Jet Propulsion Laboratory
California Institute of Technology

Real-time Quantification of Size resolved Bioaerosols and Inert Particles In Spacecraft Assembly Cleanrooms

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Planetary Protection

International treaties signed by the United Nations and The Committee on Space Research dictate NASA Planetary Protection (PP) requirements

PP Objectives

- to protect Earth, and solar system bodies from *forward contamination* and *back contamination*
- to maintain scientific integrity of a mission

Implementation

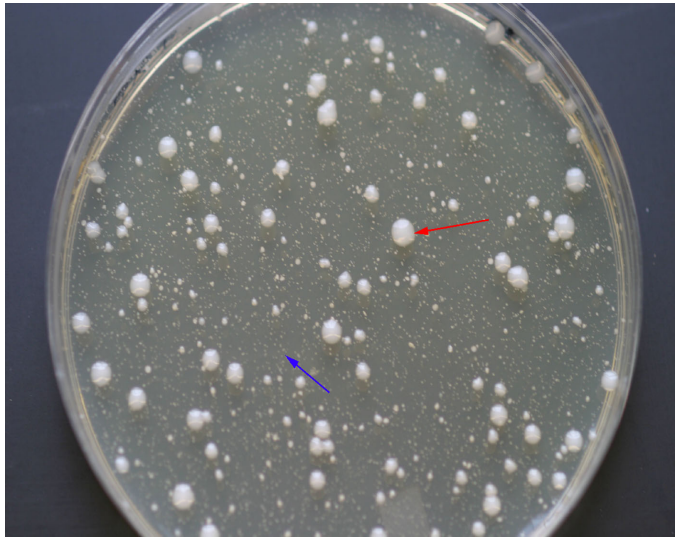
- Cleanroom Assembly
- Personal Protective Equipment
- Decontamination of flight hardware

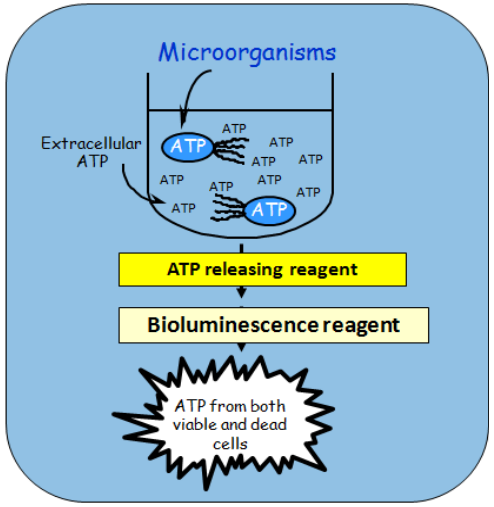
  Surfaces

  Intramural air sampling
- Microbiological Contaminant Assessment
  - Culture Based Methods: heat-shock (NSA) and other assays including non heat-shock
  - Non Culture Based methods: T-ATP, LAL

Limitations of Current Methods

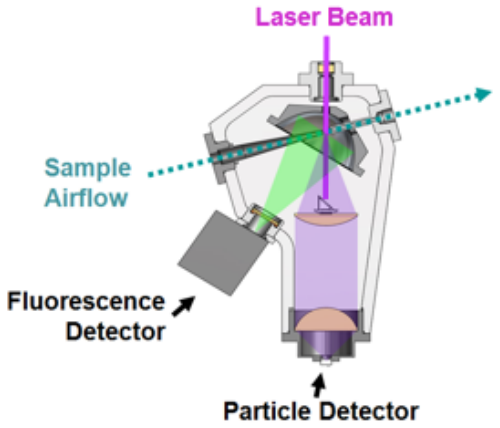
- **Culture based methods:**
  - Can be episodic, time consuming, not comprehensive
    - Samples devoid of cultivatable spores were shown to contain diverse DNA phylotypes (Cooper et al 2011)
- **Non culture based Methods:**
  - More rapid, not comprehensive
    - The total ATP assay measures the total microbial contamination (dead and alive). There is an intracellular assay, but the level of microbial burden that is too low to measure. Spores do not have ATP.(Venkateswaran et al 2003)
- **Conventional cleanroom particle counters assess particle size distribution and their abundance but cannot discern inert particles from bioaerosols**





