions; the creation of interdisciplinary microbiome educational initiatives may positively impact the future of interdisciplinary microbiome research.

With respect to the sharing of NMI information and the future of microbiome research in space, microbiome/immunome conferences, meetings, and workshops are ideal settings to introduce researchers to the unique low Earth orbit environment of the ISS National Lab and highlight the research being done onboard the ISS. It is important to help researchers understand that changing one scientific variable—microgravity—may significantly impact microbiome research as it relates to disease pathogenicity or improved immune response.

Lastly, Group I participants identified two microbiome research categories that may benefit human life on Earth: 1) microbiome research that requires microgravity, including the study of relevant disease models and the ISS mechanics and processes where microbes are typically established, such as cleaning (e.g., reclamation, filtration, and CO_2 mitigation); and 2) microbiome research that does not require microgravity, such as the study of shelf-stable meals, data management, energy production, and diagnostics. For more than ten years, NASA has collected microbial data in each category and used the information to conduct analyses that led to the identification of gaps in both knowledge and protocols. Evaluation of microbial data is ongoing, and adjustments are made to protocols, practices, and procedures as these knowledge and protocol gaps are addressed.

BREAK-OUT SESSION GROUP II

Microbiology of the Built Environment: Earth and Space

Microbiome research in a built environment such as the ISS presents researchers with several advantages but also some challenges. Microorganisms such as fungi, bacteria, and viruses are not only prevalent in nature but also often beneficial; however, left unmonitored, they can cause dangerous health consequences and environmental problems in built environments such as the ISS. Drawing attention to the similarities and differences between Earth-based built environments and the ISS may assist in furthering microbiome/immunome research, human health, and environmental safety in both settings.

Group II Participants

Karim Dabbagh
Karen Dannemiller
John Horack
Amy Jenkins
Fathi Karouia
Jennifer Martiny
Karen Mumy
Julie Robinson*
Nitin Singh
Kasthuri Venkateswaran
Liz Warren**

**Discussion Lead*
***ISS National Lab representative*

Group II Key Findings

Recommended microbiome/immunome study areas of the built environment:

- Fungal growth as it relates to corrosion and power outages on the ISS
- Biofilms, their potential use (symbiotic or pathologic), and their formation in space
- The benefits of conducting research in a closed/built environment and the opportunity to conduct comparative analysis with built environments on Earth

Recommendations on the standardization of microbiome/immunome research on the ISS:

- Standardized protocols; base protocols on those for existing closed environments to promote comparative data analysis
- Automation of sampling
- Extensive sampling of the environment on a limited basis
- Repeated sampling of ISS crew members preflight, in-flight, and postflight
- Standardized sample measurements consistently using the 16S rRNA gene

Group II Abbreviated Discussion Minutes

While extensive bacterial research has been conducted on the ISS, fungi (mushrooms, molds, and yeasts) have been comparatively less explored. Many of the fungi on the ISS are like those found on Earth; most are harmless and often one of many components of a healthy microbiome. However, the presence of fungi in a built environment (a man-made setting for human activity) such as the ISS presents potential dangers. For example, if humidity rises to a level conducive to fungal growth, both the ISS and the crew may be negatively impacted. The spacecraft could face corrosion issues, and electrical equipment may become unresponsive. Crew members may develop respiratory issues and chronic skin irritations that persist for the duration of their time on station. Therefore, maintaining as dry an environment as possible is crucial to thwarting harmful fungal growth.

Group II participants acknowledged the lack of fungal growth research on the ISS and advocated future fungi research initiatives, especially when considering the history of problematic fungal growth on the Russian space station Mir and challenges relating to corrosion on the ISS. Several participants expressed interest in learning more about the unstudied influence of fungal particle size, with some fungi displaying larger and more complex particle growth in microgravity than fungi grown on Earth.

After identifying research collection modes available on station (sampling the environment, culturing to understand aspects of the built environment, and conducting research using the external facilities), Group II participants focused on the challenges presented by the existence of fungi on the ISS in relation to these collection modes.

