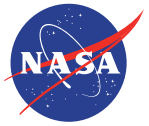

Jet Propulsion Laboratory
California Institute of Technology

The Development of a Practical Approach to Assess Cleanrooms for Biological Contamination to Minimize Hardware Recontamination Risk

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Biotechnology and Planetary Protection Group

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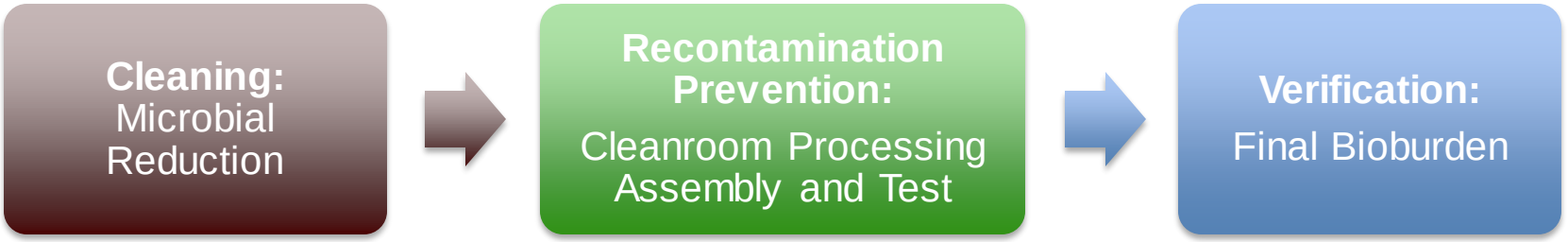
Outline

- Objective
- Mission Impacts on Biological Requirements
- Cleanroom Utilization for Biological Recontamination
- Driving Mission Requirements
- NASA Handbook Procedures
- Emerging Monitoring Techniques
  - DNA Based
  - Air Sampling
- Biological Assessment Processing Flow
- Integration of Biological Assessments in I&T
- Advantages of Biological Assessments
- Summary

Objective

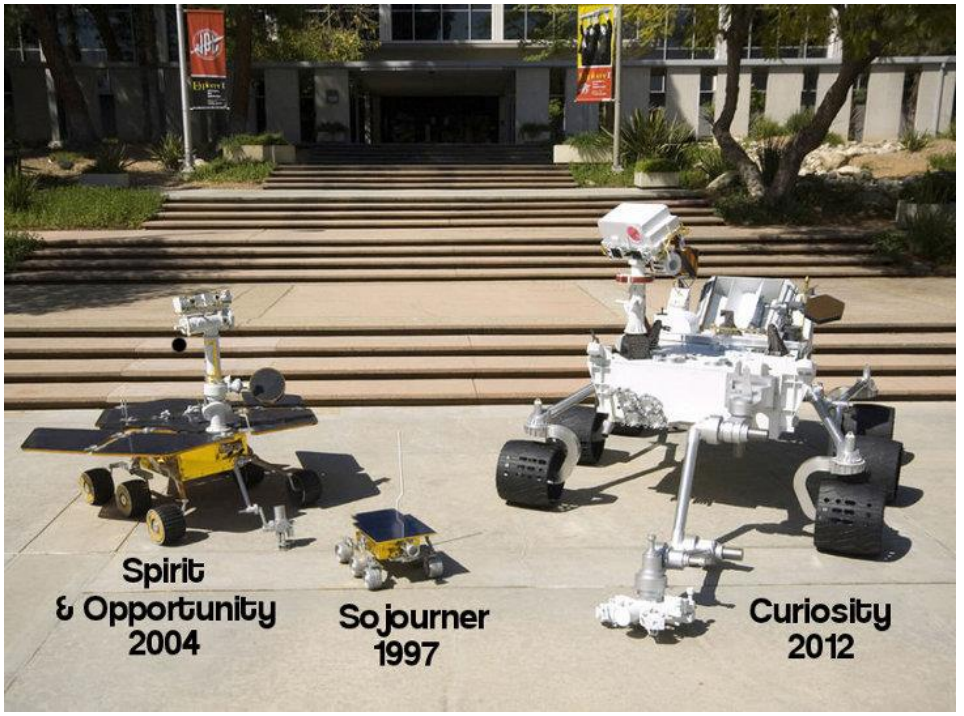
To ensure that hardware post microbial reduction is not re-contaminated by the downstream integration and testing within cleanroom environments.

