Microbial Detection and Control on the International Space Station

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Recent Advances in Microbial Control

Commercial Spaceflight

JSC Flight Medicine and Occupational Health Clinics

JSC Spaceflight Food Laboratory

Applied and Basic Research

MPCV Program

JSC Center Support

International Space Station Program

JSC Microbiology Laboratory

JSC Biosafety Review Board

Accreditations:
College of American Pathologists
American Industrial Hygiene Association
Environmental Microbiology Laboratory Accreditation Program
National Environmental Laboratory Accreditation Conference

Spaceflight Microbiology

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

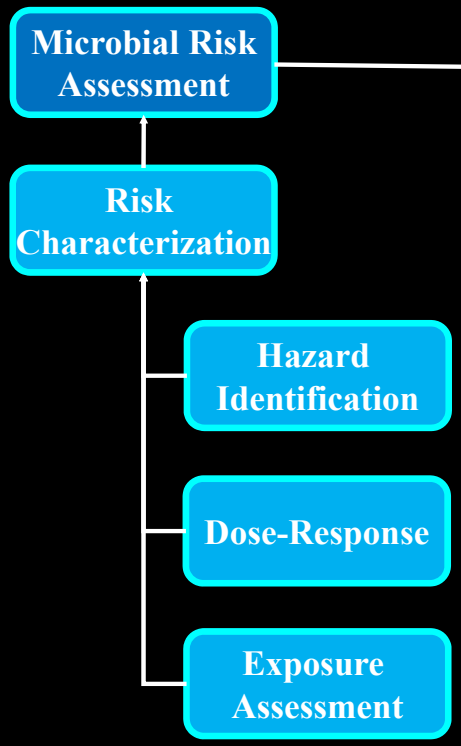
Risk Definition

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

Surface	Air	Water
10,000 cfu/cm^2 bacteria	1,000 cfu/m^3 bacteria	50 cfu/ml bacteria
100 cfu/cm^2 fungi	100 cfu/m^3 fungi	Non-detectable/100 ml fungi
		Non-detectable/100 ml coliforms

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

Mission	Illness (Crew)
Apollo 7	Upper respiratory infection (3)
Apollo 8	Viral gastroenteritis (3)
Apollo 9	Upper respiratory infection (3)
Apollo 10	Upper respiratory infection (2)
Apollo 11	
Apollo 12	Skin infection (2)
Apollo 13	Rubella (1)
Apollo 14	
Apollo 15	
Apollo 16	
Apollo 17	Skin infection (1)
Skylab-2	
Skylab-3	Skin infection (2)
Skylab-4	Skin infection (2)

Billica, Pool, Nicogossian, 1994

Vehicle Design Controls

Environment	Control
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 cm^2 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 cm^2 or fewer

Microbiological Controls

Environment	Control
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

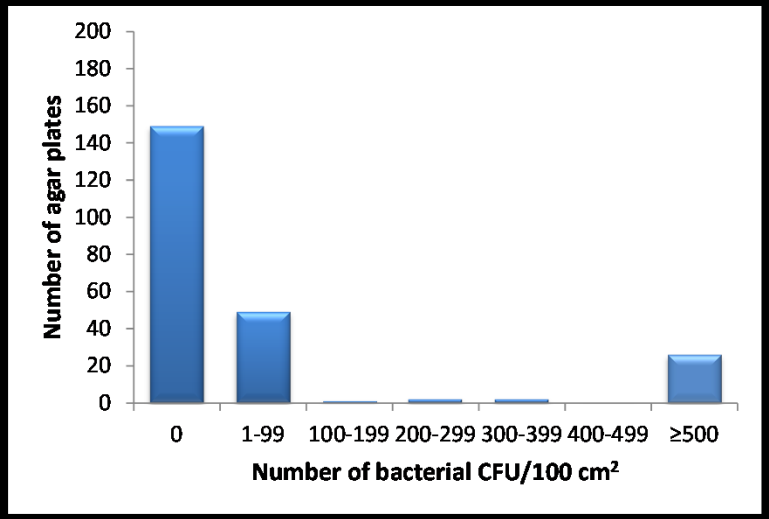
Pre-flight Microbial Monitoring: Surface and Air

- 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 cm^2 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

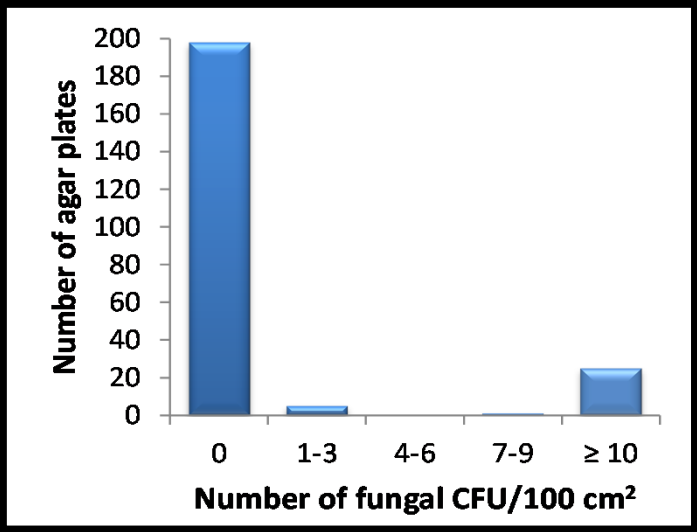
(Modules and Logistics Vehicles - ISS Construction, Expeditions 1-25)

