Microbial Detection and Control on the International Space Station

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Recent Advances in Microbial Control
Microbiology Laboratory at Johnson Space Center

• Goal: Mitigate microbial risk to crew health, safety, and performance during the human exploration of space
• Hold the requirements that dictate the microbial acceptability limits of the water, air, surfaces, and spaceflight foods
• Responsible for delivering certification of flight readiness status
• Responsible for implementing routine pre-flight and in-flight microbial monitoring practices as a check of contamination controls
• Responsible for remediation when monitoring reveals microbial loads above the acceptability limits
Spaceflight Microbiology

Risk Definition
Are pathogens present?
- Pre-flight medical exams: ↓
- Pre-flight crew quarantine: ↓
- Stringent microbiological monitoring: ↓

Will the crew be exposed?
- Limited exposure to many public health pathogens: ↓
- Small enclosed environment: ↑
- Recycled water and air: ↑

What are the odds that, if exposed, the crew will be infected?
- Healthy, well-conditioned crew: ↓
- Stressful conditions: ↑
- Immune system dysregulation: ↑
- Altered microbial characteristics, including virulence: ↑

What other factors do we need to consider?
- Medical consult throughout a mission
- Limited diagnostics and treatment on board
- Remote location with limited return options

Appropriate Requirements
- Too stringent
  - Waste resources
- Too relaxed
  - Threaten crew health
### Requirements

<table>
<thead>
<tr>
<th>Surface</th>
<th>Air</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>10,000 cfu/cm² bacteria</td>
<td>1,000 cfu/m³ bacteria</td>
<td>50 cfu/ml bacteria</td>
</tr>
<tr>
<td>100 cfu/cm² fungi</td>
<td>100 cfu/m³ fungi</td>
<td>Non-detectable/100 ml fungi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Non-detectable/100 ml coliforms</td>
</tr>
</tbody>
</table>
Sources of Microbial Contamination

Microorganisms are transported to the ISS by the spacecraft itself, cargo, food, water, and the crew.
Mitigation of Microbial Contamination through Prevention and Controls

Methods of Prevention include:
- Pre-flight monitoring of the cargo and environment
- Quarantine of the crew
- Biosafety review of payloads
- Screening of food

Methods of Control include:
- System Design
- Materials Selection
- HEPA Air Filters
- In-line Water Filters
- Water Biocides
### Prevention through Operational Controls

#### Health Stabilization Program

<table>
<thead>
<tr>
<th>Mission</th>
<th>Illness (Crew)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apollo 7</td>
<td>Upper respiratory infection (3)</td>
</tr>
<tr>
<td>Apollo 8</td>
<td>Viral gastroenteritis (3)</td>
</tr>
<tr>
<td>Apollo 9</td>
<td>Upper respiratory infection (3)</td>
</tr>
<tr>
<td>Apollo 10</td>
<td>Upper respiratory infection (2)</td>
</tr>
<tr>
<td>Apollo 12</td>
<td>Skin infection (2)</td>
</tr>
<tr>
<td>Apollo 13</td>
<td>Rubella (1)</td>
</tr>
<tr>
<td>Apollo 14</td>
<td></td>
</tr>
<tr>
<td>Apollo 15</td>
<td></td>
</tr>
<tr>
<td>Apollo 16</td>
<td></td>
</tr>
<tr>
<td>Apollo 17</td>
<td>Skin infection (1)</td>
</tr>
<tr>
<td>Skylab-2</td>
<td></td>
</tr>
<tr>
<td>Skylab-3</td>
<td>Skin infection (2)</td>
</tr>
<tr>
<td>Skylab-4</td>
<td>Skin infection (2)</td>
</tr>
</tbody>
</table>

*Billica, Pool, Nicogossian, 1994*
## Vehicle Design Controls

<table>
<thead>
<tr>
<th>Environment</th>
<th>Control</th>
</tr>
</thead>
</table>
| **Air**     | • An average continuous flow of 566.33 liters per minute (20 cubic feet per minute) or greater must be maintained per person of air that has been cleaned to have at least 99.97% of airborne particles 0.3 μm and larger in diameter/size removed  
  • ISS air systems utilize High Efficiency Particulate Air (HEPA) filter design to provide clean air |
| **Surfaces**| • The interior surfaces of the spacecraft habitable volume shall be compatible for cleaning bacterial contamination to a level of 10,000 CFU/100 cm² or fewer  
  • The interior surfaces of the spacecraft habitable volume shall be compatible for cleaning of fungal contamination to a level of 100 CFU/100 cm² or fewer |
## Microbiological Controls

<table>
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<tr>
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<th>Control</th>
</tr>
</thead>
</table>
| Water       | • Catalytic oxidizer  
              • Iodine disinfection (1-4 mg/L of iodine)  
              • In-line filtration (0.2 micron) |
Microbiological Controls

- Condensation persistence on interior surfaces shall be limited to 1 hour per day within the spacecraft habitable volume
- A means to clean up contamination within the spacecraft habitable volume shall be provided
- The spacecraft shall provide a housekeeping capability for cleaning, sanitizing, and system maintenance
Contamination Control Checks

