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Microbiological Decontamination and Analysis Capabilities

Todd Lane and Pamela Lane

Systems Biology, Sandia National Labs

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Sandia Microbiological Analysis Facilities and Capabilities

- Sandia National Labs in Livermore, CA operates a 12,000 sq. ft. BSL2 general purpose microbiology and molecular biology facility, Applied Biosciences Laboratory (ABL)
- ABL is well equipped for this kind of study
- Equipment includes:
  - Biosafety cabinets
  - 2 autoclaves
  - Equipment for microbiology and molecular biology: Incubators (25C, 30C, 37C), chambers for anaerobic growth, microscopes, PCR and qPCR machines, centrifuges, plate readers, spectrophotometers, Bioanalyzer, Nanodrop

Similar studies reported in literature

- NASA has been monitoring microbial communities on spacecraft hardware for 35 years
- NASA has devised and published standard sampling protocols
- Sampled spacecraft surfaces and clean room floors
- Live (plate) counts and identification by rDNA sequencing
- Identified and validated automated DNA preparation equipment for low biomass samples
- Purification of total DNA from environmental sample and analysis: PCR-RFLP and Sequencing

Microbiologic assay of Space Hardware. Favero, 1971.
NASA standard procedures for the microbial examination of space hardware. Anonymous 1981.
Molecular microbial diversity of a spacecraft assembly facility. Venkateswaran *et al* 2001.
Microbial Characterization of the Mars Odyssey Spacecraft and its Encapsulation Facility. LaDuc *et al* 2003.
Microbial Monitoring of Spacecraft and Associated Environments. LaDuc *et al* 2004.

Sample Processing

Based on NASA Technical Handbook:
Handbook for the Microbial Examination of Space Hardware. 2010

Swab rinse method

- Moisten sterile polyester swab (Texwipe cat # TX761) in 12mL sterile water
- Express excess moisture from swab against interior wall of tube
- Swab area 3X with change of direction, handle makes 30° angle with surface
- Return swab head to tube
- Place on vortex mixer and agitate 5-6s on high
- Suspend tube in ultrasonic bath and sonicate for 2 min ± 5s
- Plate on TSA medium

Piece-part method

- Place part in flask with sterile rinse solution at 25±5° (0.02% Tween 80 in buffered DI water pH 7.2± 0.1)
- Suspend tube in ultrasonic bath and sonicate for 2 min ± 5s
- Plate on TSA medium

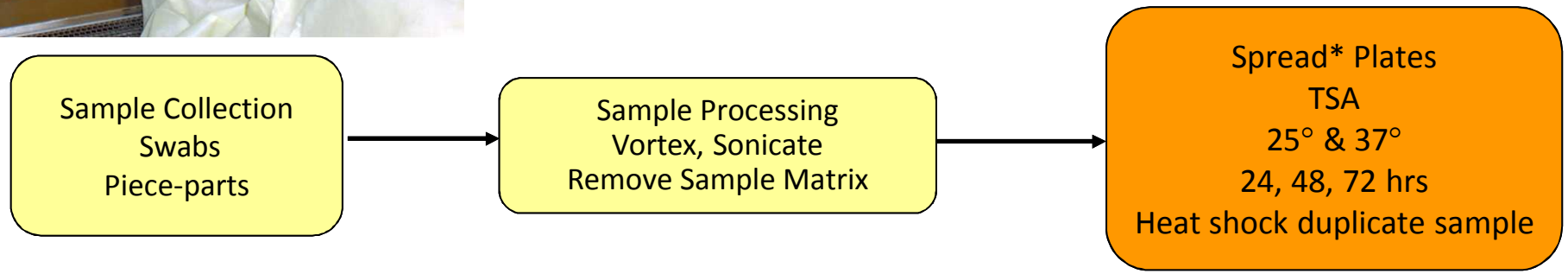
Standard Microbiological Analysis

Spread Plates

1. Plate a 10X dilution series on duplicate TSA plates
2. Incubate aerobically at 25° & 37°
3. Count colonies present at 24, 48, 72 hr
4. Grid colonies for archival purposes
5. Heat shock a duplicate sample (80 ± 2°, 15 min) plate immediately or store on ice for no longer than 45 min.