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

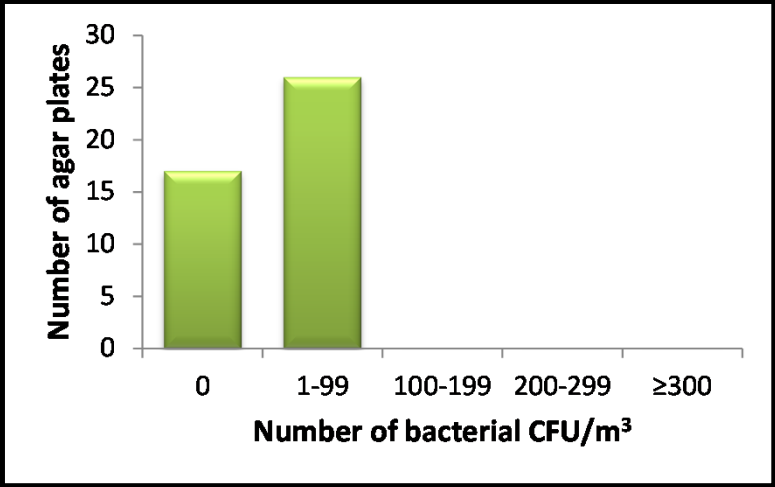


- **954 pre-flight samples collected**
- 835/954 (88%) negative for fungi
- 109/954 (11%) exceeded pre-flight specifications

Pre-flight Air Microbe Levels

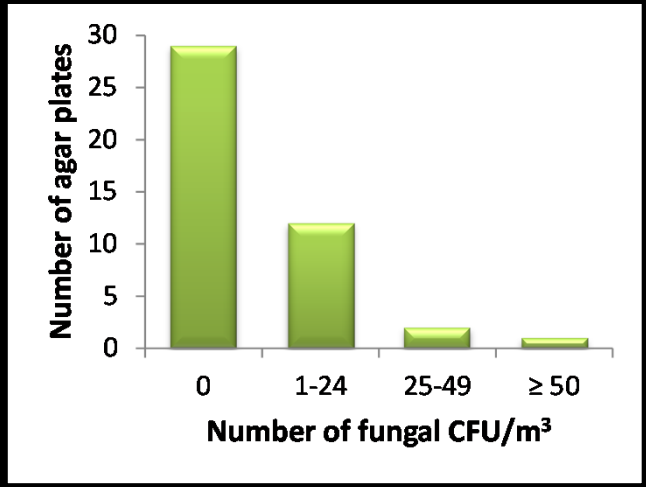
(Modules and Logistics Vehicles - ISS Construction, Expeditions 1-25)