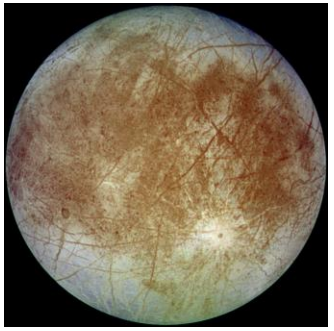


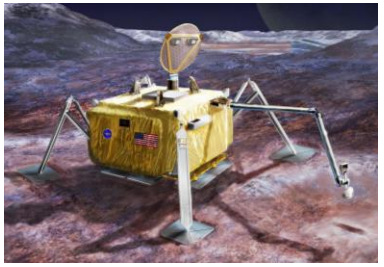
Oftentimes, microbial reduction / cleaning of hardware is easy but maintaining cleanliness throughout the hardware life cycle is the challenging part.

Mission Complexity Impacts Biological Requirements

- Bioburden requirements being applied to large (e.g. car-sized) surface area rovers to Mars.
- Return Sample Science integrity - Potential Mars Sample Return
- In-Situ life detection instruments
- Probability of Impact where low bioburden is required - Icy Moon missions (e.g. Europa)

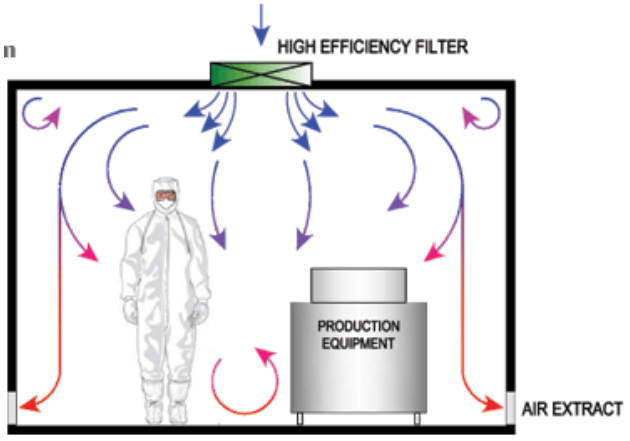






Cleanroom Utilization

- Cleanrooms are critical for maintaining and preventing recontamination of hardware that has undergone microbial reduction.
- No two cleanrooms perform the same and biological cleanliness should be evaluated on a case-by-case basis.
- Transport mechanisms – air and direct
- Many factors influence biological performance in a cleanroom:
  - Air flow
  - Protocols and gowning
  - Cleaning procedures
  - Amount of hardware
  - Seasonal variations
  - Etc.



Not all cleanrooms are treated equal –
Particulate Cleanliness DOES NOT EQUAL Biological cleanliness

Driving Mission Biological Requirements and Guidance

- NPR 8020.12
  - Hardware surface cleanliness levels
    - Defined spore numbers for Mars (e.g. 300 spores/m2, 500,000 spores at launch)
    - Project derived for Europa based on contamination modeling
  - Clean Room Requirement
    - All PP Category II, III and IV missions shall assemble and maintain spacecraft and payloads in Class 100,000 or ISO class 8 cleanrooms in the operational mode.
  - Icy Satellites 5.4.1.1 "…requiring the use of cleanroom technology…and the monitoring of spacecraft assembly facilities to understand the bioload and its microbial diversity, including specific problematic species.
  - 3.4.2. "*Following the successful application of a microbial reduction process, appropriate measures shall be taken to prevent recontamination*."
- Mission Recommendation
  - Although specific numerical limits are not imposed by planetary protection requirements, a bioburden of <2000 per m2 , on surfaces to which the spacecraft is exposed during launch.

