Final Report* – Workshop on Spaceflight Alterations in Host-Microorganism Interactions

Summary: On June 11, 2009, a workshop that included internal and external experts was convened to determine the risk of changes in microorganisms that could alter host-microorganism interactions during a mission. The evidence is based in part on multiple flight experiments which indicate altered virulence in *Salmonella typhimurium* when cultured in flight. The workshop participants were tasked to determine if adequate information was available to initiate changes in NASA's current approach to infectious disease risk assessment and medical operations. The consensus of the participants is that the current evidence was not adequate to provide direction for operational changes; however, the evidence is compelling and clearly indicates that changes to microorganisms were occurring during spaceflight and further research is required.

While preventative measures limit the presence of many medically significant microorganisms during a mission, microbial infection of crewmembers cannot be completely prevented. In 2008 the Institute of Medicine (IOM) reviewed the Human Research Program Evidence Book of the *"Risk of Crew Adverse Health Event Due to Altered Immune Response."* The IOM cited research from a flight experiment by Dr. Cheryl Nickerson aboard STS-115, which indicated that the enteric pathogen, *Salmonella enterica* serovar Typhimurium, had become more virulent when cultured during spaceflight. The IOM recommended NASA "Develop evidence books on additional risks, including alterations in microbe and host interactions…" In November 2008, a Risk entitled, "*Risk of Adverse Health Effects Due to Alterations in Host-Microorganism Interactions*," was added to the Human Research Program Integrated Research Plan with the action to convene a workshop to review the current knowledge base on alterations in host-microorganism interactions due to spaceflight and make a recommendation to the Human Research Program as to how this risk affects microbial risk assessment and subsequently our requirements and medical operations.

For this workshop, the participants were asked to provide recommendations on the following question: *Does the current evidence indicate changes in the host-pathogen relationship such that it would affect our microbial risk assessment and subsequently our requirements and medical operations?* To facilitate an answer the question, the participants were asked to provide their opinions on the following:

- Is the current evidence base adequate to answer this question?
- If the evidence base is adequate, can we define the likelihood of such an event, the most likely consequences, and the recommended requirement and operational changes (if any)?
- If the evidence base is inadequate, what specific knowledge gaps should be targeted to answer this question?

To provide information and to stimulate discussion, several oral presentations were given throughout the day.

Portions of this Final Report have been edited to remove as yet unpublished scientific and medically sensitive data.

Presentations and Discussions

Dr. Michelle Edwards (Futron/Wyle Integrated Science and Engineering Group) – Dr. Edwards discussed NASA's risk assessment and management process to clarify the context of this discussion and how it might subsequently influence current NASA operations and research.

Dr. Robert Haddon (Wyle Integrated Science and Engineering Group/NASA Space Medicine Division) – Dr. Haddon discussed NASA's approach to mitigating the likelihood of contact with pathogenic microbes and to treating infectious diseases once they occur during flight. Dr. Haddon focused on the practical aspects of infectious disease risk assessment and prevention. During a spaceflight mission, infectious disease risk cannot ever be expected to be zero, thus operational procedures only mitigate risk and must be flexible to allow for case-by-case evaluations. Dr Haddon noted that successful preventative measures included adequate sleep and exercise, preflight microbial monitoring, and engineering design, such as air filtration. Skin care and preservation of skin integrity were specifically noted as important in the prevention of microbial infections, such as those due to *Staphylococcus aureus*. Dr. Haddon also identified pharmacological agents as countermeasures against infectious disease. Pharmaceuticals were separated into two categories - definitive treatments (whether topical or oral) and temporary treatments to allow a patient to be returned to earth.

Further group discussion expanded this point to note the recent increase in the incidence of community-acquired methicillin-resistant *Staphylococcus aureus* (MRSA). Dr. Haddon pointed out, and the group concurred that while MRSA poses a distinct challenge for spaceflight, antibiotic sensitive *S. aureus* cannot be discounted as a potential threat to the crew.