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

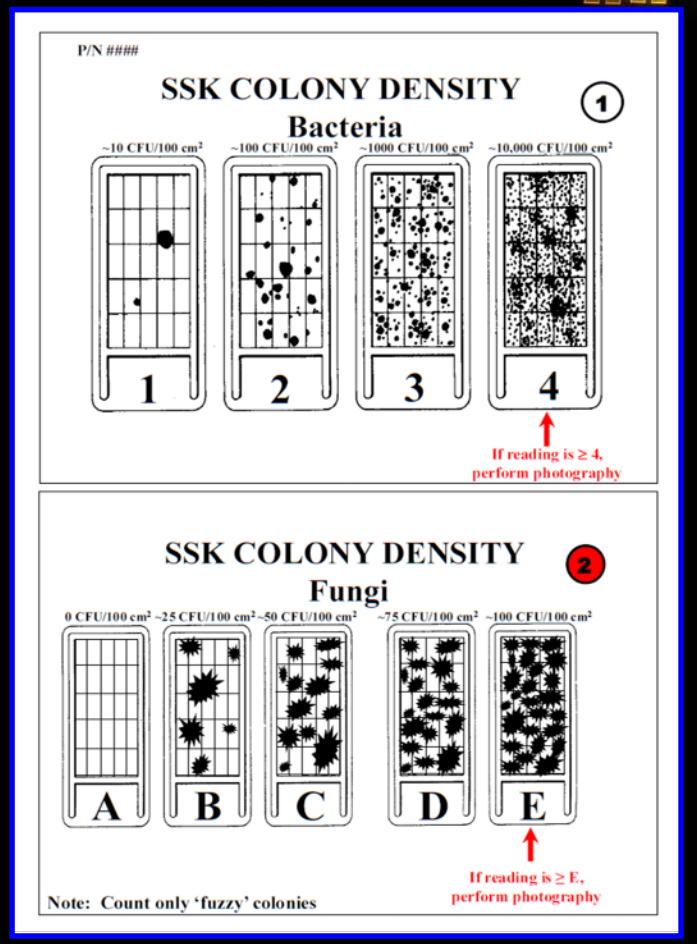
Contamination Control Checks

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

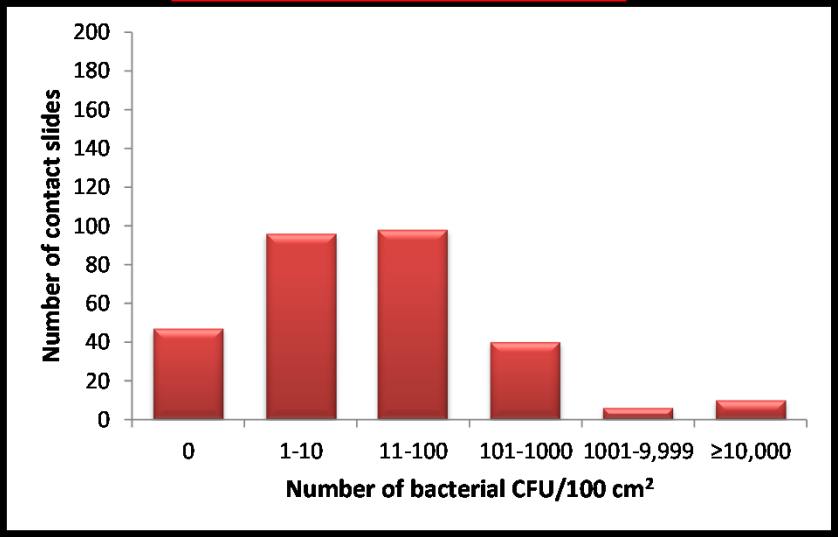
Contamination Control Checks

Surface Sampling

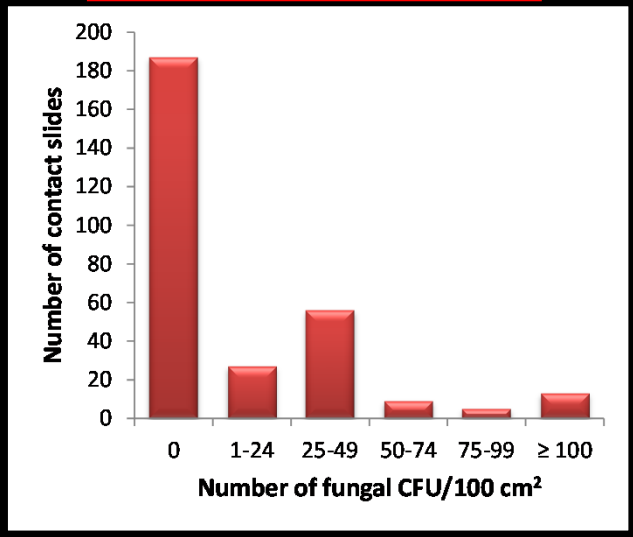


In-flight Surface Microbe Levels (Expeditions 1-29)

