Quantification of Spore-forming Bacteria Carried by Dust Particles

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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 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. This study will help in developing a better bio-contamination transport model, which in turn will help in determining forward contamination risks for future missions.

Introduction

Objective — determine the distribution of spore count per particle on surfaces of common spacecraft materials.

Challenge — no instrument can directly count particles by size and spores at the same time.

Approach — conduct separate spore and particle samplings with correlation and interpolation. Base assumption: spore distributions per particle are similar in air sampling and on surface collection.

We conduct the following experiments:

1. Sampling indoor and outdoor air using QCM 10 stage particle counter - particle size distribution in air
2. Sampling indoor and outdoor air using 6 stage cascade impactor - quantify the distribution of number of spores associated with particle sizes in air.
3. Sampling the spacecraft surfaces by coupon fallout collection using Pentagon surface particle counter - particle size distribution on surface
4. Correlate the air and surface sample studies to determine the nature of bio-particles found on spacecraft surfaces.

Sampling and Interpolation Process

Air sampling

QCM -> particle numbers for a set of particle size bins

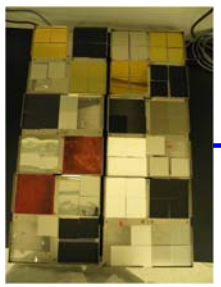
Interpolation to the same particle size bins as of the 6-stage impactor

6-stage impactor -> spore numbers for a set of partcle size bins

Spore number per particle size bin

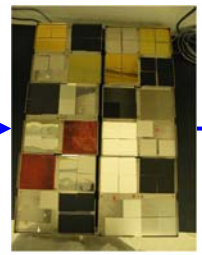
Interpolation to the same particle size bins as of the Pentagon system

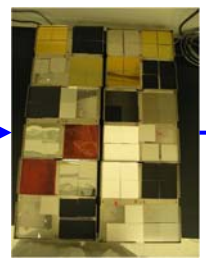
Surface sampling



Fallout collection of duplicate sets of coupons

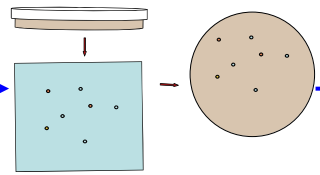
Two identical sets





Pentagon surface particle counter

Total spore count

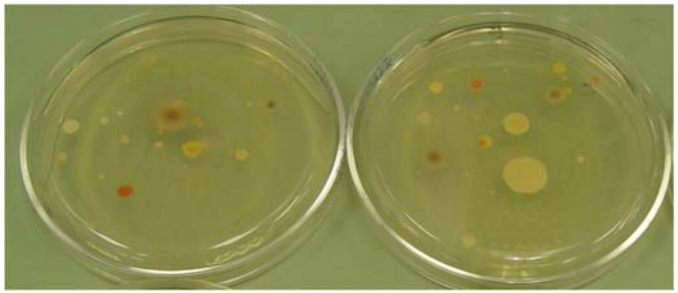


Spore distribution by particle size and estimated total spore number

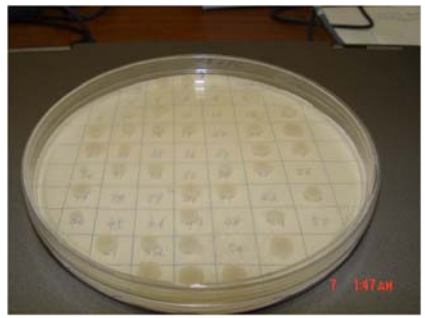
Validation comparison

Separate Spore and Non-spore Forming Bacteria

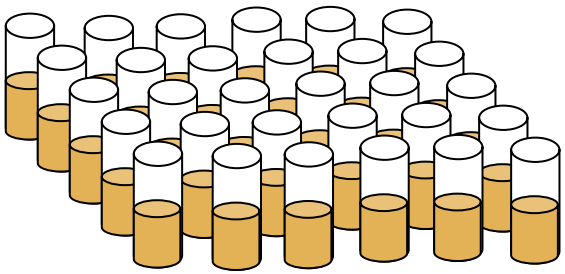
Heat shot process is used to kill non-spore forming bacteria