Pre-flight Microbial Monitoring: Surface and Air

- Surface sampling schedule and method
  - Hardware – approx. 2 months before launch
  - Modules – 15-20 days before hatch closure
  - Vehicles – 10-15 days before launch
  - 25 cm² areas sampled using polyester swabs wetted with sterile water
    - Swabs vortexed in TSB, media plated onto nutrient agars
      - TSA (bacteria)
      - SDA, SDA/chloramphenicol, and potato dextrose agar (fungi)

- Air sampling schedule and method
  - 15-20 days before hatch closure
  - SAS Super 180 (operated for 1 min @ 180 L/min)
  - TSA (bacteria) and SDA (fungi)

- Microbial Identification
  - Biochemical (VITEK)
  - Molecular (16S sequencing)
Pre-flight Surface Microbe Levels
(Modules and Logistics Vehicles - ISS Construction, Expeditions 1-25)

Bacterial counts
- 956 pre-flight surface samples from modules, Cargo Transfer Bags, and hardware
- 546/956 (57%) negative for bacteria
- 53/956 (6%) exceeded pre-flight specifications

Fungal counts
- 954 pre-flight samples collected
- 835/954 (88%) negative for fungi
- 109/954 (11%) exceeded pre-flight specifications
**Pre-flight Air Microbe Levels**  
*(Modules and Logistics Vehicles - ISS Construction, Expeditions 1-25)*

**Bacterial counts**
- 43 pre-flight samples collected in modules
- 17/43 (40%) negative for bacteria
- No sample exceeded pre-flight specifications

**Fungal counts**
- 44 pre-flight samples collected
- 29/44 (66%) negative for fungi
- 1/44 (2%) exceeded pre-flight specifications
Contamination Control Checks

Pre-flight Microbial Monitoring: Water

- Water sampling schedule and method
  - Containers/Tanks – as close to launch as possible
    - Processed by filtration (Milliflex) and plated on R2A agar
    - Enumeration by heterotrophic plate counts
    - Identification by molecular analysis (16S sequencing)
  - Coliform Detection
    - Colisure Reagent
Contamination Control Checks

**In-flight Microbial Monitoring: Surfaces**

- **SSK - Surface Sampler Kit**
  - US Segment sampled quarterly
  - TSA and SDA contact slides for flat surfaces
  - Swabs wetted with sterile saline used for uneven surfaces
  - Contact slides inoculated with swabs
Contamination Control Checks

Surface Sampling

SSK COLONY DENSITY

Bacteria

- 10 CFU/100 cm²
- 100 CFU/100 cm²
- 1,000 CFU/100 cm²
- 10,000 CFU/100 cm²

If reading is ≥ 4, perform photography.

SSK COLONY DENSITY

Fungi

- 0 CFU/100 cm²
- 2 CFU/100 cm²
- 50 CFU/100 cm²
- 100 CFU/100 cm²

Note: Count only ‘fuzzy’ colonies

If reading is ≥ E, perform photography.
In-flight Surface Microbe Levels (Expeditions 1-29)

Bacterial counts
- 47/297 (16%) negative for bacteria
- 10/297 (3%) exceeded in-flight specifications

Fungal counts
- 187/297 (63%) negative for fungi
- 13/297 (4%) exceeded in-flight specifications

297 in-flight samples collected by ISS crewmembers
Contamination Control Checks

In-flight Microbial Monitoring: Air

- MASK - Microbial Air Sampler Kit
  - 6 modules sampled quarterly
  - Burkard air sampler (~ 85 L sample)
    - TSA (bacteria) and SDA (fungi)
### Contamination Control Checks

#### Air Sampling

![Petri dishes showing bacterial colonies](image)

#### MAS Kit Colony Density

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 0 CFU/cm²</td>
<td>A 8 CFU/cm²</td>
</tr>
<tr>
<td>2 10 CFU/cm²</td>
<td>B 25 CFU/cm²</td>
</tr>
<tr>
<td>3 100 CFU/cm²</td>
<td>C 50 CFU/cm²</td>
</tr>
<tr>
<td>4 1000 CFU/cm²</td>
<td>D 5 CFU/cm²</td>
</tr>
<tr>
<td>5 &gt;1000 CFU/cm²</td>
<td>E 100 CFU/cm²</td>
</tr>
</tbody>
</table>

**NOTE:**
- Count only ‘fuzzy’ colonies
In-flight Airborne Microbe Levels (Expeditions 2-30)

**Bacterial counts**
- 121 in-flight samples collected by ISS crewmembers
- 39/121 (32%) negative for bacteria
- No sample exceeded in-flight specifications

**Fungal counts**
- 119 in-flight samples collected by ISS crewmembers
- 86/119 (72%) negative for fungi
- 6/119 (5%) exceeded in-flight specifications
Contamination Control Checks

In-flight Microbial Monitoring: Water

• Environmental Health Systems Water Kit
  • Samples are collected from the Potable Water Dispenser (PWD) on a monthly basis, alternating between hot and ambient ports
  • Archive samples are collected prior to each Soyuz return