Bacterial counts



Fungal counts



297 in-flight samples collected by ISS crewmembers

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

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

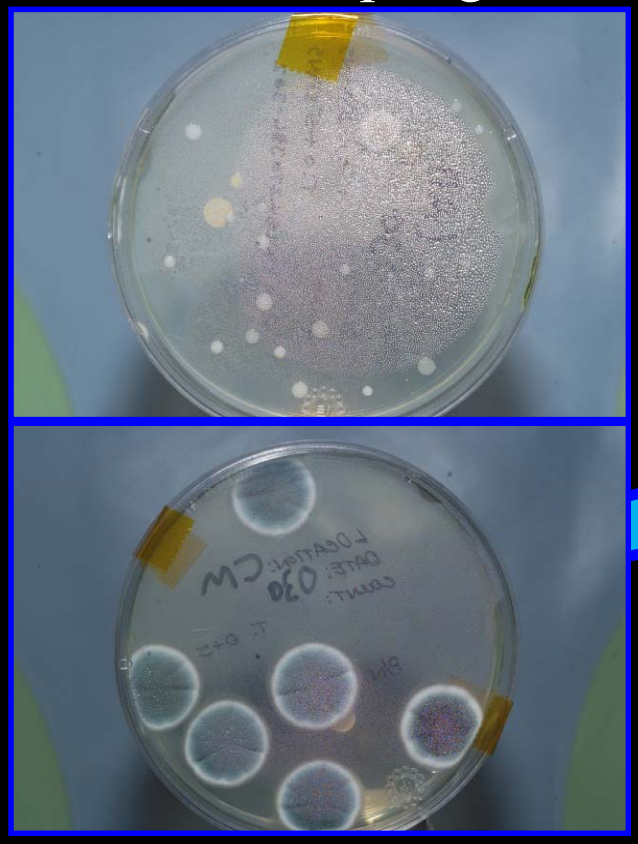
Contamination Control Checks

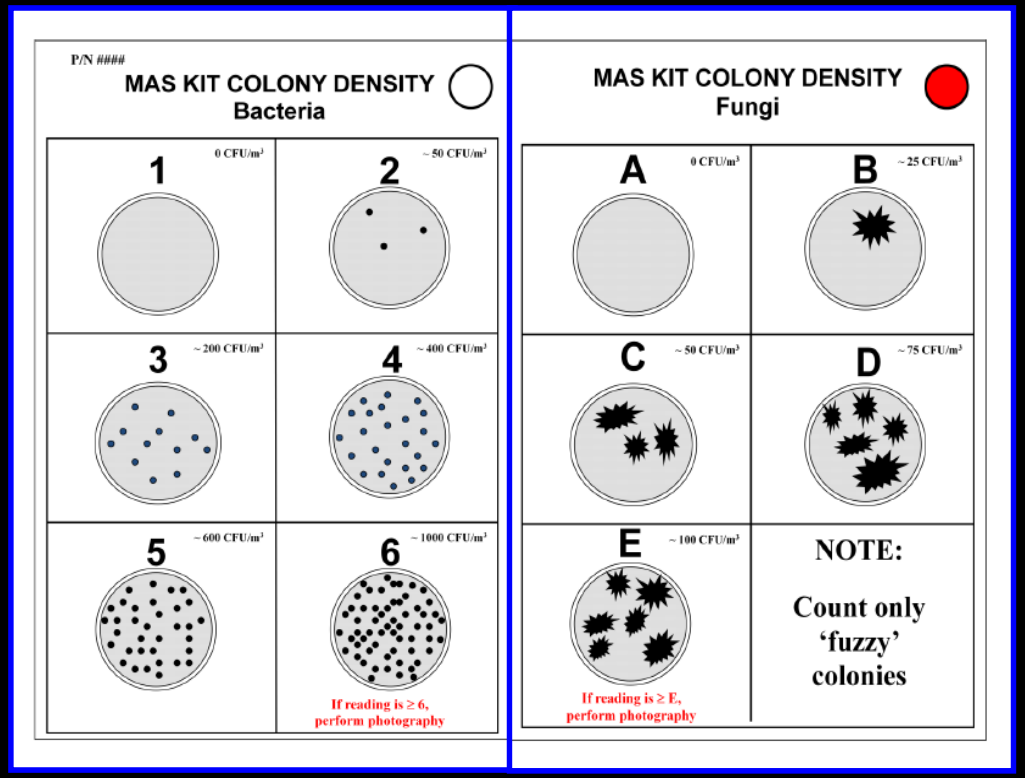
In-flight Microbial Monitoring: Air

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

Contamination Control Checks

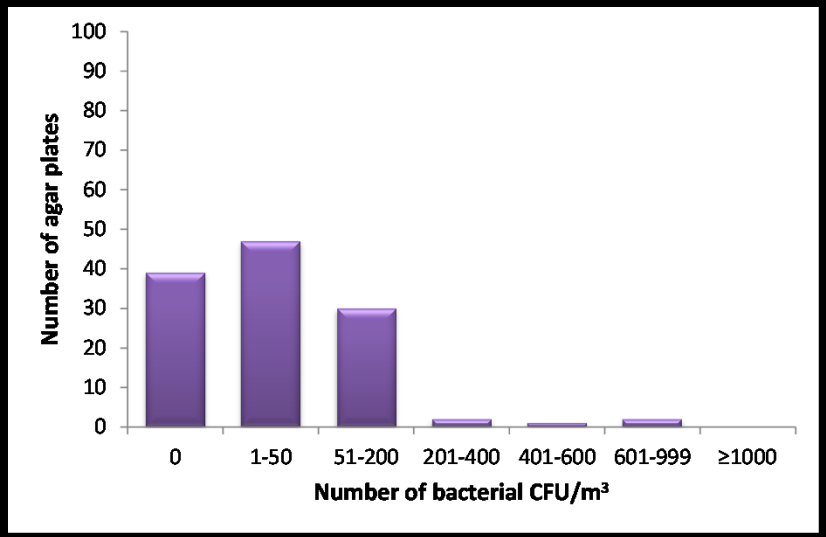
Air Sampling





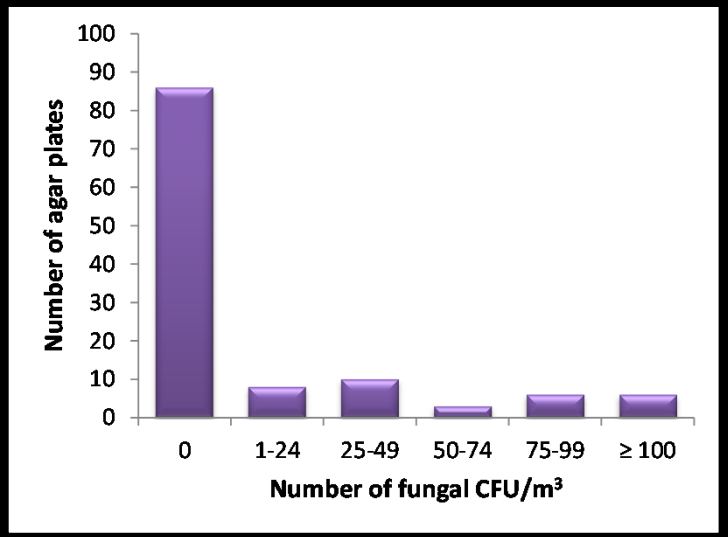
In-flight Airborne Microbe Levels (Expeditions 2-30)