ISO 8 or better environment.
No defined facility spore or microbial bioburden requirement.
Up to mission to define.

NASA HBK 6022 – Biological Assays

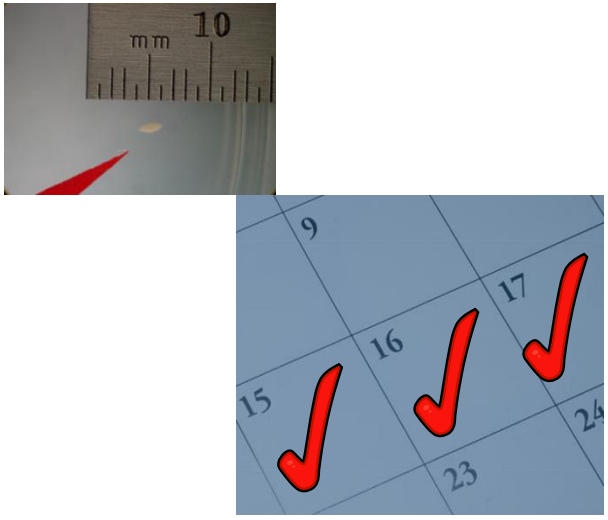
Method	Time to Results	Surface Type
Standard Assay – wipe and swab	72 hours	Hardware and facility
Teflon Fallout Ribbons	72 hours	Hardware and facility
Slit Sampler Agar Impaction Devices	72 hours	Facility air sampling
Membrane Filter Field Monitors	72 hours	Facility air sampling
Adenosine-5'-triphosphate (T-ATP)	< 2 hours	Hardware and facility
Limulus amebocyte lysate (LAL)	< 2 hours	Hardware and facility

NASA Standard Spore Assay

Clean hardware (and table or bag) with IPA wipe prior to sample or install

Assay Swab or Wipe (water is used as solvent)

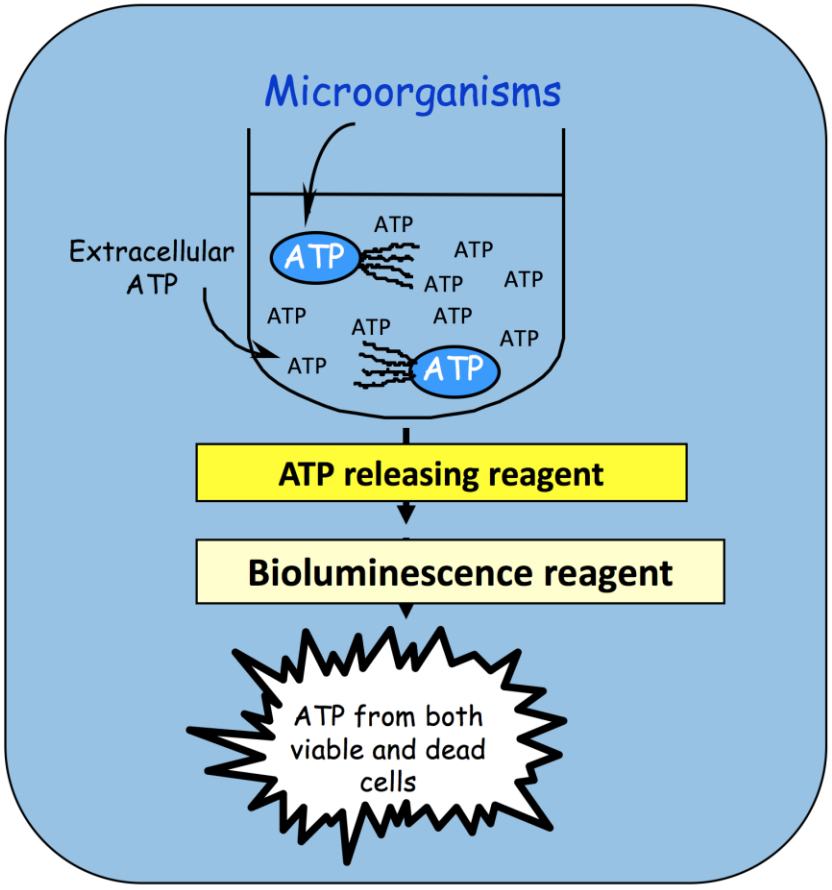
Process swabs and wipes, ~3 hours required post-assay