Precautions taken to avoid contamination

- Samples will be handled in a biosafety cabinet (BSC).
- BSC can be located in a limited access room. There is a room available that has no history of use with live agents other than mammalian tissue culture.
- Sterile disposable gloves, gowns, forceps, scalpel blades, and petri dishes will be used to manipulate samples.



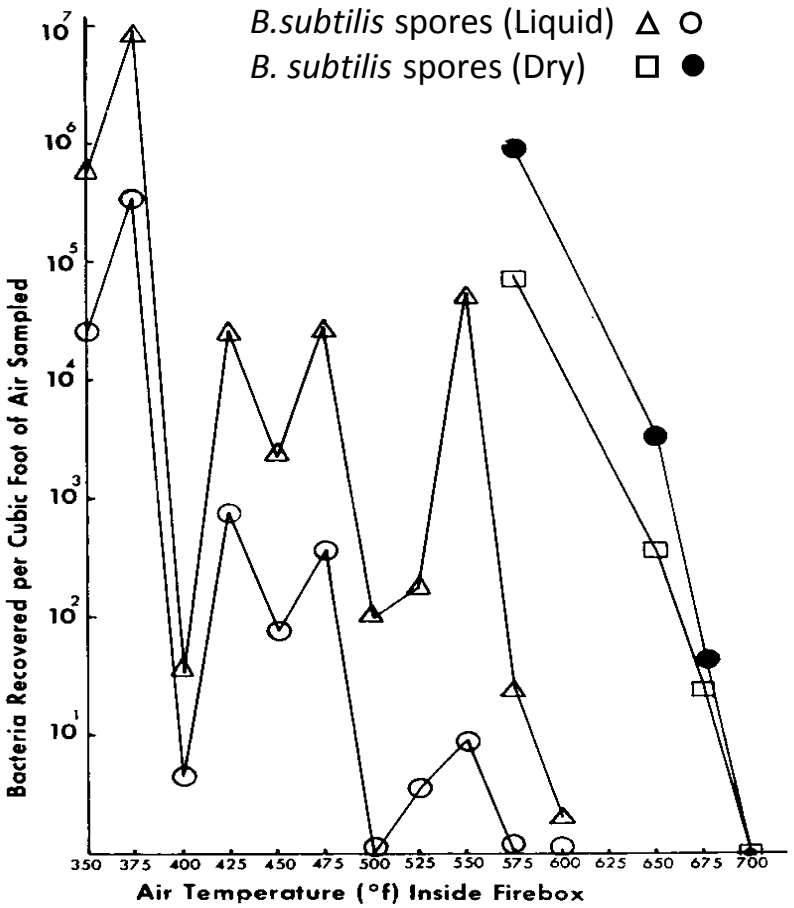
Sandia is Familiar with NASA Protocols for Microbiological Analysis

- We have carried out surface contamination monitoring using protocols developed by NASA
- We can perform the work in Livermore, CA
- We can train personnel for the work to be done elsewhere
- We can provide control organisms to test the decontamination system

Methods for Spacecraft Decontamination

- Incineration
  - 700F = 371C used to kill dry *B. subtilis* spores
- Chemical germicides
  - Nitrogen Dioxide (NO_2) gas*:
    - Rapid and effective sterilant for use against a wide range of microrganisms
    - Gas phase; Concentration is typically 1% of the saturated vapor pressure
    - Short exposure time
  - Other chemical germicides:
    - High effectiveness: 500mg/L ethylene oxide (gas) at 60C, formaldehyde+alcohol (8%+70%),
    - High-Intermediate effectiveness: 2% glutaraldehyde, hydrogen peroxide (2-30%), Iodophores (1-5g/L available Iodine)

Incineration



700F = 371C

Killed dry *B. subtilis* spores in refuse

Microbiological Safety Evaluation of an Industrial Refuse Incinerator; APPLIED MICROBIOLOGY, Feb. 1968, p. 291-295.