Johns Hopkins
Applied Physics Laboratory

Sample Collection and Processing Protocol to Prepare for Genomic Analysis

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Technical Shifts in Planetary Protection Implementation

Current Standard Culture-Based Method

Benefits:
- Heritage from previous missions
- Semi-quantitative bioburden assessment

Limitations:
- Counting-based; no identification of microbes
- Focuses on heat-resistant microbes
  - May exclude some microbes that could survive in space

VS

Genomic Inventory Methods

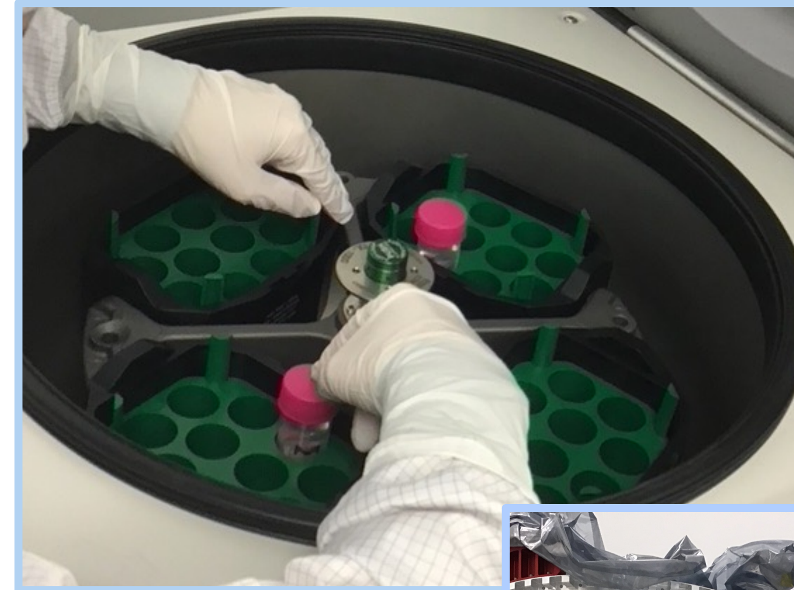
Benefits:
- Risk assessments possible
- Far more informative

Limitations:
- Low biomass in cleanrooms and on flight hardware
  - Difficulty obtaining samples with sufficient bioburden
  - Contamination at any step in the process can severely affect results

Objectives

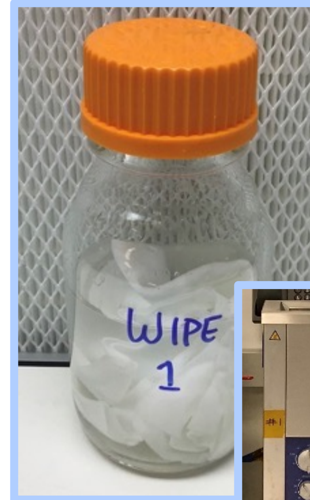
- Develop a method to collect *sufficient bioburden* from facilities and flight hardware to allow for *environmental exposures* and downstream genomic processing