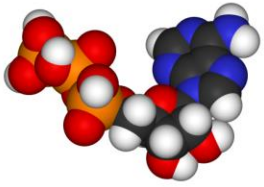


Count Plates – 24h, 48h, and 72h

Adenosine-5'-triphosphate (ATP) Assay

- Rapid turn around = <2hours
- ATP Background
  - "Molecular unit of currency" intracellular energy transfer molecule.
  - Used by all domains of life
  - ATP transports chemical energy within cells for metabolism.
  - Used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division.
- NASA HDBK 6022 "*This assay is used to pre-screen hardware for the presence of microbial contamination prior to conducting final assays….This method is useful…and can be used to assess quickly if cleaning or other processing is required prior to carrying out and reporting final assays of spacecraft surfaces.*"





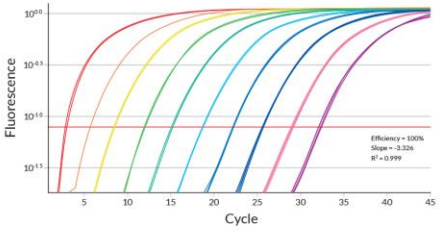
Example: ATP Same Day Vendor Evaluation Feedback

Sample Number	Sample Description	ATP (mmoles/sample)*
1	table in ISO 7	2.78536E-12
2	table in ISO 7	1.08203E-11
3	table in ISO 7	7.8977E-12
4	chair in iso 7	6.34882E-12
5	chair wheel in ISO 7	1.50421E-11
6	cable ledge on bottom of room in ISO 7	4.55322E-12
7	table in back by oven in ISO 7	5.21107E-10
8	wall in back by oven in ISO 7	9.3916E-13
9	plastic curtain in ISO 7 side of	3.77902E-12
10	control	2.66712E-12
11	marble in ISO 5	1.59268E-12
12	marble in ISO 5	3.08317E-12
13	screw table in ISO 5	7.12983E-12
14	screw table in ISO 5	4.28023E-12
15	oxygen monitor in ISO 5	2.43792E-12
16	cart in ISO 5	4.16637E-12
17	socket set in ISO 5	3.51431E-12
18	camera button in ISO 5	6.37656E-12
19	cabinet shelf in ISO 5	1.59755E-11
20	control	1.32845E-12
21	flex harness connector	9.39258E-12
22	flex harness	3.24229E-12
23	primary structure aluminum	1.0086E-11
24	primary structure aluminum	4.68802E-12
25	conformal coat on electronics	7.15757E-12
26	primary structure aluminum	1.39122E-12
27	primary structure aluminum	6.3824E-12
28	control	1.53867E-12

*KEY - Passed, Almost to suggested cleaning threshold (2.57e-11), Critical (>> required cleaning 3.57e-11)