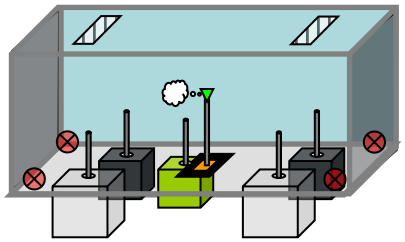
BioVigilant IMD-A® Air Monitoring System

- Laser detection of fluorophores: NADH, riboflavin, and dipicolinic acid (DPA)
- Real-time operation
- Simultaneous measurement of particle size and biologic status.
- Immediate results without a need for an operator for incubation, staining or reagents.
- Continuous monitoring, or by specified sample volume or time
- Synchronized Video and data collection
- IMD- A® 300 Meets ISO 21501-4 and suitable for certification of cleanrooms.
- IMD-A system based particle counts are comparable with existing air monitoring systems used in JPL for cleanroom certification (PMS-Lasair II 310 and the METONE 3415).
- Controlled particle dispersion test by the vendor indicates clear signal separation between each particle size with minimum or no overlap in size reporting.



BioVigilant IMD-A® Validation – Azbil

IMD-A System Validation: USP<1223> and EP 5.1.6



g-Lab Aerosol Chamber Test

(ATCC 9372)

Instruments:

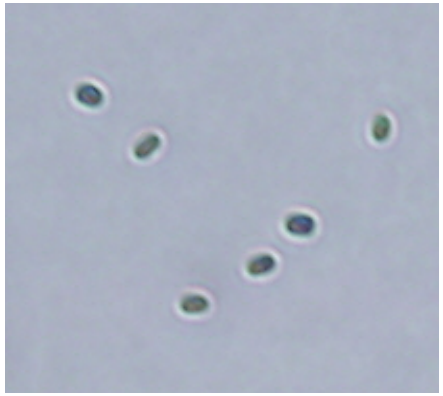
- IMD-A 300 (2)
- IMD-A 350 (2)
- SAS, SMA, MAS
- Kanomax LSAPC

Microbes:

- *B. atrophaeus*
- *C. afermentans*
- *E. coli*
- *S. epidermidis*
- *M. lylae*
- *A. niger*

Tested with additional facilities

Design of Experiment:	
9	USP <1223> Metrics
8	Instruments (4 x IMD-A)
6	Microbes
5	Concentrations
12	Replicates



Result: BioVigilant IMD-A 300, 350 has higher detection than the commercially available air particle counters tested (Azbil).

Chemical Contamination Evaluation

- Prepare Witness Plates:
  - Aluminum witness plates were fired at 500 ^{o}C for 16 hours in glass petri dishes
  - The petri dishes were wrapped in Aluminum foil after removed from the oven, and sealed in amerstat bag
- Instrument: BioVigilant IMD-A 300
- Results:
  - The contamination level on surfaces is ≤ 0.02 μg/cm^2
  - BioVigilant instrument does not add significant amount of chemical contamination to the ISO 5 cleanroom and hardware surfaces.

Monitoring of JPL Cleanrooms

Objectives:

To conduct real-time, simultaneous detection of inert and biological particles in spacecraft assembly cleanrooms

To determine the feasibility of the BioVigilant IMD-A 350 system as a supplemental source for biological contamination and monitoring in future missions