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

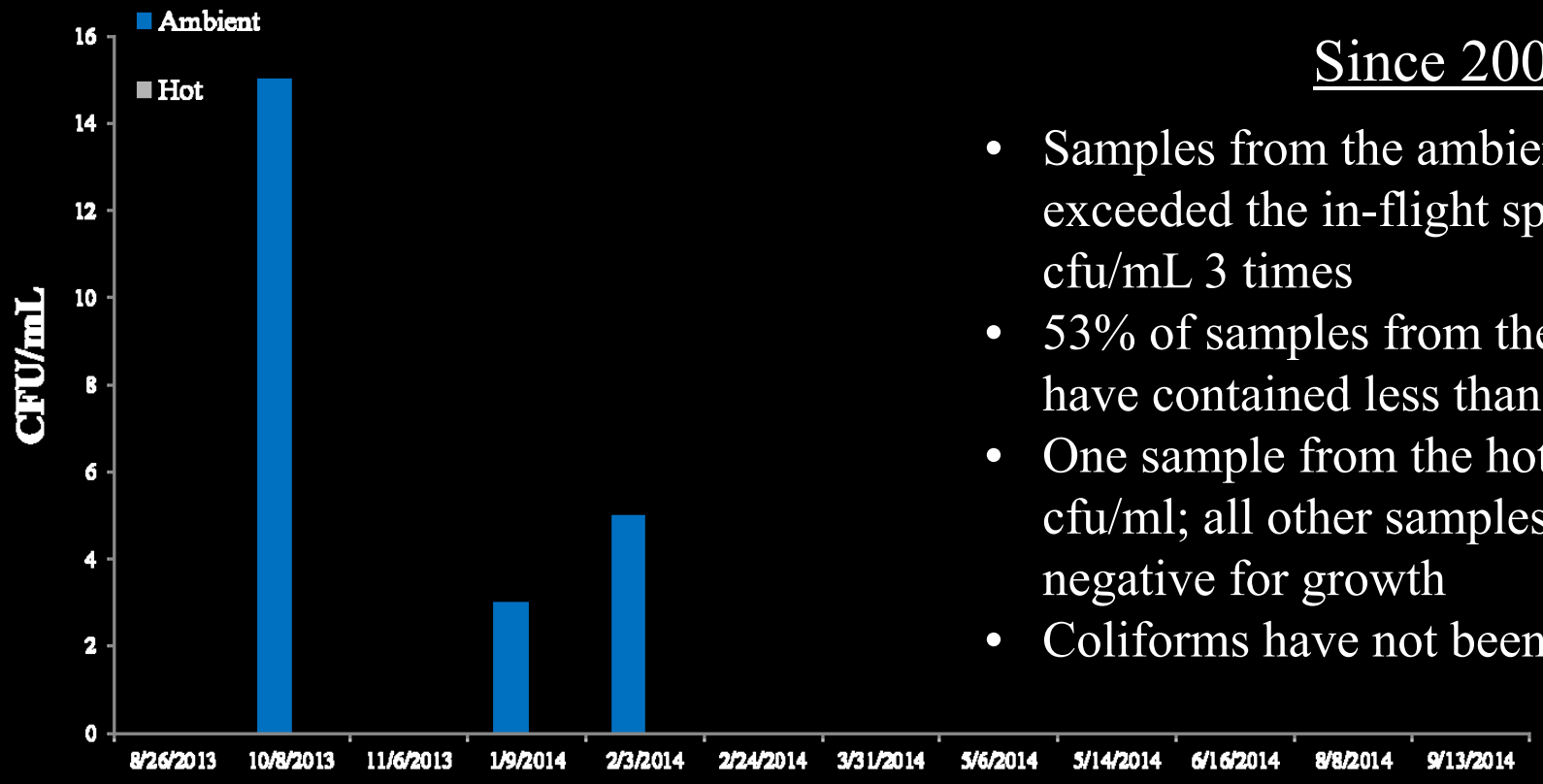
In-flight Microbial Monitoring: Water

- 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)