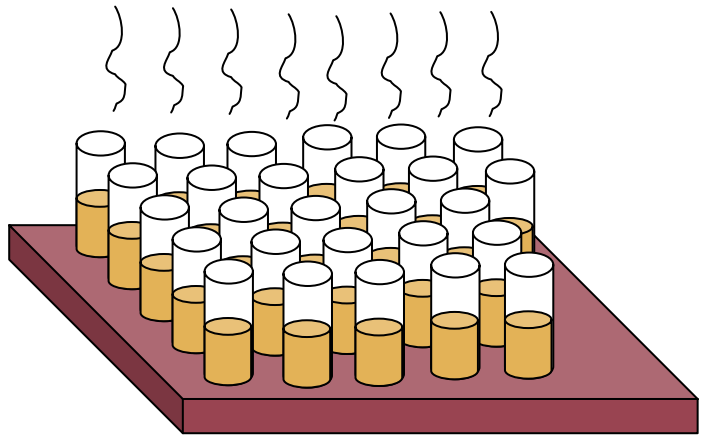
Colonies collected on Andersen Counter TSA plates



Surviving colonies are counted as spore forming bacteria

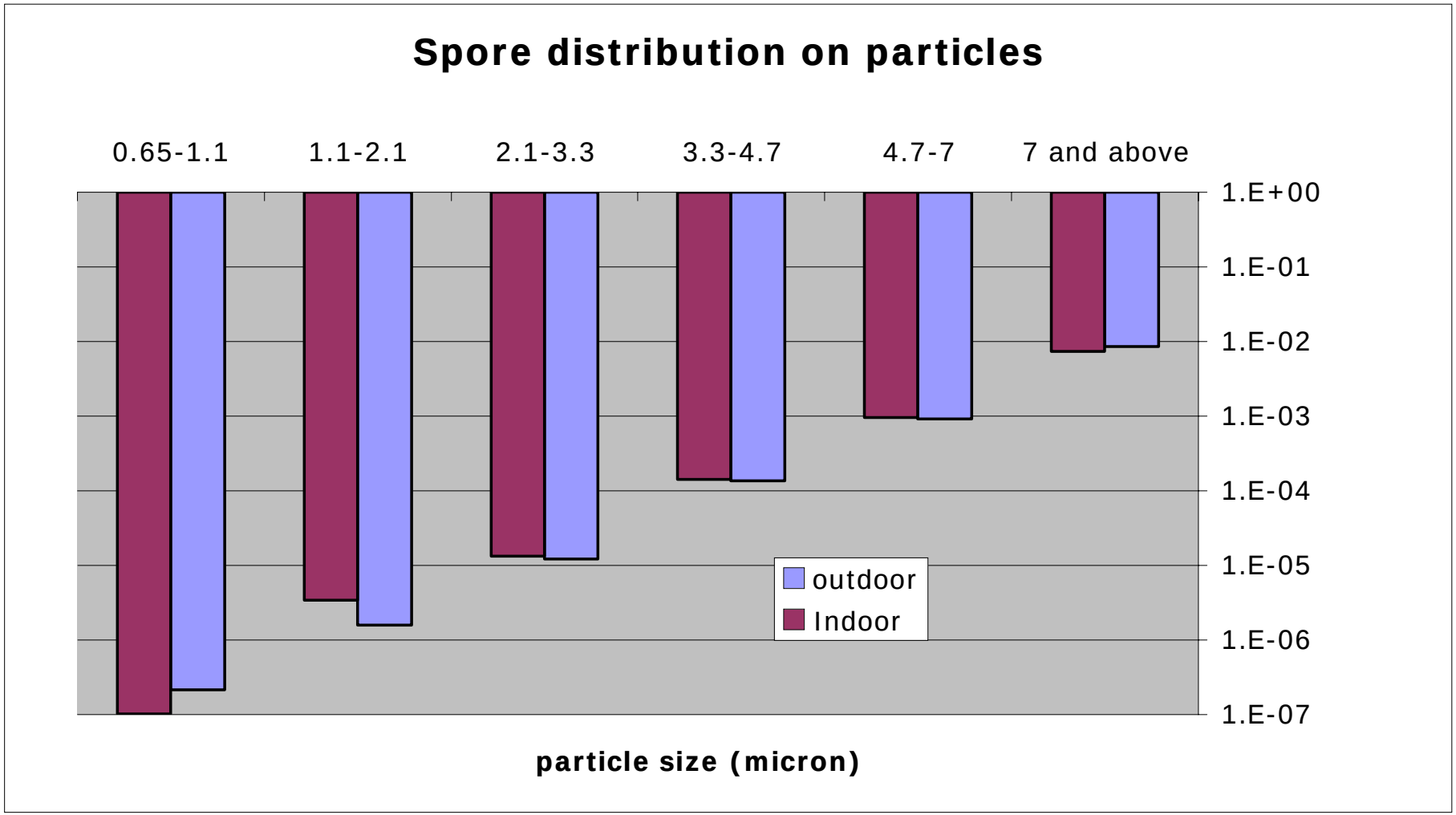


Sporulation in NSM sporulation media for 1 week



Heat shock kills non-spore forming bacteria