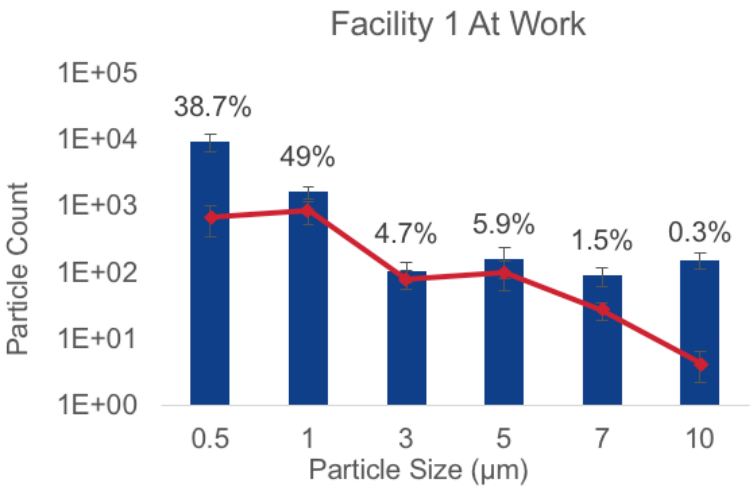
To determine the relationship between human activity and aerosolized particles

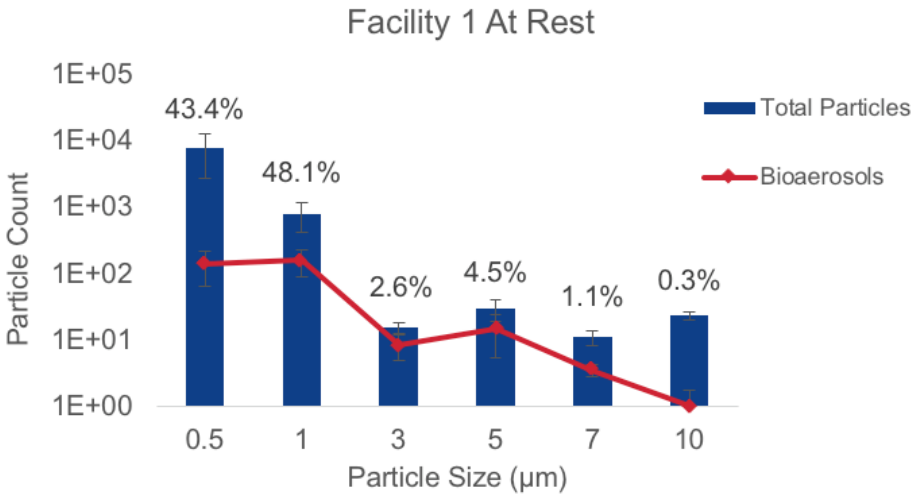
Experimental Design:

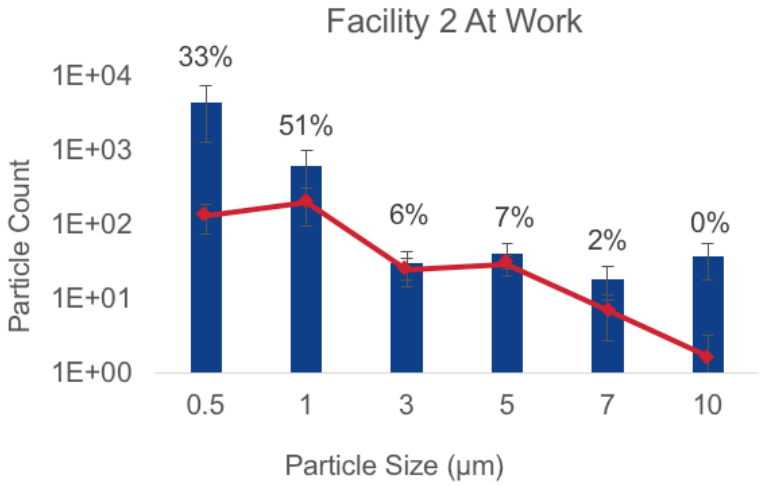
Fluorescent intensity and particle size distribution data was collected continuously for 6 hour periods during “At Work” and “At Rest” periods in ISO-8, ISO-7, and ISO-6 clean rooms in triplicate over a 3 month period

