Expanding the Planetary Protection Implementation Trade Space through Analytical Methods

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Spacecraft Biological Cleanliness Verification

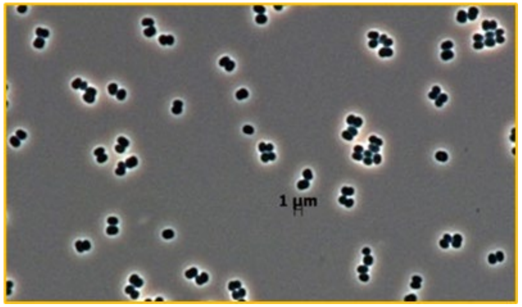
- **"Standard Spore Assay"**
  - NASA Handbook 6022 (draft)
  - ECSS-Q-ST-70-55C
- **Key Steps**
  - Directly collect potential biologicals using wipe or swab from the spacecraft surface
  - Extract potential biologicals in buffer
  - Sonicate / Vortex sampling device to remove potential biologicals
  - Heat shock to select for heat tolerant organisms (*i.e.,* spores), 80°C for 15min
  - Transfer to Petri dish, add growth media and incubate at 32°C for 72 hours
  - Enumerate colonies and apply biostats

Mihaela Ballarotto samples Europa Clipper's propulsion module for planetary protection cleanliness prior to harness installation.

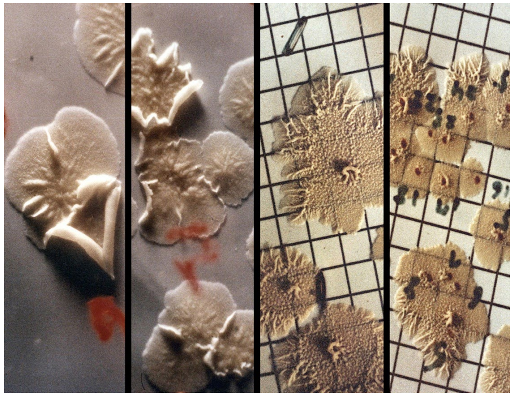
Planetary protection wipe sampling of an aeroshell.

Standard Spore Assay as the Gold Standard…

- Used on robotic spacecraft dating back to Viking.
- Current requirements based on spore biological management.
- Only approved technique on spacecraft to assess biological cleanliness.
- Spores have proven to be the most difficult form of life to eradicate
  - UV, space vacuum, radiation resistant
  - tolerance to spacecraft microbial reduction modalities (e.g., solvent cleaning and heat).



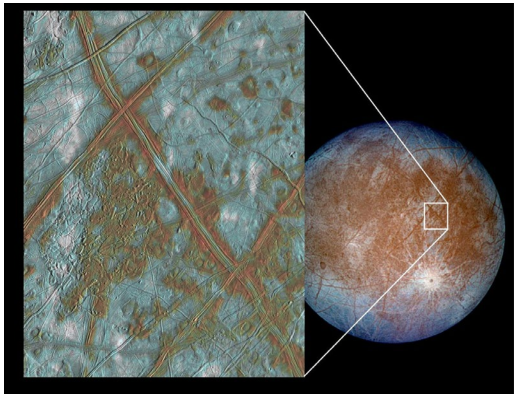
Bacterial species found in ESA and NASA cleanrooms; Credit: ESA



***Bacillus subtillus grown aboard Skylab;* Credit: NASA**

Why do we need to develop expanded verification tools now?

- Human Exploration – microbial monitoring, microbial management
- Restricted Sample Return Missions – sample safety assessment
- Parameter into risk informed decision making
  - Category III and IV sensitive icy worlds missions to be determined shall demonstrate contamination avoidance at a probability of occurrence less than 1.0×10^{-4} for a biological inoculation event into a potentially habitable aqueous environment (e.g., liquid water body, brine) for 1,000 years.
  - Life detection missions – tailored based on nature and sensitivity of science investigation.

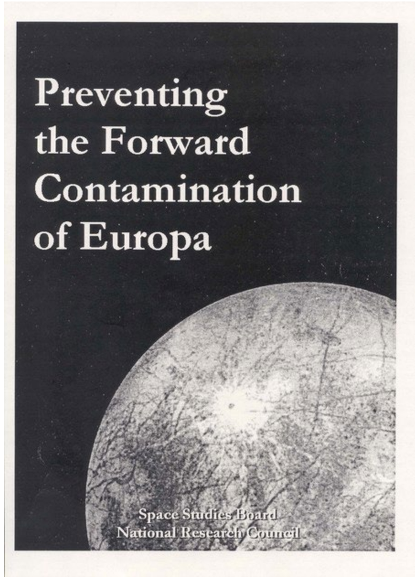


Blocks in the Europan Crust provide more evidence of Subterranean Ocean predicted by Love number.
Credit: NASA/JPL/University of Arizona