Spore Per Particle Distribution From Air Sampling



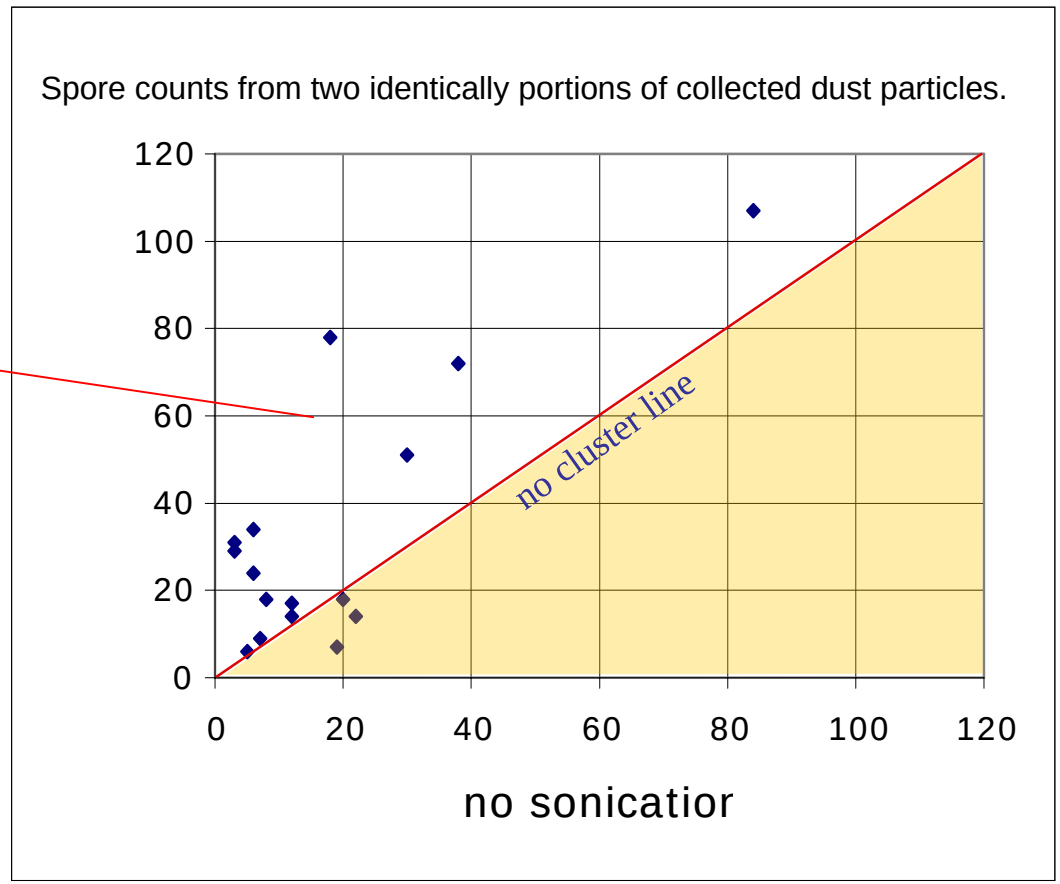
Spore per particle as a function of the particle size. The data were from indoor and outdoor air sampling. Note there is little difference in the two cases.

