Evaluation of Surface Sampling Efficiency by Swabbing

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Abstract

NASA has an ongoing research effort to introduce new technologies for the evaluation of trace biological and molecular contamination on spacecraft outbound from Earth to other planetary bodies, as well as sample return missions to Earth. Viable organisms are of particular concern for spacecraft cleanliness, yet conventional culture-based methodologies can detect only a small fraction of spores present. The overall purpose of this study is to determine the efficiency of sample swabbing and assess the quality of the bioburden recovered. A selected set of predetermined spacecraft materials were inoculated with Gram + spores or Gram – vegetative cells using the Beckman BioMek robotic dispenser. Three types of commonly used swabs were applied to study the efficiency of swabbing microbes from inoculated surfaces. A quantitative measurement of the number of microbes or molecular signals was carried out to determine the efficiency of swabbing. Two enzymatic assays were used and compared with a standard laboratory method to evaluate sampling efficiencies and coincided with a reduction in time needed to obtain results from days to minutes. Our results indicate that the swabbing efficiency of different types of swab materials is significant. Additionally, it was determined that although Gram + spores were recoverable, the recovery of Gram – vegetative cells was considerably variable from material to material. Since swab sampling is still the standard method for collecting surface environmental samples, our results can provide useful information for surface sampling.

Overview

- Select materials appropriate for study
  - Determine dimensions, quality and quantity
  - Determine and carry out cleaning and sterilization procedures
- Select swab types
  - Sterilize
- Inoculate materials with Gram – or Gram + bacteria between 1.0 and 3.0 x 10^4 CFU/mL
  - Gram - : *Escherichia coli* ATCC # 25922
  - Gram + : *Bacillus atrophaeus* ATCC # 9372
- Swab inoculated surfaces
- Determine efficiency of swabbing by assessing quality of bioburden recovered

Standard Assay

- NASA Planetary Protection Standard Assay
  - Assess degree of microbiological contamination is <300 spores/m^2
    - Spacecraft assembly and test facilities
    - Spacecraft hardware
  - Measures heterotrophic, mesophilic, aerobic and anaerobic microorganisms.
- Procedure
  - swab
  - Vortex
  - Sonicate
  - Heat shock 15 minutes at 80°C
  - Count colony forming units on TSA plates after three days

LAL Assay

- *Limulus* Amebocyte Lysate (LAL) assay
  - targets lippopolysaccharides (LPS), glucan, peptidoglycans
  - molecules linked to Gram-negative microbial cell wall
  - fairly stable

- LAL assay
  - Detects organic contamination from live or dead Gram-negative microbial cells
  - Based on enzyme cascade of microbial defense system in blood cells (i.e. amebocytes) of *Limulus polyphemus*
  - Cascade initiated by presence of LPS of Gram-negative bacteria
  - Forms a gel-clot; destroys the invading microbes
  - Exploits this principle by coupling with a chromogenic substrate
  - Widely used in pharmaceutical and semiconductor industries
  - Quantitative

ATP Bioluminescence Assay

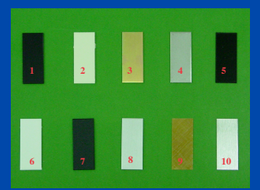
The bioliminescence reagent contains firefly luciferin and luciferase. Luciferase specifically reacts with ATP and catalyzes the following reaction.

$$\text{ATP} + \text{D-luciferin} + \text{O}^2 \xrightarrow[\text{Mg}^{2+}]{\text{Luciferase}} \text{AMP} + \text{Oxyluciferin} + \text{PP}_i + \text{CO2} + \text{light}$$

The amount of bioluminescence produced in the reaction above is in direct proportion to the amount of the ATP in the sample

CheckLite-HS set is from Kikkoman corp. Japan.

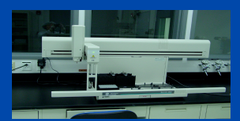
Spacecraft Materials



1. Anodized Black
2. NS43G Paint
3. Chemfilmed Aluminum 6061T6
4. Stainless Steel
5. Graphite Composite
6. White Epoxy Paint (446-21-7925)
7. Black Paint (463-3-8)
8. Anodized Clear Aluminum
9. Astroquartz
10. Bare Aluminum (6061)

Bacterial Deposition

- Gram + : Spore Suspension
  - 20nL/pin, 100pins/coupon deposited
    - 2000nL or 2μL
- Gram – : Overnight Culture
  - 25nL/pin, 81pins/coupon, repeated 3x/coupon
    - 6075nL or ~6.1μL
- Beckman-Coulter Biomek 2000 Workstation



Swab Specifications

- Chose 3 commonly used swabs
  - Puritan 6" wooden applicator cotton swab – NASA standard swab
  - Texwipe Alpha TX761 Polyester swab – recommended for LPS detection
  - Texwipe Transplex Alpha ESD-Polyester swab TX761D –compatible with engineering concerns
- Sterilized swabs
  - Autoclave
  - H_2O_2 sterilization

Swabbing Method

- Swabbed surfaces
  - Procedures from NPG 5340.1C applied for surface swabbing
    - Swabbed 26cm^2 area, at 30° angle
    - Used swab pre-moistened with sterile water
    - Vortexed and sonicated, then split into two aliquots
  - Reacted with specific substrates for each respective assay

Experimental Controls

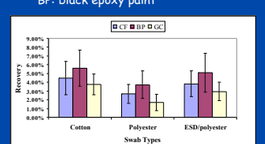
- Individual samples assayed in multiples
- Controls always included
  - Positive Controls
  - Negative Controls
  - Blanks
- Extensive calibration curves carried out in parallel for each enzymatic assay:
  - ATP: 10^{-9} moles/L to 10^{-13} moles/L
  - LAL: 50 EU/mL to 0.005 EU/mL
- NASA Standard Spore Assay
  - Positive Controls: *B. atrophaeus* ATCC #9372

Spore Study

- Three materials selected, cleaned, and sterilized
- Spores deposited with BioMek Workstation
- Coupons swabbed using three types of swab
- Samples processed following NASA Standard Assay Protocols

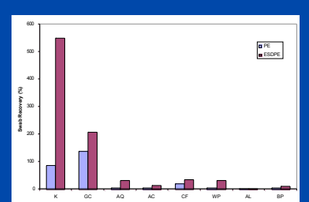
Spore Study Results

Three types of coupons:
CF: chemfilm aluminum
GC: graphite composite
BP: black epoxy paint



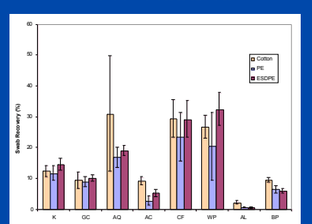
Number of spores that inoculated on each type of coupons was used as 100%.

LAL Results



K and GC had exceptionally high recoveries
Are there enhancers affecting results?

ATP Results



Amount of ATP (in the bacteria culture) inoculated onto each coupon type was used as 100%.

Conclusions

- Poor recovery of bioburden with PE
  - Spore study (3 materials; 3 swab types)
    - PE had worst recovery
    - Cotton and ESD/PE were better
  - LAL study (8 materials; 2 swab types)
    - ESD/PE was better than PE
    - Kapton exceptionally easy to treat
  - ATP study (8 materials; 3 swab types)
    - Cotton had better performance
    - 3 materials had better recoveries overall
- What did we learn?
  - Cotton or ESD/PE swabs had enhanced recovery over PE
  - Assay and material dependent
- Potential Biothreat Agent Applications Require Further Research