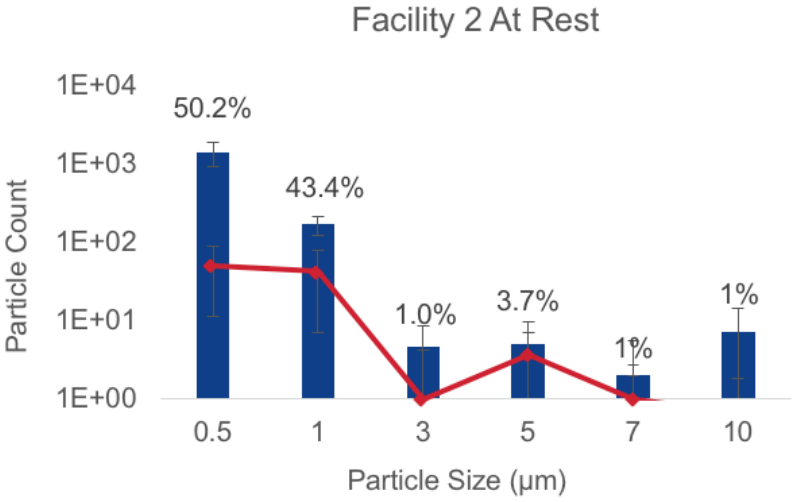
ISO 8 Facility 1 | ISO 8 Facility 2 | ISO 7 Facility 3 | ISO 7 Facility 4 | ISO 6 Facility 5 | ISO 6 Facility 6