Spore Clusters on Particles

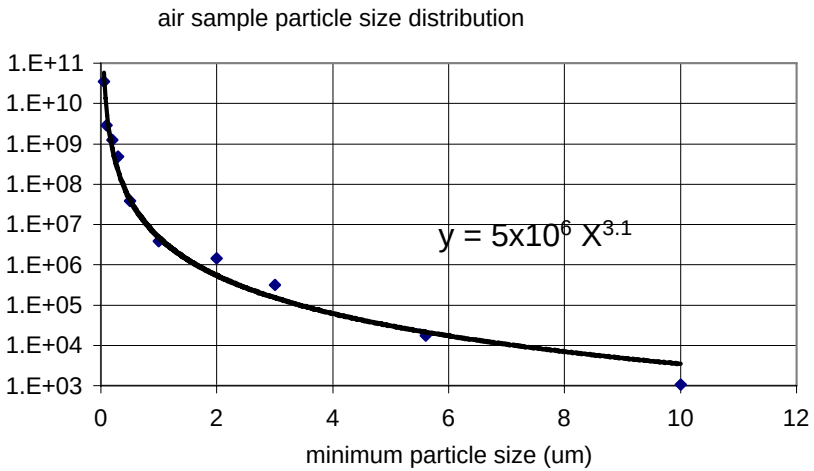
Determining if colonies formed on the plates are spore cluster or single spores

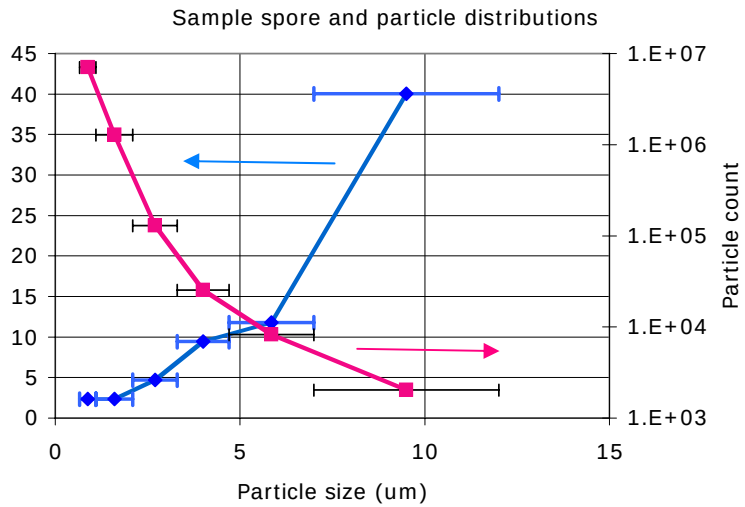
- *–Collect the particles on the 1st stage (particle size >7 micron)*
- *–Rinse out the particles and separate the sample into two equal parts*
- *–One part is subject to sonication to break up clusters*
- *–Heat shock the samples and grow on the TSA plates (pour plate instead of spread plate)*

Most of the data points fall above the no cluster line (slope of unit). This suggests that there is significant clustering of spores on dust particles.