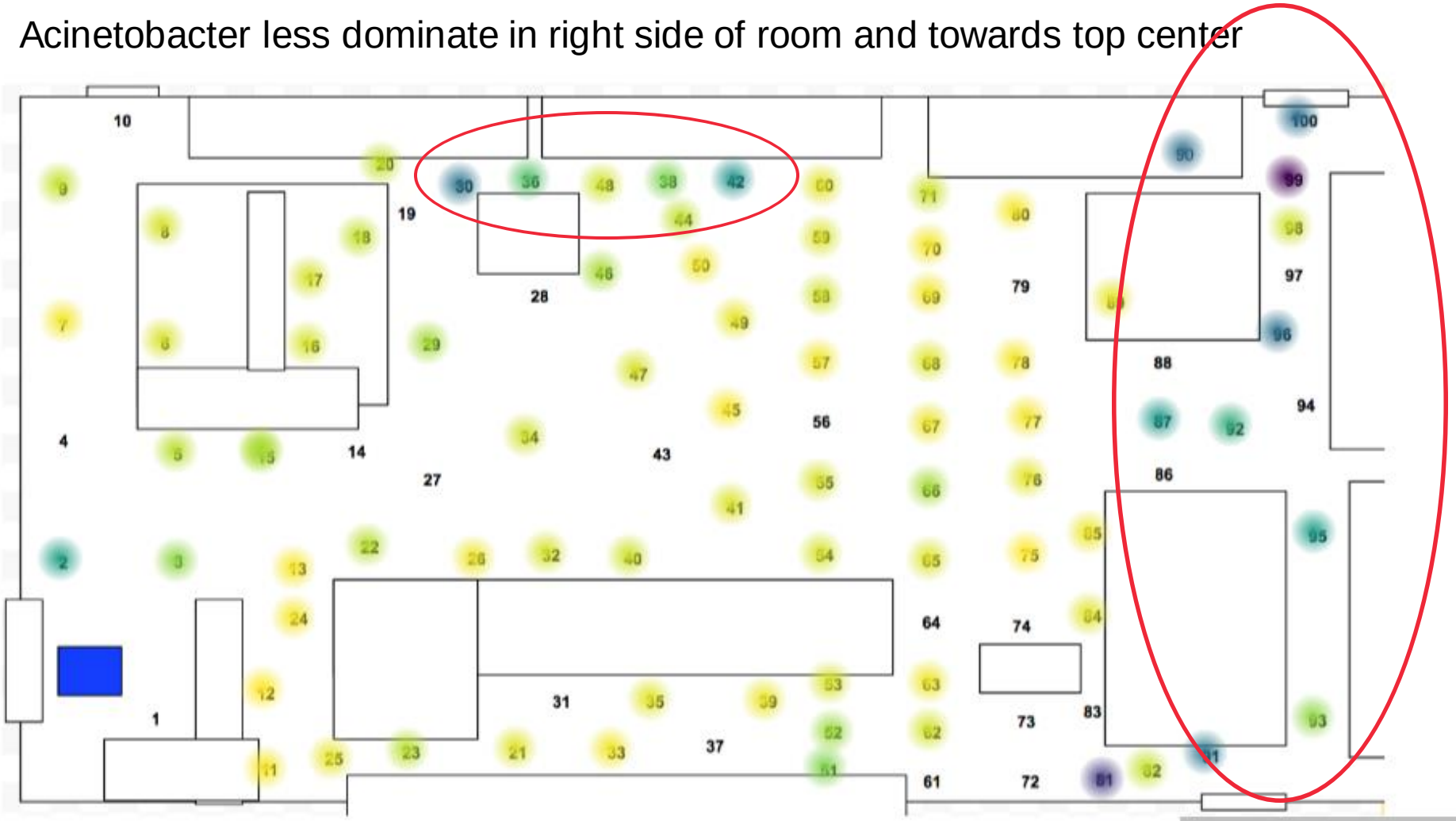
DNA Based Approaches

- DNA based surveys
  - Quantitative polymerase chain reaction approach
  - Mircobiome – what types of organisms are present (e.g. “who”)
  - Metagenomic – functionality, predictions as to what the organisms might be capable of
- Samples can come from air or surfaces.
  - Surface samples can focus on large surface area (e.g. > 1m^2) or localized (e.g. 25 cm^2) surface areas