Currently, surfaces of the ISS are frequently cleaned, the humidity level is controlled, foods are freeze-dried, the air flow system is closely monitored, and the crew are quarantined before each mission. When standardizing microbiological research in what is considered a semi-closed system (from an ecological modeling standpoint), consideration is given to surface cleanliness, the humidity level, the air flow system, and the health of the crew. Future research projects may provide opportunities to explore in greater detail how the human microbiome impacts processes and procedures regarding air flow, humidity, and crew health.

The limited number of inputs and outputs make the ISS one of the least complex human-dominated environments to study, somewhat more contained than a LEED (Leadership in Energy and Environmental Design) certified building on Earth, which has recycled air and well-sealed windows and doors specifically designed to reduce outside influence on the interior environment. The semi-closed system, acting like a “sick building” in that new air is not brought in, is an ideal research setting in its simplicity, reduced human population, and decreased interaction with outside influences. Because the ISS is a built environment and a semi-closed system, most of the microbes are human associated (living on skin, food, fabrics, surfaces, etc.). Research initiatives patterned after other ground-based microbial communities, such as closed offices and intensive care hospital units, would be a logical format to adapt when designing future comparative data analyses.

Challenges of microbiome research in general often relate to horizontal gene transfer; because of the numerous variables that exist in any given environment, determining the microbial source can often be problematic. In a built semi-closed environment, discerning the microbial source of genetic material and understanding the impact of horizontal gene transfer may be easier to assess.

Because a wide variety of experiments are conducted on the ISS, variations in extraction protocols and sampling methods are expected. Future microbiome research efforts may seek to implement specialized standard sampling measurements using genes commonly used for bacterial identification purposes. An incremental shift focusing on the collection and analysis of a common gene would provide rich data regarding which types of microorganisms persist in a semi-closed environment.

Group II participants also discussed sampling recommendations as they relate to the frequency of sampling. Current sampling regularly takes place to monitor crew health and vehicle cleanliness. There may be benefits to increasing the production of valuable microbiome and immunome data by sampling many different surfaces, environments, and microbial human communities *less frequently* to observe changes over time. Because the ISS is a semi-closed environment, frequent sampling may not necessarily identify microbial changes as well as less frequent sampling over time. The optimal sample frequency, annually or semi-annually, is still under debate. To reach statistical significance, ISS crew members should be sampled repeatedly in longitudinal studies. Such studies are currently being conducted through the Astronaut Microbiome Project, which aims to elucidate how spaceflight impacts the human microbiome/immunome.

Another important research area identified by Group II is research focused on understanding the mechanism of biofilms, the thin slimy films that stick to surfaces, and studying when and why they develop. Some evidence shows that biofilms are prone to form in space. Researchers are interested in determining whether biofilms grown on the ISS have a different thickness than those formed in nature and assessing their purpose in different environments. Group II discussed both the symbiotic and pathologic potentials of biofilms. Fluid physics is different on the ISS than it is on Earth, which may affect quorum sensing and when and how biofilms form. This information could help prevent unwanted biofilms or perhaps produce biofilms with beneficial characteristics such as UV radiation resistance or antifungal properties.

In addition to internal culturing and sampling, microbiome experiments external to the ISS could be performed. The new Materials International Space Station Experiment (MISSE) Flight Facility is a commercially available materials research facility on the ISS that does not require a spacewalk for access. More than 4,000 tests on various sample materials through the historical MISSE program have taken place since 2001, and the opportunity for microbiome research using the facility exists. Other external facilities are also available to conduct investigations outside the ISS that might enable research on radiation-resistant bacteria and microorganisms.

BREAK-OUT SESSION GROUP III

Microbiology of the Human Environment

What constitutes a healthy microbiome? Researchers have found that the human microbiome influences various aspects of human life, such as aging, behavior, and the immune system. Researchers also know that the interaction is bidirectional, and microbiome fitness can be affected by environmental changes and dietary choices. Group III discussed the relationship between the human microbiome and immunome, specifically related to research subjects, data gathering, and microbiome research controls on the ISS.