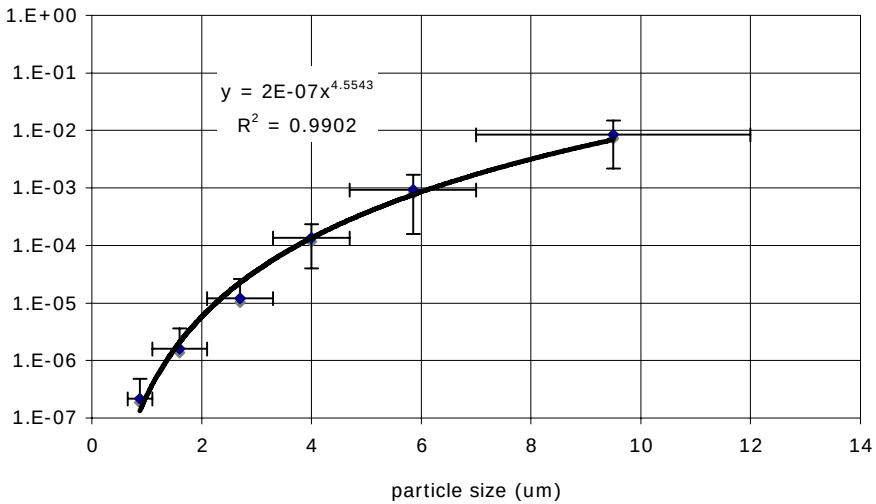


Spore per Particle from Air Sampling



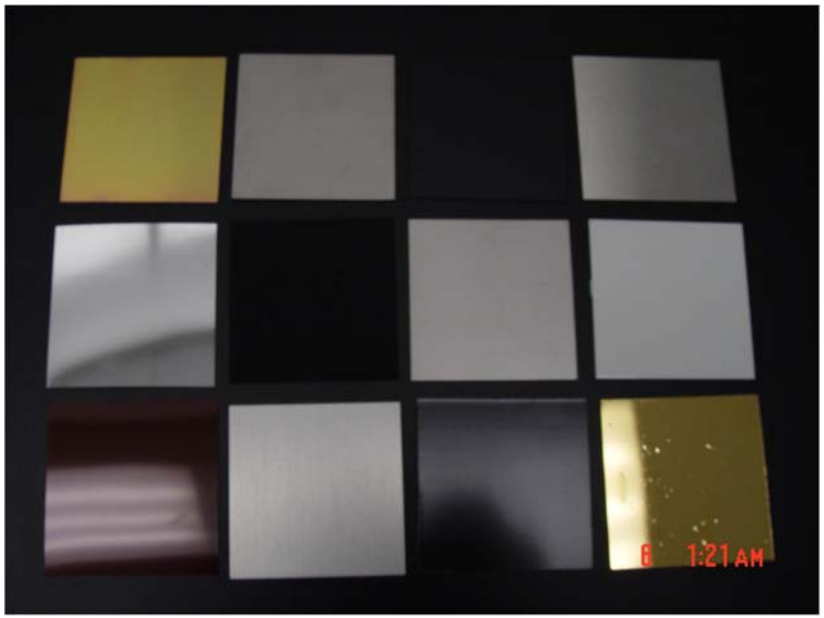


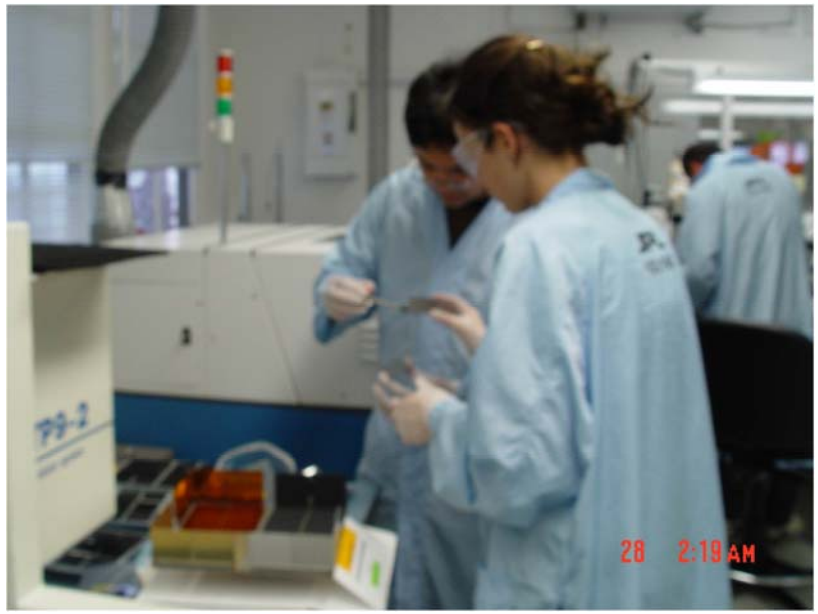
Air sampler generates an “integrated” particle count distribution function (upper left graph). Using this function, you interpolated the particle count distribution with the same particle size range (bin) as that by the spore sampler (upper right graph). From both the spore and particle distributions of the same particle sizes, we calculated the spore per particle distribution (lower graph).