Image: NASA's Perseverance rover on Sept. 7, 2021, PDT (Sept. 8, EDT), shows two holes where the rover's drill obtained chalk stick-sized samples from rock nicknamed "Rochette."
Credit: NASA/JPL-Caltech

Viable Organisms in PP Implementation

- **"Specification Value" derived from 2006 Space Studies Board Estimate**
  - **1 spore detected ≡ 50,000 viable organisms present**
- **Research Applied Value**
  - **A ratio of spore to viable organisms: a detailed case study of the JPL-SAF cleanroom estimated 1 spore detected ≡ 12,091 viable organisms present**
    - Traditional Culture Based Techniques
    - Microscopy – fluorescent-assisted cell sorting
    - Biochemical – ATP
    - Molecular techniques – quantitative polymerase chain reaction (qPCR) and propidium monoazide (PMA)-PCR



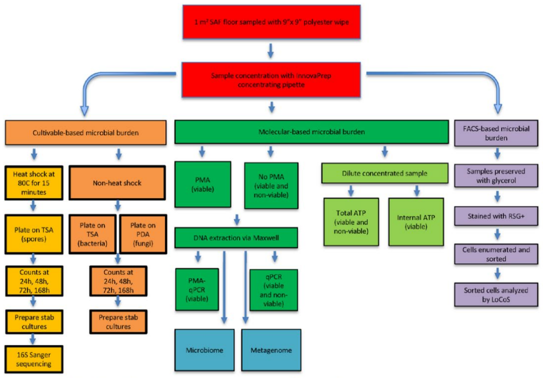
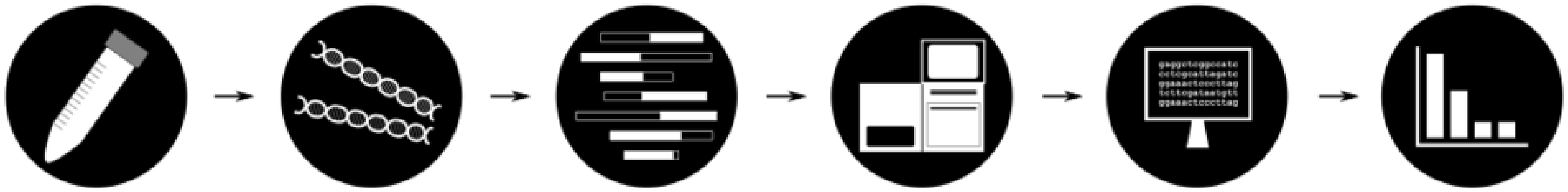


Figure 1: Workflow for collecting and processing spore to VO samples.
This schematic shows the workflow of the spore to VO from sample collection to analysis. A 1 m^2 sample was

Metagenomics in Planetary Protection – Identifying the Who and What



Credit: Jason Kralj

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Path Forward

- Evaluation of Industry Standards for Metagenomics – NIST, DOJ, etc.
- Metagenomics PP Workshop for Robotic Missions – Jan 19th
- Office of Planetary Protection to organize and lead Microbial Management Integration Team with SMD (PSD and BPS), HEOMD (SE&I and HMTA) and STMD participants.
  - Technical interface for coordination and integration of activities related to microbial management and control, particularly as related to planetary protection of crewed Moon-to-Mars activities.
  - Report and exchange information of ongoing and/or planned activities related to the PP Capability Gap areas, as well as other PP-relevant R&TD tasks
- NASA Federated Board Reporting
  - Evaluating Agency-wide annual progress on PP technology gaps
  - Balances and ensures funding responsibilities

QUESTIONS?

Abstract

The NASA Standard Assay, a direct test method for spacecraft surfaces, is the gold standard for verifying compliance with planetary protection requirements. Since the NASA Standard Assay is a proxy for overall spacecraft cleanliness more modern techniques and analytical methods are needed to understand the full breadth of organisms and their associated functional capabilities for more complex missions. Outer planets, restricted Earth return, and future crewed missions to Mars present increased mission complexities where additional biological knowledge is required to support probabilistic risk assessments and science investigations with greater sensitivity. Total viable organism assessments using microscopic, biochemical and molecular methods have been used to support planetary protection assessments for the Mars Sample Return Campaign. Molecular biological enumeration qPCR methods in combination with metagenomics are under development to support risk-informed decision making for missions to the Outer Planets and human missions to Mars. This presentation will showcase the implementation capability gap and detail the current trade space under development to advance planetary protection verification. Continued development and leveraging industrial standards for NASA missions will be essential to support more complex missions and to further enable science exploration.