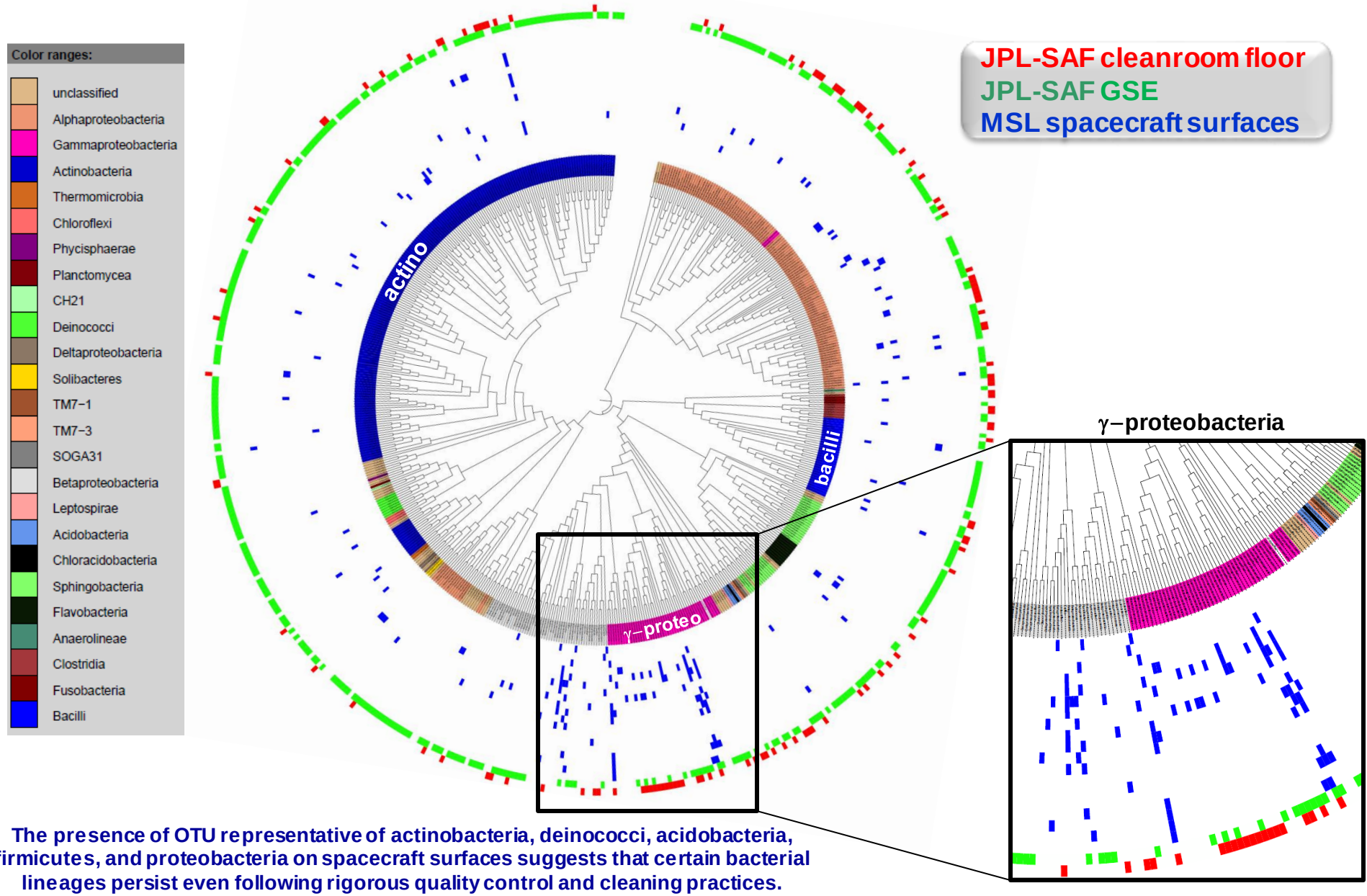


Mircobiome 2D Mapping Example

Acinetobacter less dominate in right side of room and towards top center



DNA Contamination Analysis Mapping Example



The presence of OTU representative of actinobacteria, deinococci, acidobacteria, firmicutes, and proteobacteria on spacecraft surfaces suggests that certain bacterial lineages persist even following rigorous quality control and cleaning practices.