The group also noted that the stability of antibiotics is a concern as they are the primary countermeasure after an infection occurs. Lack of knowledge of the efficacy of antibiotics on microorganisms during flight and their pharmacodynamics in the crew were also noted. One experiment that was proposed was to sample urine every 6 hours from crewmembers receiving antibiotics prescribed, such as for urinary tract infections. During group discussion, the limitations of our current in-flight diagnostic capability were considered as a potential problem, particularly with regard to our inability to definitively identify a given infectious agent, for example *S. aureus*, as the cause of an infection.

Dr. Duane Pierson (NASA Habitability and Environmental Factors Division) – Dr. Pierson discussed NASA's microbial monitoring of spacecraft, focusing on routes of infection and reservoirs of infectious agents. Dr. Pierson presented conditions occurring during spaceflight that affect infectious disease risk, including both negative factors (*e. g.*, high-stress conditions, recycled air and water, dysregulation of the immune system) and positive factors (*e. g.*, healthy crew, limited exposure to public health pathogens). He discussed, as a part of infectious disease prevention, that suspected routes or reservoirs of infectious agents are routinely monitored, including: the crew themselves; food; potable water; vehicle air; and cabin surfaces. The microorganisms that have been isolated from the ISS are primarily common environmental microorganisms, though Dr. Pierson did stress that isolates from Mir sampling demonstrated the potential for the appearance of unsuspected pathogens with extended use of flight habitats. Dr. Pierson cited reactivation of latent viruses as a potential infectious threat, and listed several types

of infections likely to occur during flight, including: urinary tract infections; skin infections; oral infections; gastrointestinal tract infections; and upper respiratory tract infections.

Group discussion on both Dr. Pierson's and Dr. Haddon's presentations began with an assessment of the extensive numbers of stakeholders affected by infectious disease risk assessment and mitigation, including: (1) NASA Engineering life support systems and vehicle and hardware design; and (2) multiple Life Science disciplines such as Pharmacology and Immunology. An even larger number of stakeholders have concerns related to microbes (including NASA Planetary Protection), and may benefit from knowledge gained in studies associated with this risk. A better understanding of the ecology and responses of microbes during a mission was recommended. The formation of a common NASA Microbiology Panel was suggested as a means to improve communication on both recent discoveries and common problems.

Dr. Cheryl Nickerson (Arizona State University Biodesign Institute) – Dr. Nickerson discussed her flight research on spaceflight-related changes in *Salmonella* genotypic and phenotypic characteristics, including microbial virulence. Dr. Nickerson's findings concerning changes in virulence in *Salmonella* aboard STS-115 prompted a recommendation by the IOM that NASA initiate a risk associated with alterations in host-microorganism interactions.

At this workshop, Dr. Nickerson presented results from ground-based experiments that led to her original STS-115 flight experiment. The interesting results from STS-115 accelerated a second flight experiment aboard STS-123, which reinforced her earlier findings and provided insight into the mechanisms behind the changes in virulence. In brief, her work showed clear changes in the virulence of *Salmonella* using a murine model of infection. Using LB medium, cultures grown during flight consistently demonstrated lower LD_{50} values and quicker times-to-death than cultures grown identically on the ground. Molecular analyses strongly suggested a role for the global regulator Hfq as a major component of the mechanism behind the response. In defining the initiator of the response, Dr. Nickerson discovered that changes in growth medium composition could eliminate differences in virulence documented between flight and ground cultures. Her work suggests ions, specifically phosphate, play a role in this change.

Dr. Nickerson identified several knowledge gaps for consideration to help to quantify this risk. She made it clear that the practical application of her research does not inherently imply a threat to the crew; however, the information provided by this research may lead to operational techniques to mitigate the risk of infection during a mission.

Dr. Brian Crucian (Wyle Integrated Science and Engineering Group/NASA Human Adaptation and Countermeasures Division) – Dr. Crucian discussed a summary of spaceflight-related changes in the immune system focusing on data from their recent flight experiment. Dr. Crucian's presentation provided insight into changes in the host response in host-microbe interactions. Study of the human immune system during spaceflight is limited primarily to samples collected preflight and post-flight with a small number of exceptions, including the current Integrated Immune spaceflight experiment. These studies provide several pieces of information that suggests dysregulation of the human immune system during flight, though the full impact of altered immune function on either host-microbe interactions specifically, or on infectious disease risk more generally remains unclear.