Validation Assay Results - ISO 8

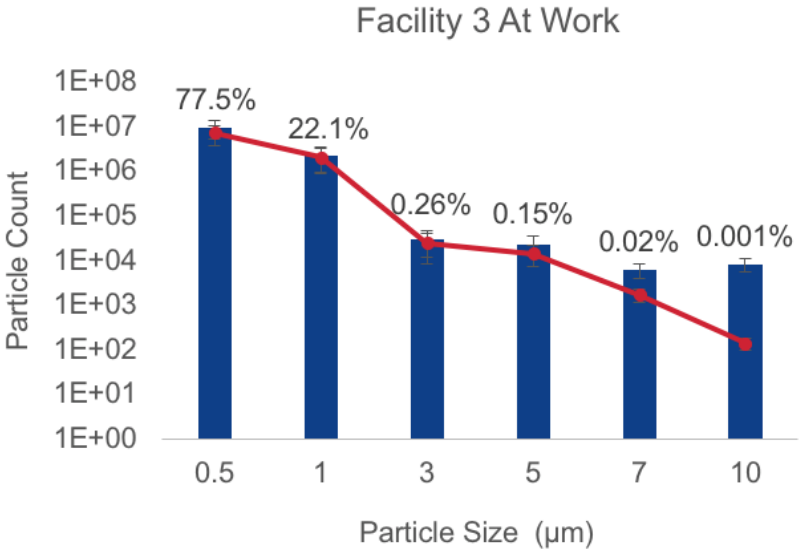


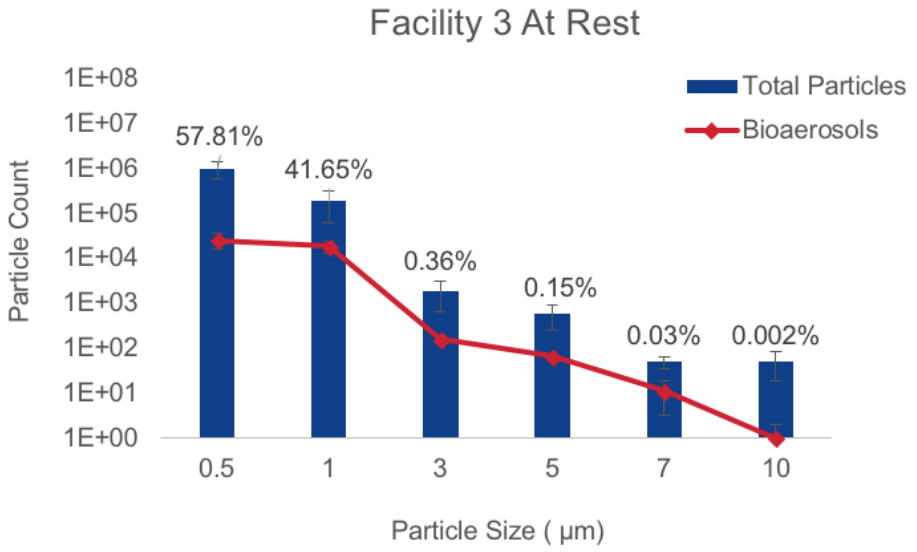


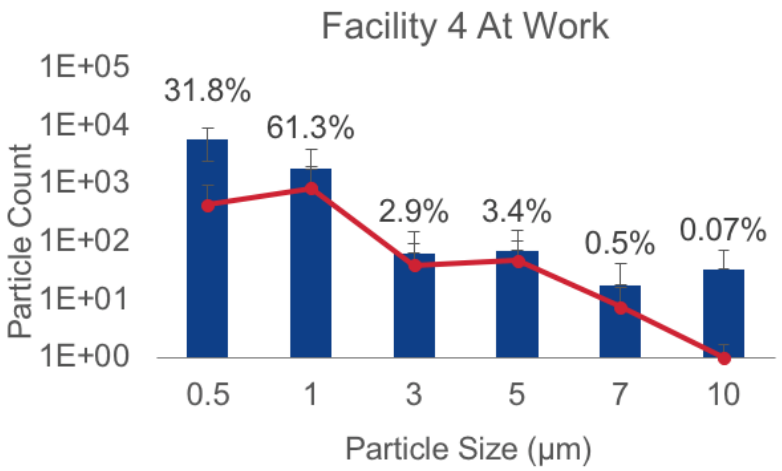


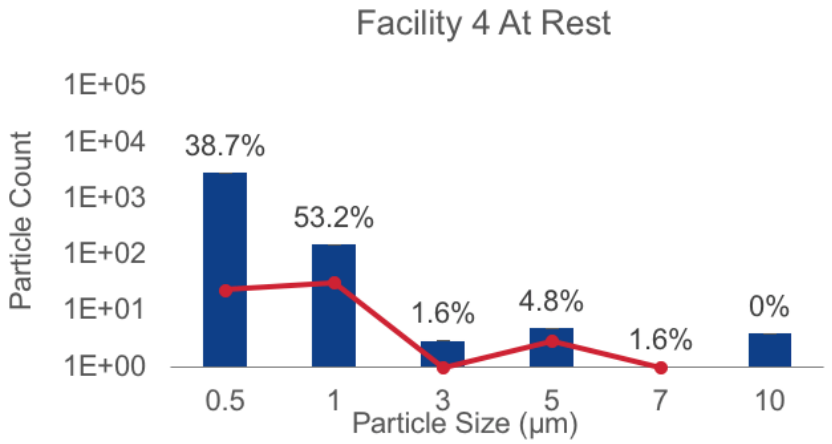


Validation Assay Results - ISO 7

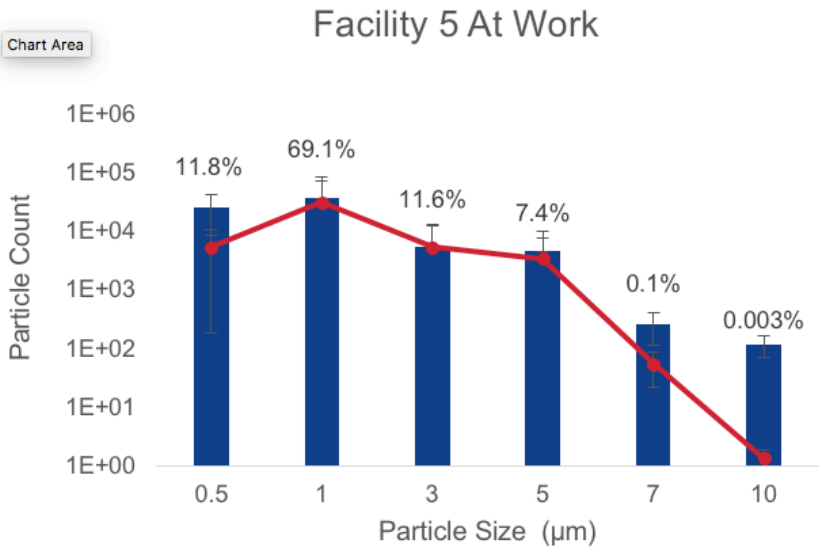


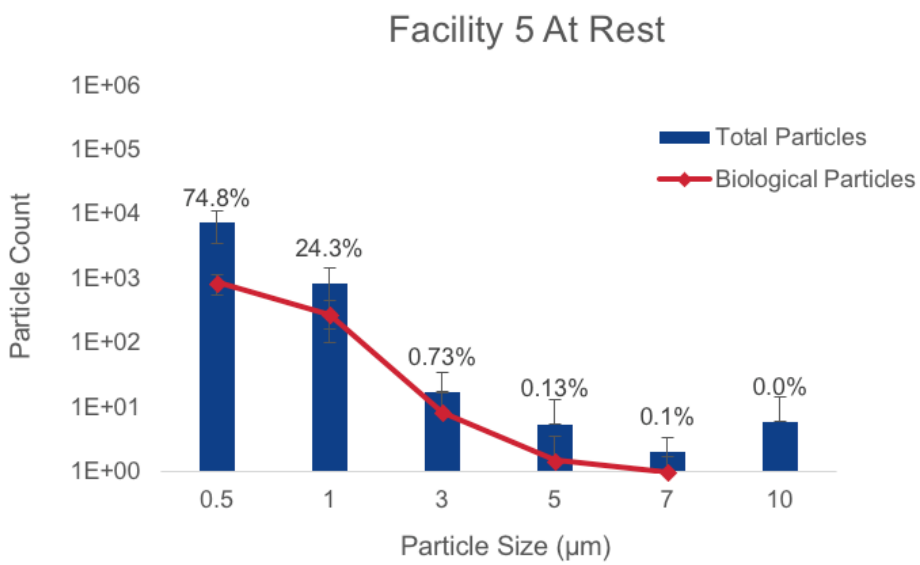


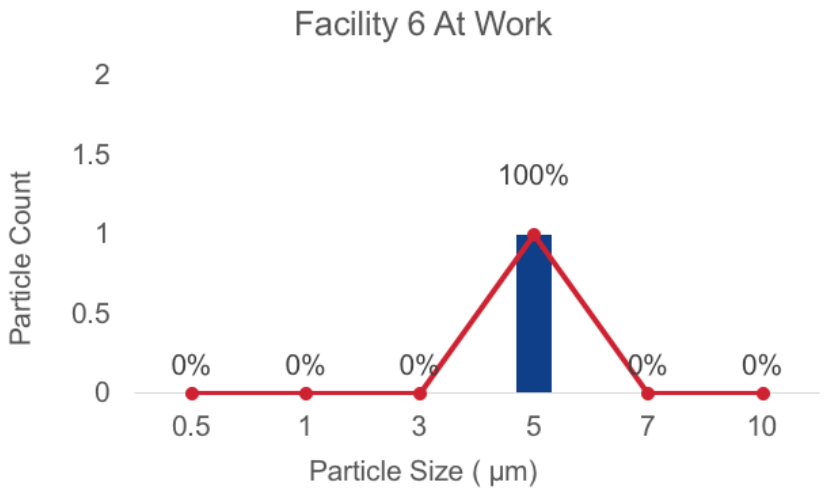


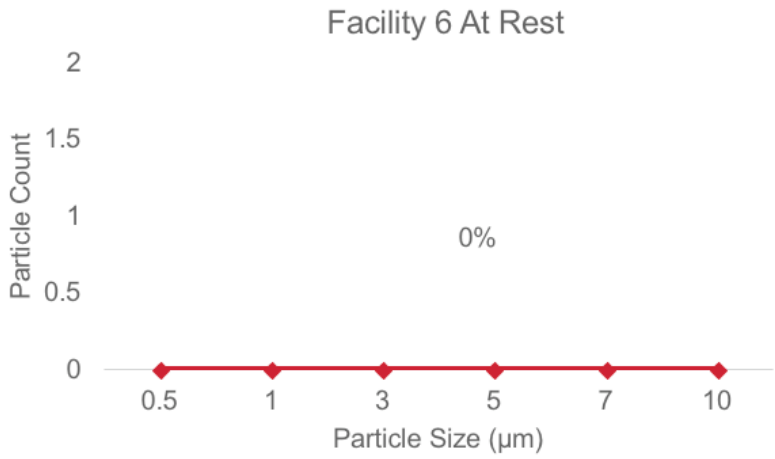


Validation Assay Results - ISO 6

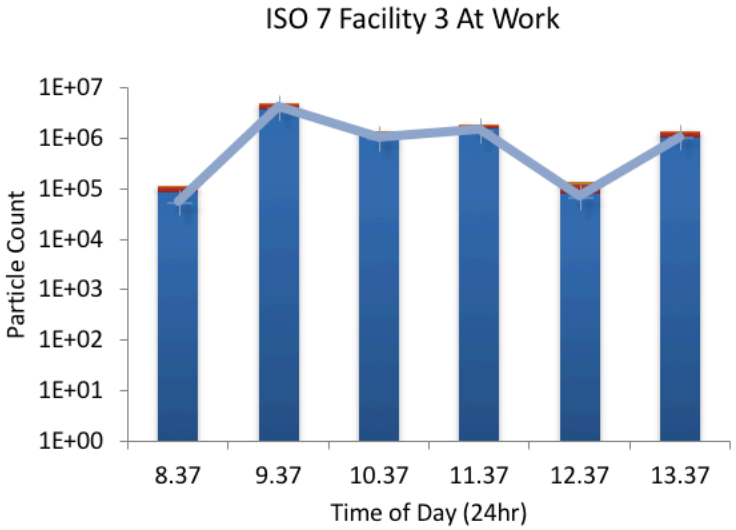


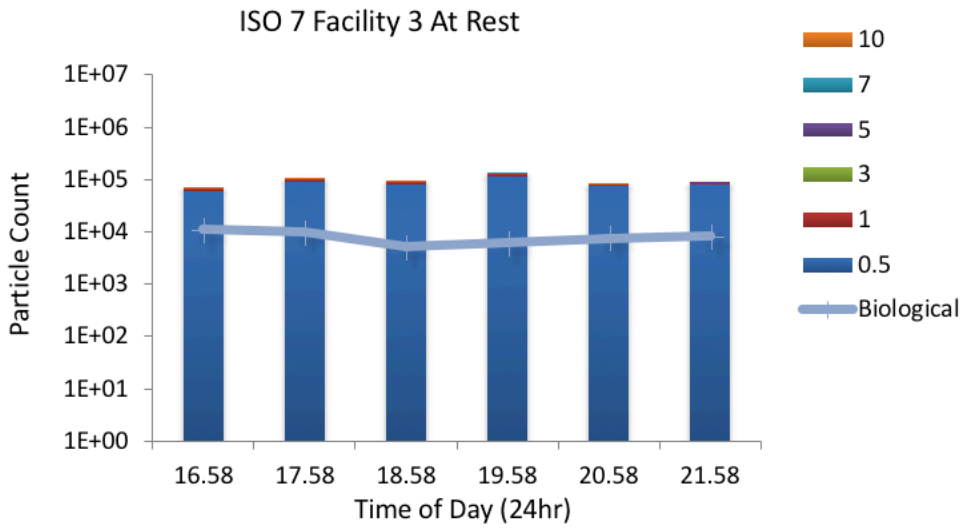


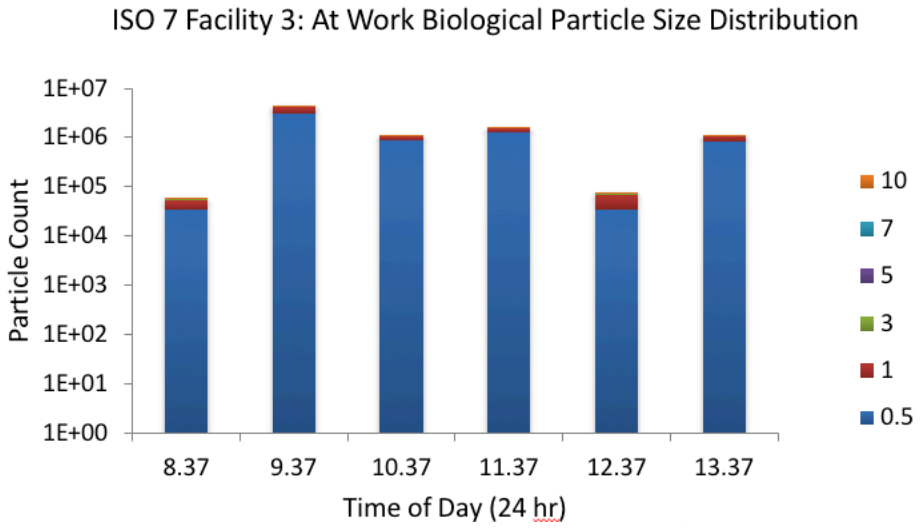




Results: Human Impact on Particle Distribution





