Humans living and working in the harsh environment of space present many challenges for habitability engineers and microbiologists. Spacecraft must provide an internal environment in which physical (gas composition, pressure, temperature, and humidity), chemical, and biological environmental parameters are maintained at safe levels. Microorganisms are ubiquitous and will accompany all human-occupied spacecraft, but if biological contamination were to reach unacceptable levels, long-term human space flight would be impossible. Prevention of microbiological problems, therefore, must have a high priority.

Historically, prevention of infectious disease in the crew has been the highest priority, but experience gained from the NASA-Mir program showed that microbial contamination of vehicle and life-support systems, such as biofouling of water and food, are of equal importance. The major sources of microbiological risk factors for astronauts include food, drinking water, air, surfaces, payloads, research animals, crew members, and personnel in close contact with the astronauts. In our efforts to eliminate or mitigate the negative effects of microorganisms in spacecraft, the National Aeronautics and Space Administration (NASA) implemented comprehensive microbial analyses of the major risk factors. This included the establishment of acceptability requirements for food, water, air, surfaces, and crew members. A robust monitoring program was then implemented to verify that the risks were within acceptable limits.

Prevention of microbiological problems is preferred over mitigation of problems during flight, and preventive steps must begin very early in the design phase. Spacecraft development must include requirements to control free water from humidity, condensate, hygiene activities, and other releases. If water is available, microbes are likely to grow because sufficient nutrients are potentially available. Materials selected for the spacecraft must not promote or support microbial growth. Air filtration can dramatically reduce the number of airborne bacteria, fungi, and particulates in spacecraft breathing air. Waterborne bacteria can be reduced to acceptable levels by thermal inactivation of bacteria during water processing, along with a residual
biocide, and filtration at the point of use can ensure safety. System design must include onboard capability to achieve recovery of the system from contamination. Robust housekeeping procedures that include periodic cleaning and disinfection will prevent high levels of microbial growth on surfaces. Food for consumption in space must be thoroughly tested for excessive microbial content and pathogens before launch. Thorough preflight examination of flight crews, consumables, payloads, and the environment can greatly reduce pathogens in spacecraft.

Many of the lessons learned from the Space Shuttle and previous programs were applied in the early design phase of the International Space Station, resulting in the safest space habitat to date. This presentation describes the monitoring program for the International Space Station and will summarize results from preflight and on-orbit monitoring.

Microbial Monitoring of the International Space Station

Cherie M. Oubre, Ph.D.

Wyle, Senior Scientist

Microbiology Laboratory - NASA Johnson Space Center
Book Chapter

The Impact of Infectious Disease

<table>
<thead>
<tr>
<th>Mission</th>
<th>Published Incident</th>
<th>Consequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apollo 7</td>
<td>Upper respiratory infection in 2 crewmembers prior to flight. Influenza symptoms within a few days after return</td>
<td>Preflight antibiotic therapy</td>
</tr>
<tr>
<td>Apollo 8</td>
<td>All crewmembers experienced preflight viral gastroenteritis which was</td>
<td>Believed to cause one crewmember’s vomiting and GI distress during flight</td>
</tr>
<tr>
<td>Apollo 9</td>
<td>Rhinitis and pharyngitis in one crewmember</td>
<td>3 day launch delay</td>
</tr>
<tr>
<td>Apollo 13</td>
<td>Urinary tract infection during flight</td>
<td>Incapacitation</td>
</tr>
<tr>
<td>STS- 36</td>
<td>Upper respiratory infection</td>
<td>4 day launch delay</td>
</tr>
<tr>
<td></td>
<td>Shingles (thoracic zoster) in 47-year-old healthy astronaut 2 days prior to flight</td>
<td></td>
</tr>
</tbody>
</table>
NASA Microbiological Monitoring

- Preflight
  - Clinical
  - Food
  - Potable water
  - Vehicle surfaces
  - Vehicle air
  - Cargo