The horizontal error bars indicate the particle bin size range of the size bin; the vertical error bars indicate the scattering of particle count among all different materials (twelve common spacecraft materials were used.)

Coupon Fallout Collection

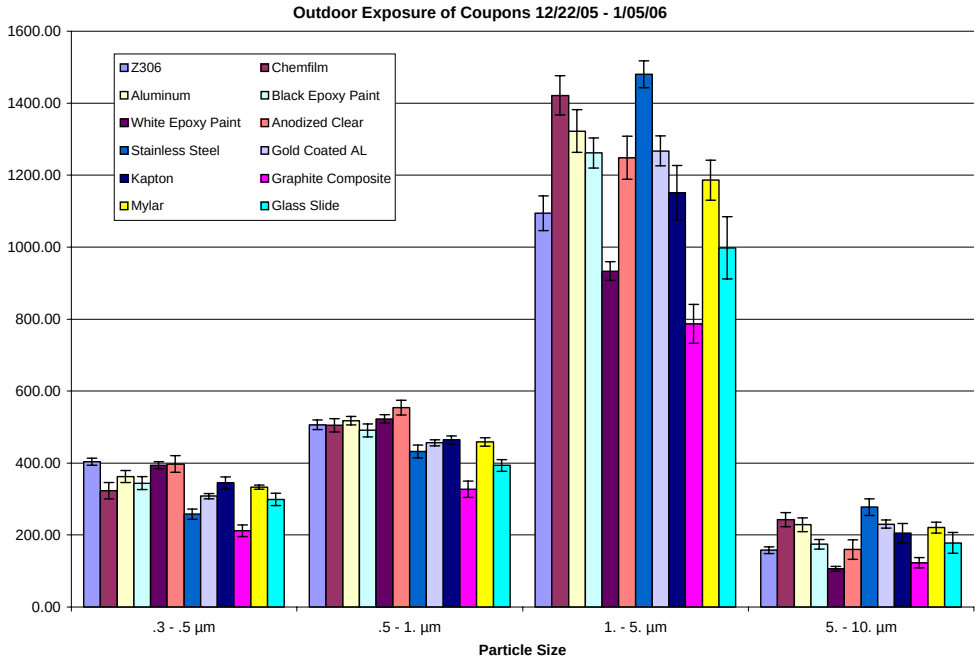




- 12 coupon materials* are precision-cleaned at JPL facility.
- The particle counts are measured using Pentagon QIII particle counter
- The JPL precision cleaning procedure can not reduce particle counts to the acceptable level.
- Multiple samplings were used to “vacuum” clean the surface to a minimum particle counts.