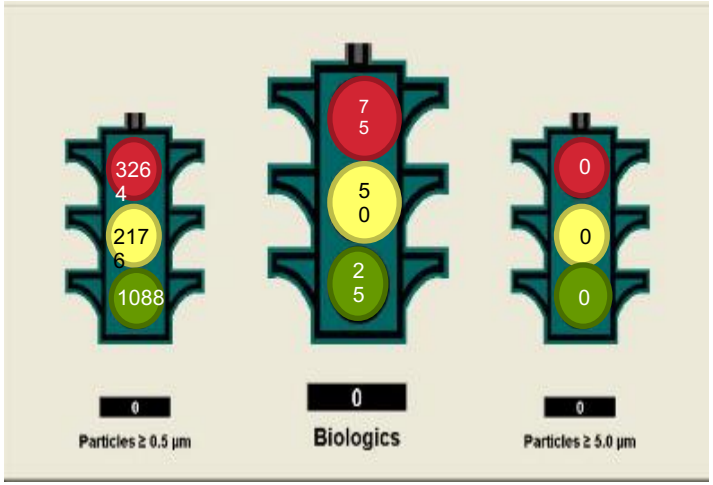


Monitoring of JPL Cleanrooms

- **Preliminary Results:**
  - 0.5 and 1 μm size particles dominate the cleanroom air environments.
  - Relatively high percentage (90.98%) of biological particles are associated with 0.5 and 1 um size particles.
  - We observed a correlation between human activity and total and biological particle counts.
  - JPL cleanrooms tested during this study were well within the limits of ISO standards.

Future Work

- BioVigilant IMD-A air monitoring system is currently deployed for continuous air monitoring during critical hardware assembly process for the Sample Caching System
- More comprehensive bioburden assessment in future missions
  - Europa Lander Concept
- Based on ISO certification levels, BioVigilant Action and Alert levels will inform engineers about cleanroom air bioburden status in real time
  - **Point source contamination** identified quickly, to clean and continue build
- BioVigilant can indirectly supplement the NSA to better understand biological contamination sources and risk



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Abbreviated References

- NASA Handbook 6022
- COSPAR Planetary Protection Policy, 20 October 2002, as amended.
- NASA Policy Directive, NPD 8020.7G “Biological Contamination Control for Outbound and Inbound Planetary Spacecraft (Revalidated 05/17/13 w/change 1) Accessed on 07/20/2017 at https://nodis3.gsfc.nasa.gov/displayDir.cfm?Internal_ID=N_PD_8020_007G_&page_name=main&search_term=8020%2E7G
- NASA Procedural Requirements, NPR 8020.12D “Planetary Protection Provisions for Robotic Extraterrestrial Missions” Accessed on 07/20/2017 at https://nodis3.gsfc.nasa.gov/displayDir.cfm?Internal_ID=N_PR_8020_012D_&page_name=AppendixD
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