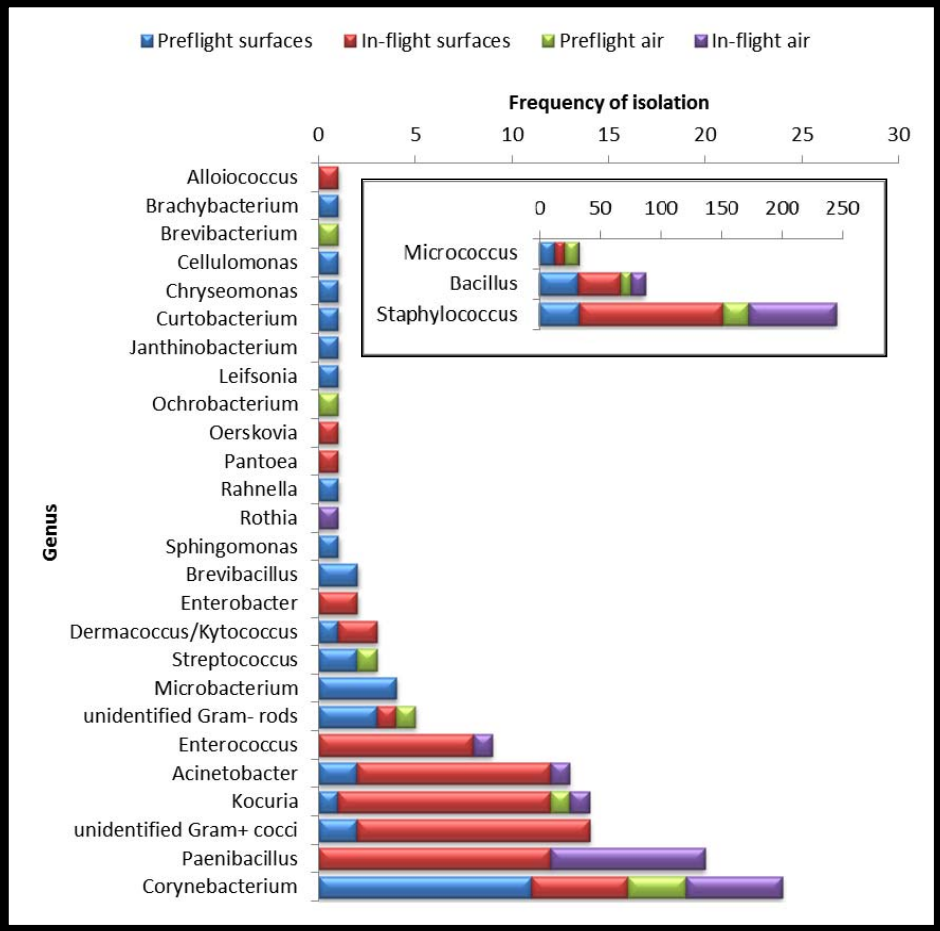


Since 2009

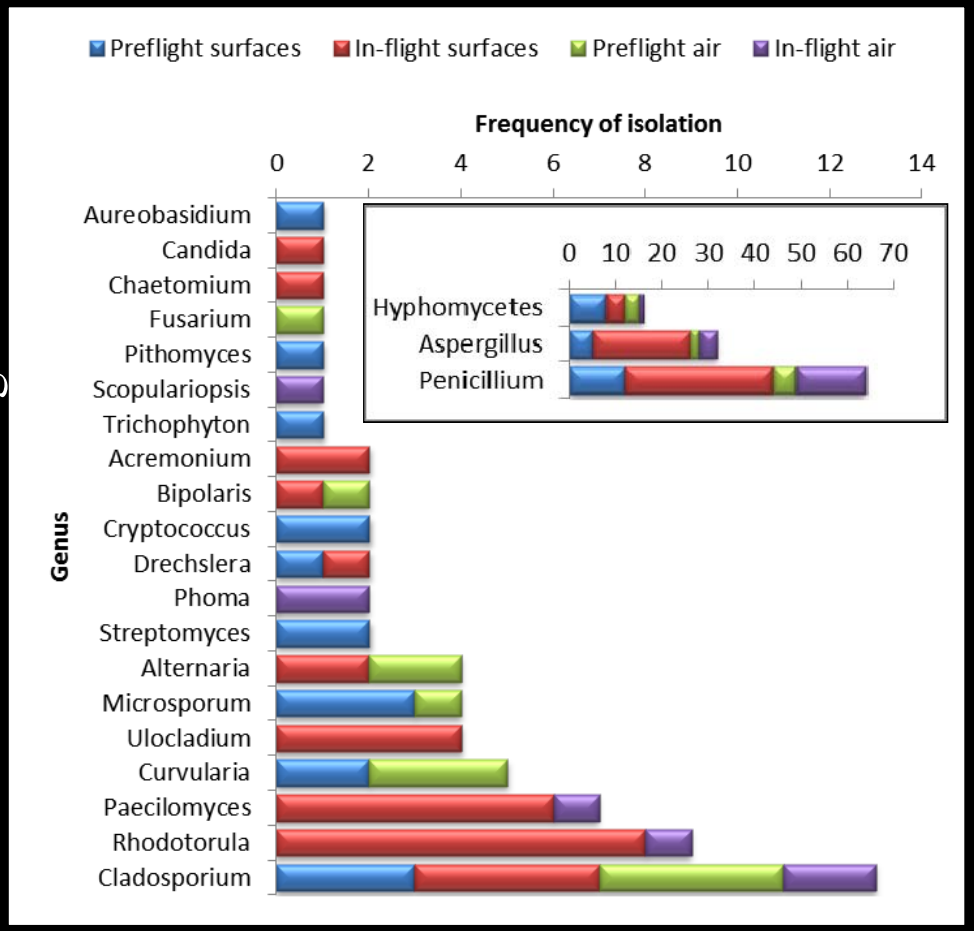
- Samples from the ambient loop have exceeded the in-flight specification of 50 cfu/mL 3 times
- 53% of samples from the ambient loop have contained less than 2 cfu/ml
- One sample from the hot loop revealed 1 cfu/ml; all other samples have been negative for growth
- Coliforms have not been detected