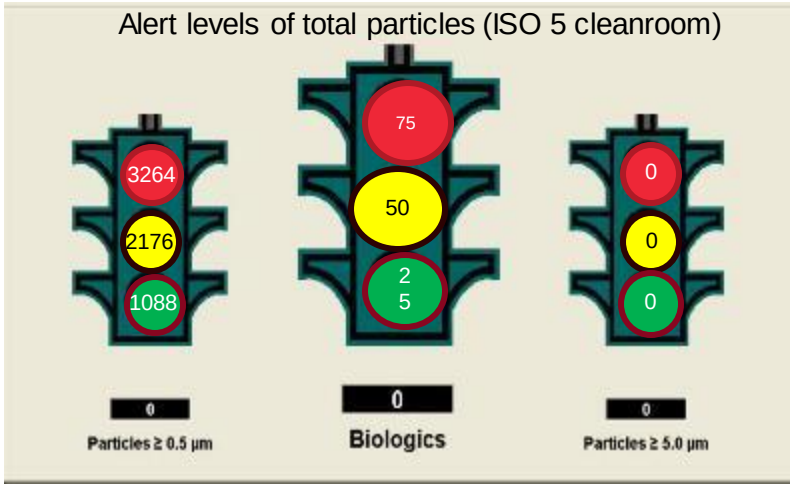
Air Sampling Overview

- Direct agar impingement – MAS-100, total colony forming units.
- Direct buffer impingement – Bertin Coriolis, total counts, spore counts, DNA extraction, etc.
- Application of USDA Guidelines on Sterile Drug Products for mission air requirements.

Clean Area Classification	ISO Class	USFDA Guidelines on Sterile Drug Products (cfu/m^3)	WHO 2002 (cfu/m^3)	NFS90-351 (French Standard, cfu/m^3)
100	5	<3	<1	<10
1000	6	<7	5	-
10000	7	<18	100	<10
100000	8	<88	500	<100

BioVigilant Real-Time Air Monitoring

- BioVigilant IMD-A air monitoring system is ready for deployment for continuous air monitoring during critical hardware assembly process.
- Based on the ISO air particle requirements of the cleanroom, Biovigilant based Action and Alert levels will inform the engineers about the real-time cleanroom air bioburden status.
- Biovigilant will capture air particle data every 10 sec. and any potential 'point source contamination' could be detection real-time.
- Cruz et al. (2017) "Simultaneous Quantification of Bioaerosols and Inert Particles along with their Size Distribution In Spacecraft Assembly Cleanrooms", In preparation.



Biological Assessment Process Flow (1 of 2)

KEY
Standard
Full Characterization

Sample collection

Wipe Sampling +
Air Sampling
Bertin Coriolis

Suspension in buffer

InnovaPrep
concentrating pipette

Concentration of samples

Molecular based microbial burden

Maxwell automated
DNA extraction
system

Microbiome and Metagenome

Cultivable based microbial burden

Sample collection

Direct Agar Impingement

Biological Assessment Process Flow (2 of 2)

KEY
Standard
Full Characterization

Sample collection

↓

Physical Characterization

Particulate Sampling
BioVigalent Sampling

Biological Sampling

Integration of Biological Assessments

- Mars IVa implementation (e.g. MSL, InSight)
  - Project requirements
    - 1,000 spores/m^2 on cleanroom and ground support equipment surfaces **not** within direct contact with flight hardware
    - 300 spores/m^2 on cleanroom and ground support equipment surfaces within direct contact with flight hardware

