# Quantification of Spore-forming Bacteria Carried by Dust Particles

Ying Lin, Tanya Cholakian, Shariff Osman, and Jack Barengoltz
Jet Propulsion Laboratory, California Institute of Technology
4800 Oak Grove Drive
Pasadena, CA 91109
818-393-6381
ying.lin@jpl.nasa.gov

***Abstract-In order to establish a biological contamination transport model for predicting the cross contamination risk during spacecraft assembly and upon landing on Mars, it is important to understand the relationship between spore-forming bacteria and their carrier particles. We have conducted air and surface sampling in indoor, outdoor, and cleanroom environments to determine the ratio of spore forming bacteria to their dust particle carriers of different sizes. The number of spore forming bacteria was determined from various size groups of particles in a given environment. Our data also confirms the existence of multiple spores on a single particle and spore clumps. The results of this study provide realistic inputs in developing the bio-contamination transport model, which in turn will help in better determining forward contamination risks for future missions.***

## I. Introduction

Microorganisms such as bacterial spores and fungi are almost always present in air. They can be present in large numbers in clouds of dust such as in the agriculture environment around combine harvesters or near barns housing swine [1-2]. On the other hand, microbial contaminations in cleanroom air are in small numbers, making it difficult to quantify. Previous study showed that higher particle counts usually correlate with higher microbial counts. Most, if not all, airborne microorganisms are associated with dust particles of a variety of sizes [3-7]. However, few details are given on how many of particle at what sized range contains most of viable particles. It is important to quantify the viable particle size distribution and to establish the correlation between the viable and non-viable particle counts in a given environment. If such correlation exists, it can help us predict microbial contamination level based on the particle concentration and size distribution without the need of tedious and time-consuming microbiology lab work. This is particularly useful for microbial contamination prediction in cleanroom where particulate counts are readily available but the microbial counts are difficult to obtain. For planetary protection purpose, we use NASA standard assay, which quantifies spore-forming bacteria as our reference in measuring the biological contamination levels in clearnroom. Spore forming bacteria are gram-positive bacteria that can survive 82 °C heat shock for a period of 15 minutes [8-11]. For convenience, we use term spore for the spore-forming bacteria in this paper.

The objective of this study is to determine the quantity and sizes of spore containing microbes on the surface of spacecraft in cleanroom. The number of spores associated with particles as a function of particle size and the adhesion forces, are essential for developing biological contamination transport models for predicting the cross contamination risk during spacecraft assembly and upon landing on Mars.

## II. Methods

In order to determine the number and size distribution of spores containing particles on surfaces of spacecraft in cleanroom environment, it requires one to measure the quantity and size of the particle contamination and biological contamination on a surface simultaneously. Since there is no such instrument available, an alternative strategy, with multiple instruments has been used. The low abundance in biological and particle contaminants and small particle size (1-10 micron) in a cleanroom environment pose additional challenges for the measurement. The traditional Andersen six-stage particle counter method could not produce meaningful counts due to the extremely low biological particles. We conducted air samplings in relatively dirtier environment such as non-classified indoor and outdoor environment and extrapolate the ratio of viable and nonviable particle counts to cleanroom. We also made an initial assumption that the spores on particle ratio on cleanroom surface is the same as in air samples. In other words, most if not all particle contamination on surfaces are direct result from particle fallout from air in cleanroom. This assumption was then verified by our experiment later.

### *A. Air sampling*

Two instruments are used for conducting air samplings. An ten-stage air particle analyzer QCM (Quartz Crystal Microbalance) cascade impactor [12] was used to quantify particle sizes from 0.1 micron to >7 micron. A six-stage Andersen counter was used to separate air particles into six stages based on the size of the particle. We then quantify the viable and cultivable particles in each size group by counting the colonies on TSA plates placed at each stage [13]. However, the total particles of each stage cannot be quantified using this instrument. Those data has to come from The QCM particle counter. By running the two types of particle counter side by side simultaneously we obtained both total particle amount of each size range and the percentage of those particles containing spores.

To differentiate whether the colonies of Andersen TSA plates are spore-forming bacteria, every colony was inoculated into a sporulation medium and incubated for seven days. The colonies, which survived heat shock protocol, are identified as the spore forming bacteria.

The air sampling was conducted periodically for over 9 months. Each sampling takes 5-20 minutes for particle sampling and 30 to 90 minutes for spore sampling, depending on the sampling environment and the amount of particles in each environment. Three consecutive samplings are performed at the same location for each data point.

It should be noted that the two instruments both separate particles by size, the size ranges for each stage are different. To incorporate data from the two instruments, we have to interpolate the data from one instrument to match the particle size range of the other. In this way, we obtained the spore containing particle population at each particle size range in air samples. The results are shown in Fig. 1.