- Biosafety review of payloads
  - In-flight
  - Potable water
  - Vehicle surfaces
  - Vehicle air
NASA Environmental Monitoring

- Air assessments
- Surface assessments
  - Multiple locations within the vehicles
- Hardware assessments
  - Random samples to ensure quality control
- Payload assessments
  - Biosafety Review Board
Preflight Sample Collection

- **Surface**
  - Randomly selected hardware items
  - Habitable environment of vehicles or modules 10 – 15 days before launch

- **Air**
  - Habitable environment of vehicles or modules 10 – 15 days before launch

- **Water**

**Sample collection**
- Surface (25 cm² area)
- Air - SAS Super 180 Air Sampler (1 min. at 180 L/min flow rate)
- Water – 100 mL sample

**Growth**
- Trypticase Soy Agar (Bacteria)
- Sabouraud dextrose agar (Fungi)
- Heterotrophic Plate counts (CFU/100 cm²)

**Identification**
- Vitek® analysis
- 16s rDNA sequencing
- Microscopy (fungi)
# Preflight acceptability limits

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Acceptability Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td>Air</td>
<td>300 CFU/m$^3$</td>
</tr>
<tr>
<td>Surface</td>
<td>500 CFU/100 cm$^2$</td>
</tr>
<tr>
<td>Water</td>
<td>50 CFU/mL</td>
</tr>
</tbody>
</table>
ISS In-Flight Monitoring

Surfaces

Air

Water

Quantified in-flight and returned to JSC for identification
Inflight acceptability limits

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Acceptability Limit</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Bacteria</strong></td>
</tr>
<tr>
<td>Air</td>
<td>1000 CFU/m³</td>
</tr>
<tr>
<td>Surface</td>
<td>10,000 CFU/100 cm²</td>
</tr>
<tr>
<td>Water</td>
<td>50 CFU/mL</td>
</tr>
<tr>
<td></td>
<td>No detectable coliforms</td>
</tr>
</tbody>
</table>

* As defined in the ISS Medical Operations Requirements Document, NASA, 2003
Inflight Air Sample Collection
Inflight Surface Sample Collection
Surface and Air Sample Analysis

MAS KIT COLONY DENSITY

Bacteria

Fungi

P/N #

0 CFU/m³

~ 50 CFU/m³

~ 200 CFU/m³

~ 400 CFU/m³

~ 600 CFU/m³

~ 1000 CFU/m³

~ 25 CFU/m³

~ 50 CFU/m³

~ 75 CFU/m³

~ 100 CFU/m³

NOTE:
Count only ‘fuzzy’ colonies

If reading is ≥ 5, perform photography

If reading is ≥ E, perform photography
Inflight Water Sample Collection

http://www.youtube.com/watch?v=mu1z153Zs8U
Inflight Water Sample Collection
Air and Surface Results - Bacteria

- The inset illustrates the top 3 most frequently isolated genera of bacteria.

[Diagram showing the frequency of isolation of different genera of bacteria, with Micrococcus, Bacillus, and Staphylococcus being the top 3.]
Air and Surface Results - Fungi


- The inset illustrates the top 3 most frequently isolated genera of bacteria.
ISS Bacterial Isolates – Potable Water