Group III Participants

Richard Arnold
Maria Dominguez-Bello
Susan Erdman
David Fothergill
Marc Giulianotti**
Alexander Khoruts
Andrew Lee
Camilla Mauzy
C. Mark Ott*
Michael Strong
Camilla Urbaniak

**Discussion Lead*
***ISS National Lab representative*

Group III Key Findings

Recommended research subjects:

- ISS crew members
- Rodents
- Other model systems

Recommended data sources:

- Feces
- Urine
- Saliva
- Blood
- Skin
- The live/work environment

Recommended controls:

- ISS crew members
- Citizen scientists
- Recommended entities for data evaluation:
- U.S. Department of Defense
- Multiple agencies including NASA, U.S. Department of Agriculture (USDA), and NIH

Group III Abbreviated Discussion Minutes

Microbiome research subjects on the ISS include ISS crew members, rodents, and other model systems. More than 230 astronauts from 18 countries have flown to the ISS (including Expedition 52). The current ISS crew member sample size is six; because of this small sample size, Group III agreed that as much data as possible should be gathered from the crew members, including dietary, medication, and mood-related data. Feces, urine, saliva, and blood samples could also be frozen or fixed. NASA's Human Research Program (HRP) is conducting ongoing microbiome investigations based on this data. Rodents also serve as research subjects, and sampling should include their feces and the areas around their enclosures.

There may be an opportunity to capture aspects of astronaut behavior/mood, but the policy regarding the anonymization of astronaut data may limit or exclude the use of such data. Barring challenges to astronaut data, behavior or mood could be quantified with a mental skills test or behavioral health assessment to evaluate cognitive ability and emotional state. Crew members could serve as their own controls by having data gathered preflight, in-flight, and postflight, and Group III participants recommended citizen-scientists (amateur or nonprofessional scientists) or volunteers to contribute behavioral/mood data as a control group.

Rodent research on the ISS may also provide a context for behavioral analysis. Current research in this area focuses on the sleeping patterns and location for rest, the group behavior of the rodents in the habitat as it relates to their activity level, and their dietary history. Additional opportunities to collect rodent research data are in development, as the standard variables are more clearly defined by experts in the field.

Both rodents and humans experience increased stress onboard the ISS. The Army and Defense Advanced Research Projects Agency (DARPA) is interested in observing changes in the human microbiome when humans are in stressful environments. Collaborative research could be conducted across multiple agencies—including NASA, DARPA, the U.S. Army, USDA, and NIH—regarding ISS crew member behavioral health and physical health and their relationship to the astronaut's environment.

Finally, Group III participants were asked a hypothetical question: What would you study if you were given nine ISS crew members? The group agreed that they would continue to expand on current microbiome research projects on the ISS while conducting additional tests related to crew member behavioral health. One of the most important aspects of spaceflight research is the control—the experiments or observations designed to minimize the effects of variables other than an independent variable. Future controls must be similar to previous studies to accurately interpret new data. While a standard control for spaceflight does not currently exist, chamber studies, stress-based studies, Antarctic studies, and nutritional studies all provide examples of current research that would successfully offset different aspects of the ISS crew member experience onboard the ISS and, therefore, assist in defining controls going forward.

BREAK-OUT SESSION GROUP IV

What's Next for Microbiome Research in Space?

The U.S. Congress extended operations of the ISS National Lab through 2024, leaving seven years to conduct additional experimental research on the ISS National Lab in its present form. Timely identification of potential investigations and partnerships for microbiome/immunome research will assist in focusing future efforts to take advantage of the unique capabilities of the ISS National Lab. Researchers can utilize the stress-inducing environment of the ISS—a semi-closed system in microgravity with increased exposure to radiation—to investigate how the human microbiome responds to these stressors. Group IV participants identified opportunities to harness the microbiome to benefit life on Earth and made recommendations on how to improve current microbiome/immunome research methods on the ISS and on the ground.