- Identify microorganisms present in facilities and on flight hardware at APL

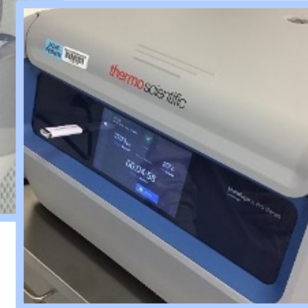


Sample Collection and Processing Flow

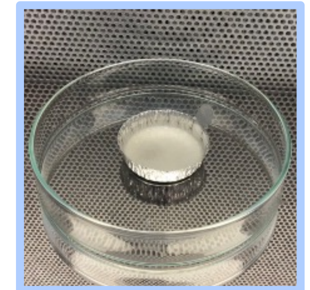
Sample Collection

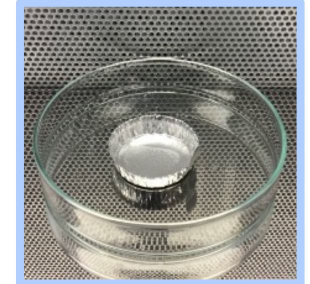


Sample Released in Buffer Solution



Concentrated Sample

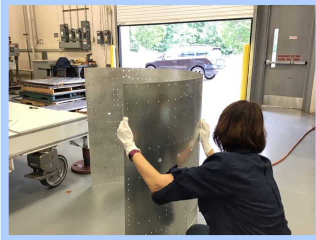




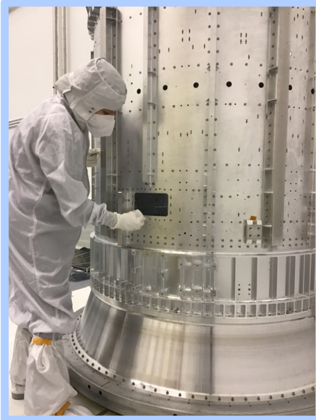
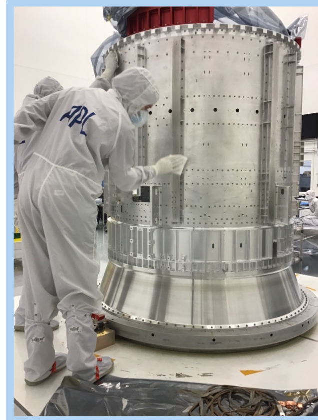
Dried Concentrated Samples in Al Boats

Transfer of microorganisms from facilities onto aluminum boats allows for environmental exposure (vacuum exposure, radiation exposure)

Sample Collection



Area: 2.361 m^2 Area: 2.358 m^2 Area: 3.186 m^2

Is sampling the flight hardware, before precision cleaning, a viable alternative for extracting qualitative information about the microbial community?

Sampling "biologically clean" flight hardware requires extensive effort due to very low biomass

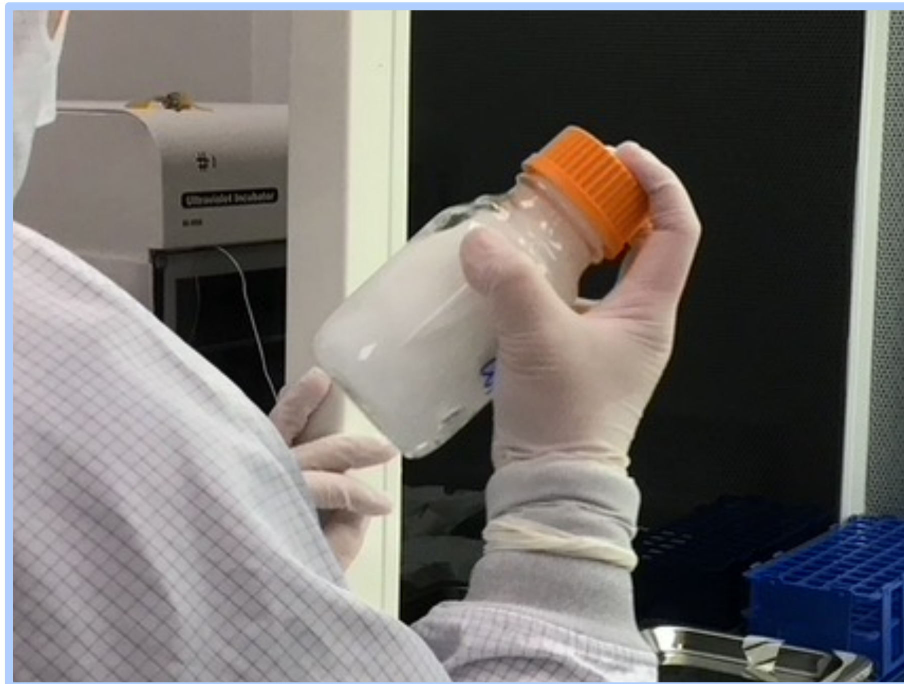
- Large number of swab/wipe samples required
- Labor-intensive sample processing