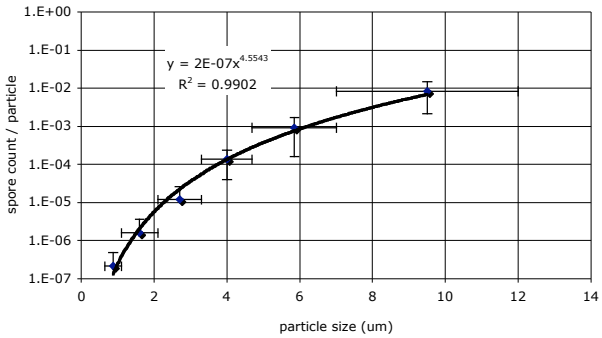


Fig. 1. Spore per particle distribution at different size range obtained by air sample. The vertical error bars are statistical variance of three sampling and the horizontal error bar merely indicate the size range.

## *B. Surface sampling*

Surface samples are collected by placing particle-collecting coupons on stands to collect fallout particles. The three environmental conditions selected for surfacing samplings are outdoor, indoor, and class 100,000 cleanroom. The collection times ranged form one week in outdoor environment to four months in cleanroom environment. Twelve common spacecraft materials were used for this study. All materials, with the except of glass, were manufactured and processed based on the same specification of spacecraft hardware. The coupons are 2”x2” in size. The materials are Bare aluminum, Anodized clear aluminum, Stainless steel, Black epoxy paint, White epoxy paint, Black pain (Z306), Chemfilm, Gold coated aluminum, Mylar, Kapton, and Graphite composite. Sample material coupons are shown in Fig. 2. All coupons are cleaned by JPL precision cleaning process and then further cleaned until minimum particle counts for each material are reached.

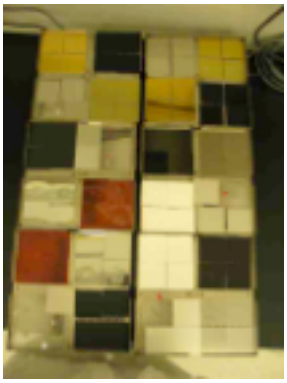

Fig. 2. Coupons of the twelve materials on a collecting tray.

To ensure that there will be no microbial contamination, coupons are also exposed under UV 254 nm for 2 minutes before set out for particle collection. A Pentagon QIII+ surface particle counter [14] was used to quantify particle size distribution on fallout particles collected on surfaces. Using spore per particle distribution from the air sampling, we calculated spore distribution on surfaces in each environment using our previously established assumption. Spore distribution on coupons from outdoor collection is illustrated in Fig. 3 as an example.

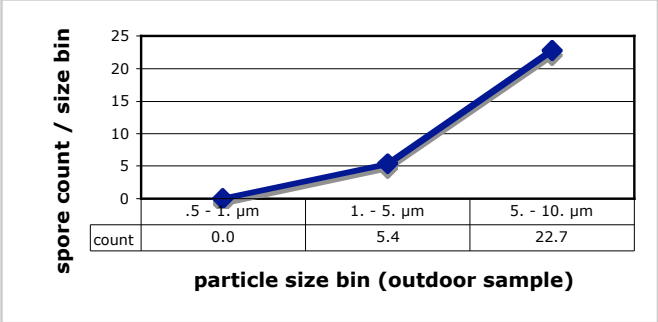


Fig. 3. The spore counts from the Pentagon QIII surface particle measurements using the air sample spore per particle distribution.

To independently verify the validity of the assumption, a separate method was used to determine the amount of spore carrying particles on surface. Contact TSA plates are used to lift off the particle containing spores from the coupon surfaces and counted [15]. This direct spore count result is compared with the derived data from the calculation based on the surface particle counts and the air sampling data. The results are shown in Table I.

TABLE I
SURFACE SPORE COUNT COMPARISON

| | Outdoor | Indoor | Cleanroom |
|---|---|---|---|
| Direct measure | 174 | 33 | 51 |
| Calculated | 28 | 5 | 10 |

## III. Results and Discussion

We conducted a series of air sampling and fallout coupon collection experiments to better understand the spore and particle associations in air and on spacecraft surface. Since there is no instrument capable of direct measurements, a combination of correlation and interpolation are used to determine the spore per particle distribution.

Our result shows that spore-forming bacteria are associated mostly with larger particles. The number of spore per particle appears to be a strong function of the particle volume. The scaling stronger than the particle volume suggests the existence of spore clustering on larger particles. No strong material dependence was observed. Using the air sampling data, we have generated the spore on particle distribution on fallout collections. The analysis shows that the calculated spore distribution based on the air sample result is consistent with the direct spore culture measurement in all three different environments.

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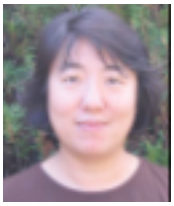
## Biography



Dr. Ying Lin is a senior member technical staff at JPL's biotechnology and planetary protection group. She has a BS in chemical engineering from Tsinghua University and a Ph.D. in Chemistry from University of Arizona. She has extensive research experience in chemistry, biochemistry and chemical engineering. She is the PI and Co-I of several proposals funded by JPL and NASA on planetary protection. She is currently a technical lead for the planetary protection tasks for Mars Technology Program.

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