Event	Surface Sampling NASA Standard Assay	Surface Sampling ATP	Air Sampling Bertin Coriolis or Direct Agar
Vendor Evaluation		✓	
> 1 month prior to cleanroom occupancy	✓		✓
<1 week prior to cleanroom occupancy	✓		✓
GSE Move In	✓	✓	✓
Hardware Move In	✓	✓	✓
Hardware Processing	✓	✓	✓

Integration of Biological Assessments for Critical Hardware Environments

- Mars IVb or Icy Moon implementation
  - Facility requirements; tailored but at least that of Mars IVa project reqs

Event	Surface Sampling NASA Standard Assay	Surface Sampling ATP	Surface Sampling DNA Based	Air Sampling Bertin Coriolis or Direct Agar	Air Sampling BioVigalent
Vendor Evaluation		✓	✓		Possibly
> 1 month prior to cleanroom occupancy	✓		✓	✓	✓
<1 week prior to cleanroom occupancy	✓		✓	✓	✓
GSE Move In	✓	✓	✓	✓	✓
Hardware Move In	✓	✓	✓	✓	✓
Hardware Processing	✓	✓	✓	✓	✓

Advantages of Biological Assessments

- Direct feedback into recontamination risk – risk mitigation.
- Establishes facility cleanliness prior to flight hardware and ground support equipment.
- Regularly scheduled / real-time assessments lead to hardware bio-loading predictions, process changes and increased cleanliness
  - Vendor evaluation and discussion
  - Reduced hardware cleaning time
  - More predictable hardware surface cleanliness

Summary

- Hardware recontamination in a cleanroom can occur from air or direct surface transfer. Thus, multiple modes of biological monitoring have to be employed to include both air and surface bioassays.
- Traditional microbiology and real-time monitoring essential to minimizing biological cleanroom contamination.
- The ATP assay has proven to be useful in the management of hardware and facility bioburden for multiple missions (e.g. MER, MSL, InSight, M2020). Sampling and processing easily conducted at vendor location.
- When devising a implementation strategy understanding the hardware I&T flow is critical in assessing cleanrooms where monitoring needs to take place → feeds into project PP risk mitigation strategy.
- Critical cleanrooms should be evaluated prior to hardware coming into cleanroom and throughout the I&T process.

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Backup

Abstract

Planetary Protection sensitive missions with bioburden requirements must undergo extensive bioburden reduction and verification steps. While hardware microbial reduction modalities (e.g. solvent cleaning, heat microbial reduction, etc) are important in achieving hardware allocation values, hardware recontamination is a key implementation consideration in order to maintain the hardware in a biologically clean state. One of the most essential areas for recontamination is the environment in which the hardware is assembled, integrated and tested. While NASA provides PP spore/m^2 specifications for hardware based on the cleanroom environment, there is a wide interpretation of cleanroom implementation and practices across the flight hardware industry. As such, the bioburden of the same cleanroom ISO class may vary and PP engineering guidance should be considered as a standard project practice where sensitive hardware is being processed. At JPL from 2001 to present day, PP engineering has increased the overall stringency of the environmental bioburden guidance from 2,000 to 1,000 spore/m^2 to account for more complex and larger represented spacecraft surfaces and have adopted Food and Drug Administration Guidelines on Sterile Drug Products for air sampling (e.g. ISO 8; <88 cfu/m^3).

The portable, rapid adenosine triphosphate assay has been implemented post 2005 to make assessments where processing in a PP lab is not possible. PP research has also infused technologies into the evaluation of cleanrooms by introducing state-of-the art sampling devices (e.g. Bertin Coriolis and BioVigilant) and metagenomics. Presented herein, will be an account of the changes in technologies and philosophies of recontamination prevention from the cleanroom environment.