Sample Collection

- All samples collected using sterile gloves in accordance with NSA standard procedures
- Control taken at end of sampling event
- TX3211 wipes with Falcon sterile 50 mL conicals and sterile, molecular-biology grade deionized water
- Samples stored at -80°C

Sample Processing

- Molecular biology grade Phosphate buffered saline, pH 7.4, was used as a buffer to release microbes from the sample wipes
- Samples were shaken with PBS for 15 seconds in 150-165 mL of PBS and then vortexed for 15 seconds, followed by sonication at 25 kHz for 2 minutes



Sample Concentration

- 2 Amicon filters (50 kDa cutoff, 15 mL max volume) used per wipe sample
  - Concentration performed per manufacturer's instructions
  - Multiple spins per wipe sample and per Amicon filter to process total of 150 mL of PBS
- Sample concentrates pooled in single glassware and then distributed among clean sample boats placed in covered petri dishes
- All samples should be moved to -80 C freezer within 1 hour of collection
  - "Gentle freeze"

Downstream Processing

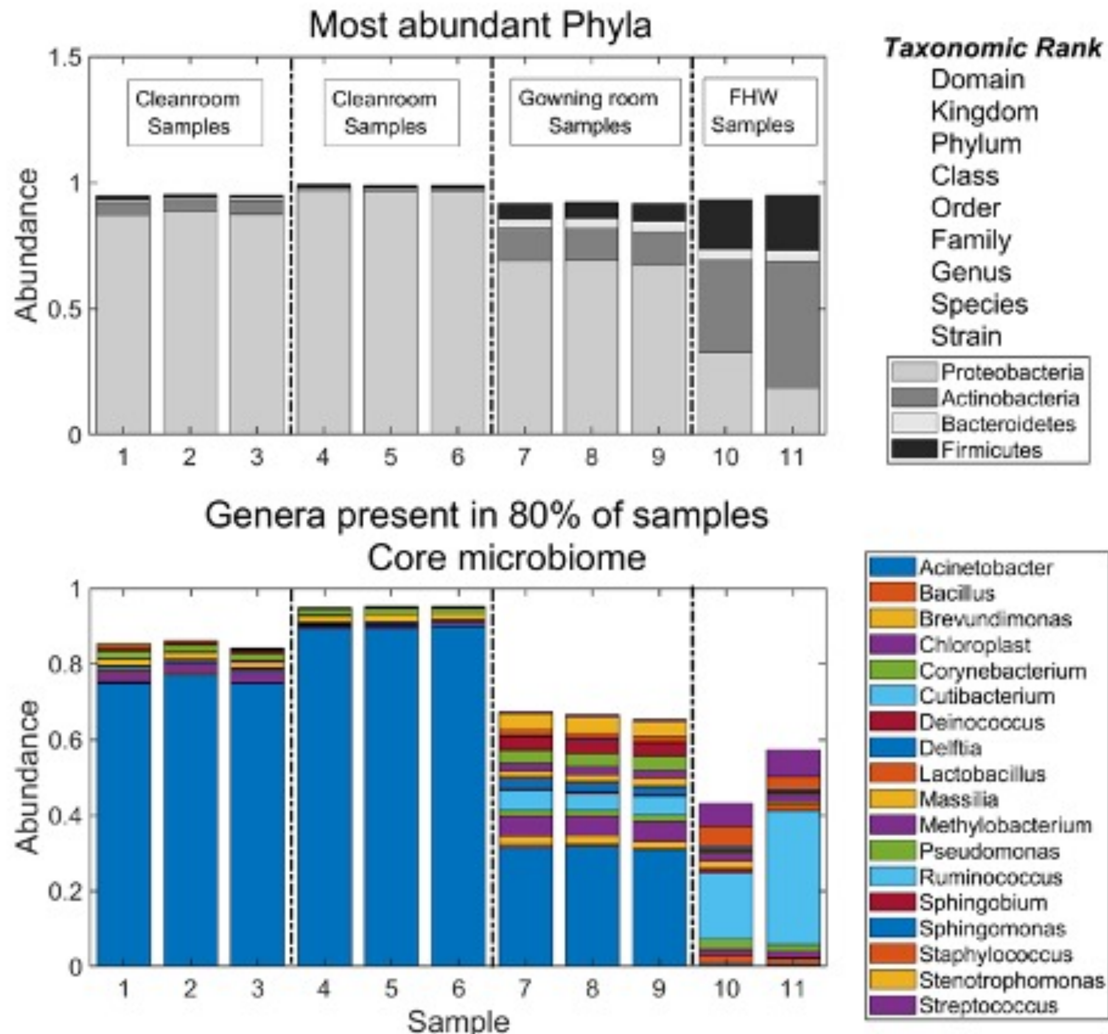
- Partnership with J. Craig Venter Institute (JCVI)
- DNA extraction performed using UCP Mini Pathogen kit (QIAGEN)
- 16S sequencing performed
- Data analyzed to give phyla and genus