Survey of Bacterial and Fungal Genera: Surfaces and Air

Bacteria

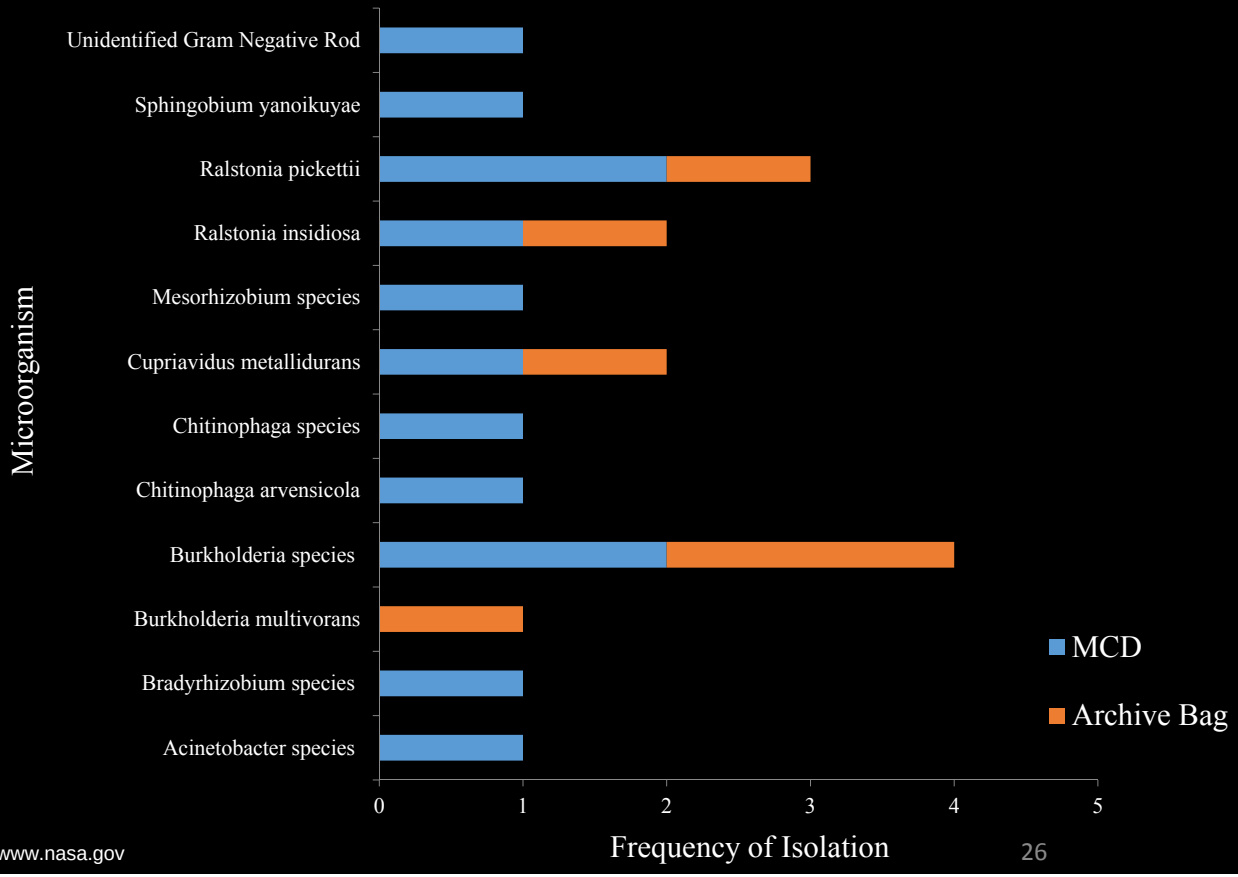


Fungi



Survey of Microbes: ISS Water

Potable Water Dispenser Archive Data – Expeditions 34 to 39

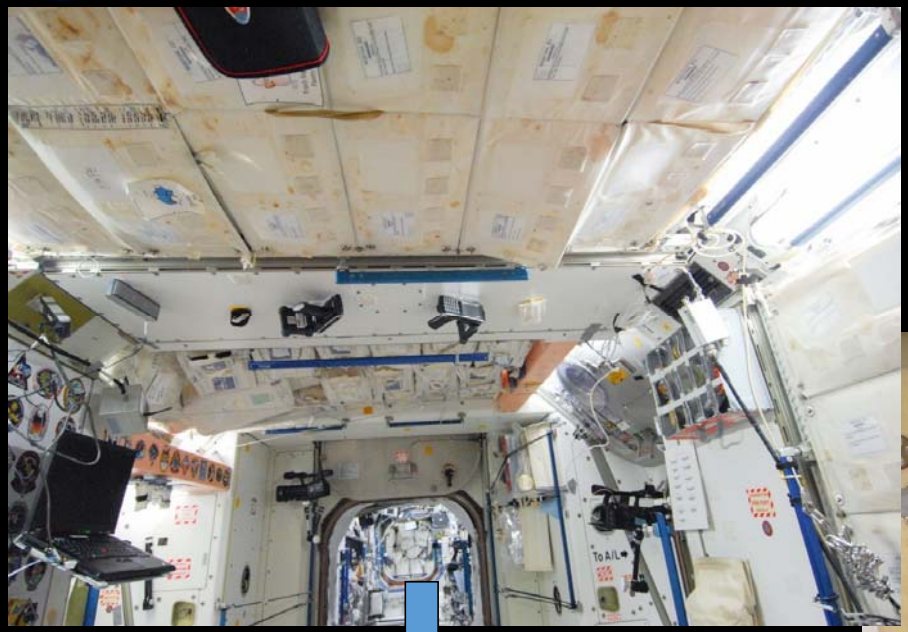


Most Common isolates (historically)

Ralstonia pickettii
Burkholderia multivorans
Sphingomonas sanguinis
Cupriavidas metallidurans