One key question that the research of Dr. Crucian and colleagues addresses is the effect of long-duration spaceflight on the immune system not connected to those changes induced by launch and landing. While a large number of samples have yet to be processed, their work suggests that immune dysregulation persists after launch, providing new insight into whether the crew are under extended, consistent stress or if the stress and potential immune dysfunction result from events such as launch, landing, and EVAs.

General Discussion

Following Dr. Crucian's presentation, the participants continued an extended discussion based upon the workshop presentations. Key points of that discussion include:

- Infectious disease risk is a factor in many aspects of spaceflight, including vehicle design. One example was the potential of a latent virus, e.g. cytomegalovirus, being present in urine and then passing to other crewmembers through a regenerative water system;
- Much of the discussion centered on the extent to which the data type (*e.g.*, dose-response characteristics; antibiotic resistance; flora identified) affected the resulting assessment of risk. The answer to this has not been fully investigated even here on Earth, though some interesting information has been obtained. For example, in considering dose-response characteristics in microbial risk assessment, several computer models suggest changes in this variable may not have as dramatic an effect on microbial risk, as had originally been predicted;
- The extent of alterations in host-microbe interactions may be influenced by the impact of radiation on both the host and the microorganism. Much of the potential impact of this wild-card may depend on the nature of the mechanism behind the alterations in each;
- Immune dysregulation may play an important role in impacting the host-microbe interaction; the time from immune dysregulation to disease is not necessarily immediate, however, and in fact may take considerable time to be manifested;
- The key to many terrestrial analyses of host-microorganism interactions is animal testing, which has the potential to be of tremendous benefit in better understanding this risk. The participants all agreed that additional analytical capabilities in space, including more robust animal experimentation capabilities, would be beneficial;
- Additional investigation into routes of transmission of infectious diseases is required to better understand this risk. This is especially important with transmission routes that are unique to spaceflight, such as droplets from a sneeze that remain suspended for extended periods of time. How, for example, might the absence of settling of such droplets impact the transmissibility of *S. pneumoniae* infections?;
- While the effect of true microgravity on host-microorganism interactions is being studied, almost no information is available concerning the effects of partial gravity, and no such studies are in progress.

Panel Findings:

The consensus of the participants was that the current evidence was not adequate to provide direction for making operational changes. However, the evidence is compelling and clearly indicates that culture in spaceflight does induce a response by microorganisms, and this response is not limited to *Salmonella*. The cause and extent of the response, including its effect on

interactions with a host with possible immune dysfunction, are unclear and require additional research before an operational decision can possibly be made.

Several concerns and areas of research were cited to determine the cause and extent of alterations in host-microorganism interactions and their effect on current risk assessment and countermeasures. These recommendations are encapsulated in the following four knowledge gaps.

Knowledge Gap: Determination of the genotypic and phenotypic alterations of microorganisms in response to spaceflight, the mechanisms behind this response, and its conservation in prokaryotes, including both opportunistic pathogens and commensal organisms.

Knowledge Gap: Determination of specific host-pathogen alterations that occur during spaceflight.

Knowledge Gap: Determination of the efficacy of current countermeasures, including both antibiotic treatment and engineering control, to prevent exposure.

Knowledge Gap: Determination of changes in host susceptibility that occur during a mission, including dysregulation of immune function, composition of normal microbial flora (*e.g.*, mouth, gastrointestinal tract, and skin), and physical and biochemical changes in crewmembers (*e.g.*, skin breaks or changes in cellular ion levels).

Participants

Chair – C. Mark Ott
Michelle Edwards
Robert Haddon
Duane Pierson
Cheryl Nickerson
Brian Crucian
David Watson
Kristina Mena
Clarence Sams
Diane Younker
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Tom Goodwin
Barbara Woolford
Deborah Harm
Sarah Castro
Jacob Cohen
Mayra Nelman

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