• Analyses
  • Enumeration
    • Microbial Capture Device (MCD) with modified R3A broth
  • Coliform Detection
    • Colisure Reagent
Contamination Control Checks

Water Sampling
In-flight Water Microbe Levels (Expeditions 36-40)

Since 2009

- Samples from the ambient loop have exceeded the in-flight specification of 50 cfu/mL 3 times
- 53% of samples from the ambient loop have contained less than 2 cfu/ml
- One sample from the hot loop revealed 1 cfu/ml; all other samples have been negative for growth
- Coliforms have not been detected
Survey of Bacterial and Fungal Genera: Surfaces and Air

**Bacteria**
- Alloccocus
- Brachybacterium
- Brevibacterium
- Cellulomonas
- Chryseomonas
- Curtobacterium
- Janthinobacterium
- Leifsonia
- Ochrobacterium
- Oerskovia
- Pantoea
- Rahnella
- Rothia
- Sphingomonas
- Brevibacillus
- Enterobacter
- Dermacoccus/Nyctococcus
- Streptococcus
- Microbacterium
- unidentified Gram+ rods
- Enterococcus
- Acinetobacter
- Kocuria
- unidentified Gram+ cocci
- Paenibacillus
- Corynebacterium

**Fungi**
- Aureobasidium
- Candida
- Chaetomium
- Fusarium
- Pithomyces
- Scopulariopsis
- Trichophyton
- Acremonium
- Bipolaris
- Cryptococcus
- Drechslera
- Phoma
- Streptomyces
- Alternaria
- Microsporum
- Ulocladium
- Curvularia
- Paecilomyces
- Rhodotorula
- Cladosporium
Survey of Microbes: ISS Water

Potable Water Dispenser Archive Data – Expeditions 34 to 39

Most Common isolates (historically)

- Ralstonia pickettii
- Burkholderia multivorans
- Sphingomonas sanguinis
- Cupriavidas metallidurans

Microorganism: Unidentified Gram Negative Rod, Sphingobium yanoikuyae, Ralstonia pickettii, Ralstonia insidiosa, Mesorhizobium species, Cupriavidus metallidurans, Chitinophaga species, Chitinophaga arvensicola, Burkholderia species, Burkholderia multivorans, Bradyrhizobium species, Acinetobacter species

Frequency of Isolation

- MCD
- Archive Bag
Contamination Events
Contamination Events

Acceptability Limit Exceeded
Pre-flight Remediation

**Vacuum with HEPA Filtration**
- All cargo bags are vacuumed inside and out for the removal of particulates

**Disinfection Wipes**
- All cargo bags are disinfected with 6% hydrogen peroxide
  - Method was developed based on the European Space Agency’s recommended procedures
1. Determine the cause of the contamination and alter activities accordingly
2. Surfaces
   • Disinfectant Wipes – Benzalkonium chloride (BZK)
3. Air
   • Assess HEPA filters
   • Attempt to identify sources of contamination in the proximity of where the air sampling occurred
4. Water
   • Addition of biocide
Antimicrobial Technology for Long Duration Spaceflight

- Antimicrobial Materials for Next Generation Space Suits
  - Evaluations to determine various antimicrobial materials’ resistance to microbial colonization
  - Copper-doped fiber, silver coated fiber, and silver salt fabric surface treatments

![Image of astronaut in space suit]

**Figure 1.** *Escherichia coli* concentrations (CFU/mL) from the Consortium on Antimicrobial Materials
Antimicrobial Technology for Long Duration Spaceflight

- **Next Generation Water System**
  - Use of beneficial microbes for utilization of waste water
- **Synthetic Biology**
  - An engineered bacteriophage system
- **Electrochemical Disinfection**
  - Contaminated water is part of the biocide-generation process, resulting in biocides being produced in situ
    - Hydrogen Peroxide ($\text{H}_2\text{O}_2$ or HP)
    - Sodium Hypochlorite (NaOCl or Hypochlorite)
    - Peracetic Acid (PAA)
    - Ozone
Antimicrobial Technology for Long Duration Spaceflight

• Ongoing Investigations
  • Antimicrobial compound incorporation into nonporous materials during vehicle design
    • self-disinfecting materials
    • Super hydrophobic materials
  • Assessments of alternate disinfectants
    • chlorhexidine gluconate
    • quaternary ammonium compounds
  • Human factors engineering
    • new methods for housekeeping and food preparation
    • washable keyboards
Does the spaceflight environment alter microbial responses?

Conclusions

- Current methods of microbial control have been historically effective in mitigating infectious disease and biodeterioration risks for spaceflight missions
- Routine microbial monitoring is appropriate as a validation of our contamination controls
- Lessons learned from previous space programs drove design of microbial monitoring efforts for ISS
- Microbial levels in the ISS environment have been, and remain, low due to numerous design and procedural specifications
- Microbes identified during sampling reflect those found in typical terrestrial environments
- Obligate pathogens are generally excluded from the ISS environment
- NASA is seeking to develop new microbial detection hardware and next generation sustainable antimicrobial technologies for long duration spaceflight
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