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

On-orbit Remediation

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

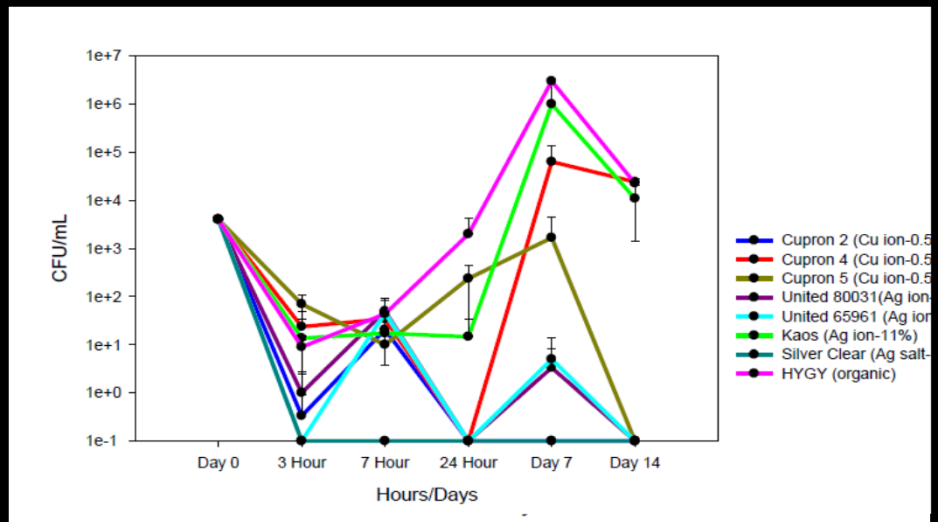
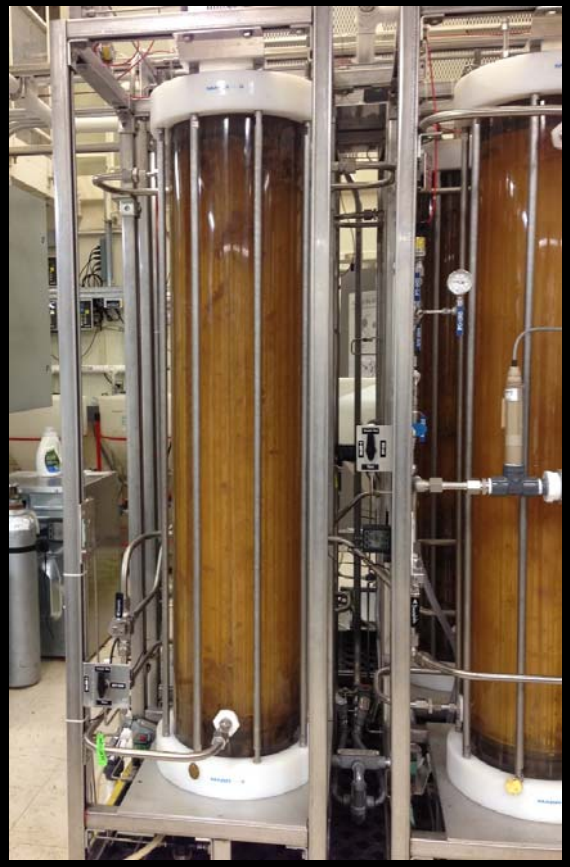


Figure 1. ***Escherichia coli*** **concentrations (CFU/mL) from the Consortium on Antimicrobial Materials**

Antimicrobial Technology for Long Duration Spaceflight

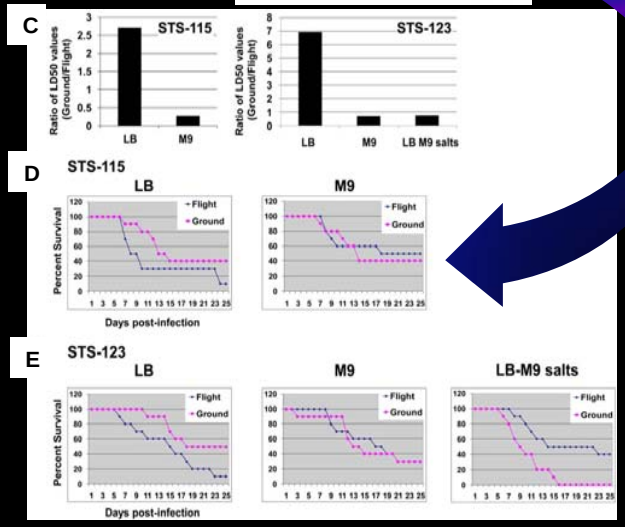
- Next Generation Water System
  - Use of beneficial microbes for utilization of waste water
  - Synthetic Biology
    - An engineered bacteriophage system
  - Electrochemical Disinfection
    - contaminated water is part of the biocide-generation process, resulting in biocides being produced in situ
      - Hydrogen Peroxide (H_2O_2 or HP)
      - Sodium Hypochlorite (NaOCl or Hypcholorite)
      - Peracetic Acid (PAA)
      - Ozone



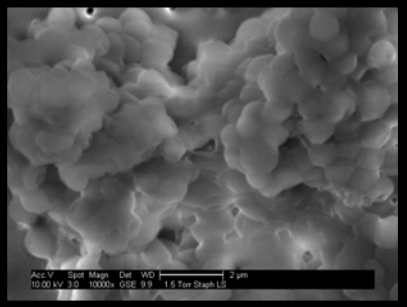
Antimicrobial Technology for Long Duration Spaceflight

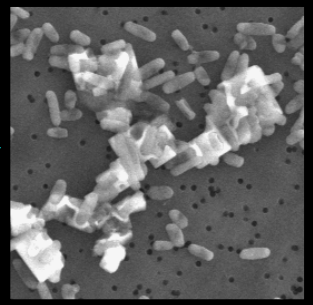
- Ongoing Investigations
  - Antimicrobial compound incorporation into nonporous materials during vehicle design
    - self-disinfecting materials
    - Super hydrophobic materials
  - Assessments of alternate disinfectants
    - chlorhexidine gluconate
    - quaternary ammonium compounds
  - Human factors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

Does the spaceflight environment alter microbial responses?



(Wilson et al. 2007, Wilson et al. 2008)





(Wilson et al. 2007, Crabbe et al. 2008, Castro et al. 2011)

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

Acknowledgements

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