DNA Extraction Results for Select Samples

Collection	Sample	Sample Type	Surface Area	DNA concentration per Qubit
1	1	Facility – Cleanroom	4 m^2	0.1850 ng/µL
	2		4 m^2	0.0720 ng/µL
	3		4 m^2	0.0923 ng/µL
3	4	Facility – Cleanroom	4 m^2	0.0249 ng/µL
	5		4 m^2	0.0202 ng/µL
	6		4 m^2	0.0281 ng/µL
2	7	Facility – Gowning Room	4 m^2	0.0762 ng/µL
	8		4 m^2	0.0946 ng/µL
	9		4 m^2	0.0689 ng/µL
FHW1	10	FHW (before precision cleaning)	7.91 m^2	0.0057 ng/µL
	11	FHW (after precision cleaning)	7.91 m^2	low*
all (-)s	multiple	Negative Controls	N/A	too low

* Sample was still processed via 16S sequencing successfully

16S rRNA Analysis for Bacterial Diversity in APL Cleanroom



- Presence of DNA confirmed using spectrophotometric techniques (Qubit / Nanodrop)
- Taxonomic profiles from flight hardware (FHW) are significantly different from cleanroom and gowning room samples
  - FHW samples are richer in Firmicutes and Actinobacteria
  - Acinetobacteria is almost absent in FHW sample, most abundant in facility samples
- Survival in space is associated with resistance to cold, radiation, and desiccation - properties found in microbes forming spores, biofilms, or found in extreme locations on Earth
  - High risk passengers for Planetary protection: *Bacillus, Deinococcus*
- *Deinococcus radiodurans* at APL? - most radiation-resistant organisms; It can survive cold, dehydration, vacuum, acid, the world's toughest known bacterium.

Conclusions

- Sampling the flight hardware before precision cleaning might be a viable alternative for extracting qualitative information about the microbial community, without such an extensive labor effort
- Genomic sequencing is necessary for effective risk assessment
  - 16S does not provide sufficient detail; no species or targeted gene information
  - Impossible to determine presence or absence of environmental resistance genes
- Essential to collect samples from both facility and flight hardware
  - The distribution of phyla and genus differs substantially between the two; both must be analyzed
- Sampling FHW before and after is important for qualitative information – distribution of phyla and genus are similar before and after cleaning
- **The method outlined here is an effective way of collecting and processing samples from cleanroom facilities and FHW to prepare for downstream genomic assessment**
- Work to go: evaluate environmental exposure effect on microbial viability
  - PMA treatment before sequencing

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