<table>
<thead>
<tr>
<th>SRV-K Hot</th>
<th>SRV-K Warm</th>
<th>SVO-ZV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidovorax temperans</td>
<td>Acidovorax temperans</td>
<td>Acinetobacter lwaffil</td>
</tr>
<tr>
<td>Acinetobacter radiorestitens</td>
<td>Burkholderia gladioli</td>
<td>Bradyrhizobium betae</td>
</tr>
<tr>
<td>Burkholderia gladioli</td>
<td>Caulobacter leidyi</td>
<td>Brevundimonas species</td>
</tr>
<tr>
<td>Caulobacter vibrioides</td>
<td>Comamonas testosteroni</td>
<td>Caulobacter species</td>
</tr>
<tr>
<td>Comamonas testosteroni</td>
<td>Corynebacterium species</td>
<td>Chryseobacterium gleum</td>
</tr>
<tr>
<td>Cupriavidus eutropha</td>
<td>Cupriavidus eutropha</td>
<td>Comamonas testosteroni</td>
</tr>
<tr>
<td>Flavobacterium ferrugineum</td>
<td>Cupriavidus metalldurans</td>
<td>Cupriavidus paucula</td>
</tr>
<tr>
<td>Methylobacterium lusitanum</td>
<td>Dechlorosoma sullum</td>
<td>Leifsonia xyl</td>
</tr>
<tr>
<td>Methylocystis species</td>
<td>Flexibacter species</td>
<td>Methylbacterium fugisawaense</td>
</tr>
<tr>
<td>Sphingomonas paucimobilis</td>
<td>Methylobacterium lusitanum</td>
<td>Methylbacterium lusitanum</td>
</tr>
<tr>
<td>Sphingomonas stygiatis</td>
<td>Methylobacterium podarum</td>
<td>Methylbacterium species</td>
</tr>
<tr>
<td>Staphylococcus species</td>
<td>Microbacterium species</td>
<td>Proteobacterium, alpha-subgroup</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>Pseudomonas fluorescens</td>
</tr>
<tr>
<td></td>
<td>Ralstonia mannitolytica</td>
<td>Ralstonia pickettii</td>
</tr>
<tr>
<td></td>
<td>Ralstonia pickettii</td>
<td>Rhizobium radiobacter</td>
</tr>
<tr>
<td></td>
<td>Sphingomonas capsulata</td>
<td>Sphingomonas capsulata</td>
</tr>
<tr>
<td></td>
<td>Sphingomonas cloacae</td>
<td>Sphingomonas capsulata</td>
</tr>
<tr>
<td></td>
<td>Sphingomonas paucimobilis</td>
<td>Sphingomonas paucimobilis</td>
</tr>
<tr>
<td></td>
<td>Sphingomonas species</td>
<td>Sphingomonas species</td>
</tr>
<tr>
<td></td>
<td>Sphingomonas stygiatis</td>
<td>Sphingomonas stygiatis</td>
</tr>
<tr>
<td></td>
<td>Sphingomonas xenophaga</td>
<td>Sphingomonas yanolkuyae</td>
</tr>
<tr>
<td></td>
<td>Sphingomonas yanolkuyae</td>
<td>Staphylococcus warneri</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stenotrophomonas maltophilia</td>
</tr>
</tbody>
</table>

Predominant genera

- Methylobacterium
- Sphingomonas
- Cupriavidus/Ralstonia
Anomalies

- Fungal contamination of Russian smoke detector (~2001)
- Suspected fungal contamination on SM structure in TVIS pit area (March 2002)
- Fungal contamination of panel fronts in ‘hygiene area’ of FGB; remediation performed (2004)
- Clogged lines in Russian condensate recovery system suspected to be of microbial composition (2005, 2007)
Anomalies (continued)

- High bacteria counts noted in archive samples from CWCs (2007 - 2008); silver-resistant Cupriavidas species isolated
  
- Suspected fungal contamination on Russian air conditioning system ductwork and BOK-3 Command Processing Unit; some condensation pooling behind panels reported (August 2007)

- Fungal contamination on Payloads Water Reservoir (April 2008)
Future Goals

- Evaluating new microbial monitoring technologies for flight implementation
  
  Molecular-based microbial monitoring (MiDAS, MMS, WetLab 2, etc)

  Paper-based technology

- Updating requirements to allow for new monitoring technologies

- Lifetime Surveillance of Astronaut Health
JSC Microbiology Laboratory

Goal: Mitigate microbial risk to crew health, safety, and performance during the human exploration of space

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NASA Microbiologist

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Tom Molina
Melanie Smith
Airan Yoets