Group IV Participants

Cathy Abidin**
Aaron Mills
David A. Mills
Andrew Moeller
Michael Roberts*
Poorani Subramanian
Fred Turek
Martha Vitaterna
Alexander Voorhies
Renee Wegrzyn

**Discussion Lead*
***ISS National Lab representative*

Group IV Key Findings

Suggested future microbiome/immunome research areas:

- Studying the biofilm structure change exhibited by *Pseudomonas aeruginosa* (a common disease-causing Gram-negative bacterium)
- Observing microbiome assembly and sources
- Using bacteria to transform waste into useful products or things that humans need, such as vitamin D
- Furthering the development of a portable system that can rapidly detect organisms and generate data on an ecosystem
- Using the microbiome as a "living foundry" to manufacture beneficial products
- Assessing the effect of the stressful environment of the ISS on transference of the microbiome from parent to offspring
- Conducting Lenski *Escherichia coli* long-term evolution experiments (LTEE)
- Measuring how microbiomes change while on the ISS and then again once back on Earth
- Identifying phages to combat multidrug use
- Gauging the impact of the low sheer force of the ISS on viral oncogenesis
- Analyzing the interplay between worsening innate immunity on the ISS and increased pathogenesis of certain organisms

(Continued) Suggested future microbiome/immunome research areas:

- Informing studies on cancer-causing viruses that simulate oncogenesis, the formation of cancer cells, and preadaptation to cancerous versus noncancerous cells that allow viruses to track (or not track) cancer progression
- Testing the effectiveness of a drug or probiotic that targets the microbiome community associated with tumor growth/various types of cancer
- Observing microbial changes through multigenerational studies of plants and rodents in a space environment; informing potential future multigenerational studies of humans
- Studying how bone loss and muscle atrophy are affected by the combination of stress and changes in the microbiome caused by living in space
- Monitoring circadian rhythms and circadian medicines

General recommendations to improve microbiome/immunome research in space:

- Rather than waiting for a return to Earth to analyze data, begin analyses on the ISS, allowing for real-time changes to experimental parameters; provided samples are saved from each variation
- Approach ISS research from a migration viewpoint, rather than a genetic diversity viewpoint
- Hardware automation is preferred to citizen-scientists (amateur/nonprofessional scientists; often volunteers)
- Inbred strains of mice should be used for experiments rather than mice with increased genetic diversity
- New data collection methods to assess microbiome- and immunome-related data, such as pin pricks and “chip” devices to continuously monitor rodent/human health in a semi-closed environment

Group IV Abbreviated Discussion Minutes

Interest in understanding the functionality and relationship between humans and their microbiome is a relatively new area of study. Mining existing data from previous experiments to conduct novel microbiome research promotes microbiome research without much effort and serves as a bridge to research specifically directed toward microbiome research on the ISS. ISS-based experiments on viral transfer, latent virus activation, rodent models, and wound healing are just a few of the completed or current projects that could provide robust microbiome-related data. Current and future experiments here on Earth and on the ISS have the potential to expand the scope of research to include the collection of microbial data. Agencies, initiatives, and individual principal investigators conducting microbial research welcome Interagency collaboration efforts around the concept of sharing data.

Researchers are invited to take advantage of the unique aspects of the space environment (microgravity, radiation exposure, etc.) when contemplating research opportunities related to the microbiome and immune function. Relevance should not be determined solely by what researchers consider to be the most important environmental aspect of ISS research. Because of the uncommon nature of space and the unknown elements of the human microbiome/immunome, relevant data may come from unidentified or understudied research areas, and the parameters of research conducted on the ISS should accommodate for that possibility.

Ideally, future microbiome/immunome studies will continue to take advantage of the minimal external interference offered by the semi-closed system on the ISS and expand on current research strategies and concepts. A few such analogs exist on Earth (e.g., the South Pole or NASA's Extreme Environment Mission Operations [NEEMO]), but none are quite as closed as the ISS National Lab. The low level of outside interference—limited to the exchange of materials from outside the ISS—means better control of microbial inputs. That alone may increase knowledge of how environments influence microbial ecology.

Group IV participants also discussed overcoming research challenges on the ISS, such as limits on the time allotted to conduct research, limits to physical space for the experiments themselves, and the difficulties associated with achieving statistical significance. Researchers are often faced with difficult decisions whenever they conduct research in a singular and complex environment such as the ISS National Lab. Therefore, collaboration efforts and joint project goals should be determined before implementation.

Lastly, low Earth orbit is only the first step in the journey to Mars or expansion on the Moon. The continuation of research on ISS crew member health in relationship to the built semi-closed environment of the ISS may prove essential in future space endeavors.

Potential Research Opportunities
In addition to the funding opportunities offered by the agency-affiliated microbiome research projects mentioned earlier—the Human Microbiome Project and the National Microbiome Initiative—opportunities in the field of microbiome/immunome research are advancing:

- The ISS National Lab has several projects related to the human microbiome and the immunome that include wound healing and the stability of the human virome (collection of viruses in and on the human body) in spaceflight.
- NASA and the Alfred. P. Sloan Foundation are collaborating to promote microbiology research on the ISS. Postdoctoral fellowships were awarded for ISS research that will promote human health here on Earth and inform our understanding of the microbiome in space. Using NASA's existing microbial data collection to study various aspects of the microbiome, researchers will explore the microbiome, including genetic changes resulting from spaceflight, microbial virulence and resistance to antimicrobial agents in the built environment, and relevance to future design and development of human-occupied exploration vehicles.

(list continued on next page)

- The newest division of DARPA, the Biological Technologies Office (BTO) has recognized biology as a core science in the future development of defense technology. DARPA's Living Foundries project has two components: The first component, now complete, focused on the development of tools and capabilities for engineering biological systems, while the second component builds upon those advancements to develop a prototype infrastructure for engineering biology experimentation that has yet to be invented.
- Solicitations that employ biological systems to enable and inform the design of new technologies will be promoted. The work done at BTO will expand on work in other DARPA offices that was already branching out into neuroscience, microsystems, and other diverse disciplines.
- Current grant funding exists through NIH to advance mechanistic, translational, and clinical probiotic/prebiotic and human microbiome research.
- The National Science Foundation (NSF) and the USDA recently awarded more than $2 million in grants to support early-stage microbiome research through the Early-concept Grants for Exploratory Research (EAGER) program.

The list of opportunities outlined above is not exhaustive; interest in and demand for microbiome/immunome research is widespread and will only increase as current studies publish statistically significant research warranting further study.

Microbiome and Agriculture

Plants and microorganisms have a similar relationship to that of humans and microorganisms. Beneficial bacteria, fungi, and other microbes live in and on plants and, for the most part, enhance growth and boost disease resistance. Agricultural microbiome research on the ground and in space will promote plant health and productivity here on Earth and, in turn, inform the development of future food production methods and techniques needed to grow food on station and during extended spaceflight expeditions.

Research on the microbiome as it relates to human health and agriculture will help identify opportunities for the private sector and academia to make meaningful contributions to future research efforts. Investigations studying how microbial communities impact plant growth will inform the development of techniques and technologies and may lead to improved consistency regarding qualitative and quantitative metrics of agricultural microbiome research. Clarifying biological systems and answering questions, such as "What is a healthy microbiome?" will propel microbiome/immunome research forward.

THE PATH FORWARD

The Future of Microbiome/Immunome Research in Space

The ISS National Lab is dedicated to managing next-generation microbiome/immunome projects that will benefit life on Earth and promote continued research and collaboration on the ISS. Empowered by NASA, the ISS National Lab helps researchers meet their unique research and development objectives through the unparalleled research environment of space. To ensure project success, the ISS National Lab coordinates with a cadre of implementation partners to provide specialized services from preflight to postflight.

The results of this initial workshop produced meaningful discussion regarding the microbiome/immunome and disease, provided the current state of microbiome research, and highlighted a fraction of the potential research opportunities available onboard the ISS National Lab. The information collected here will be promoted among federal agencies, in addition to NASA, to build interest in microbiome research, further interagency collaboration, and generate sponsored research programs that focus on the human microbiome/immunome in microgravity and on Earth.

For more information on the microbiome/immunome workshop and access to related ISS National Lab activities and content, visit www.issnationallab.org/workshops/microbiome.

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