* Materials studied: BP, Al, SS, WP, KP, GL, Z306, AC, GC, GA, MY

Outdoor Fallout Surface Sampling Data

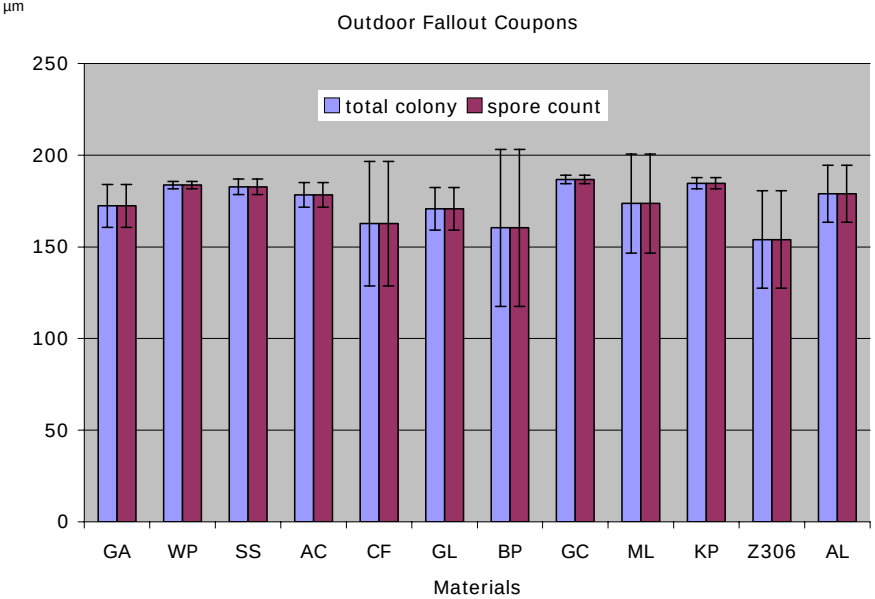


Particle distributions of the fallout coupon collection from various materials.

The dust particle size is peaked in the 1 to 5 um size bin. Material dependence is not strong.

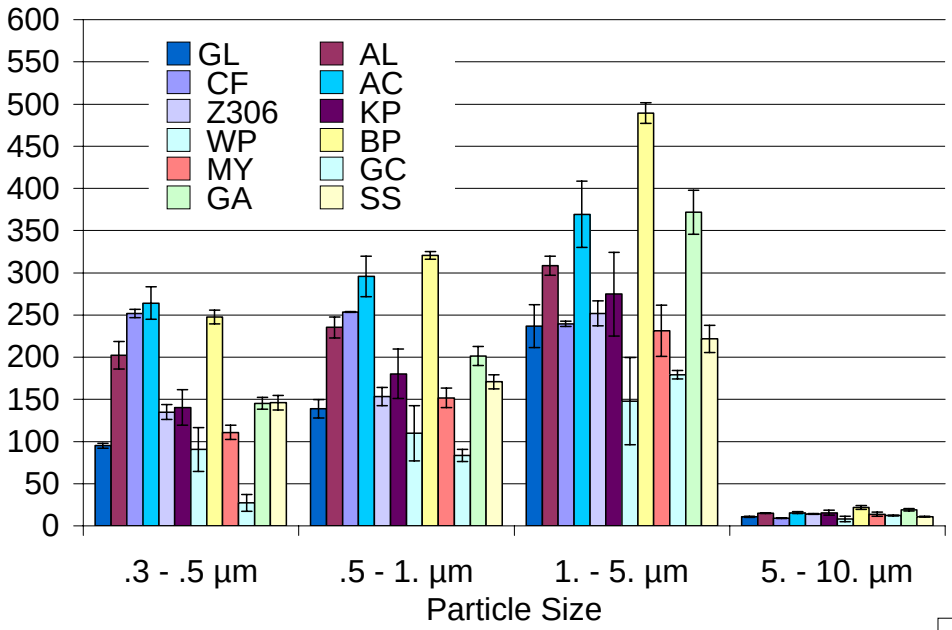
Spore distributions of the fallout coupon collection from various materials.

Errors are from three identical coupons



Indoor Fallout Surface Sampling Data

Building 98 Indoor Exposure of Coupons 5/01/06 to

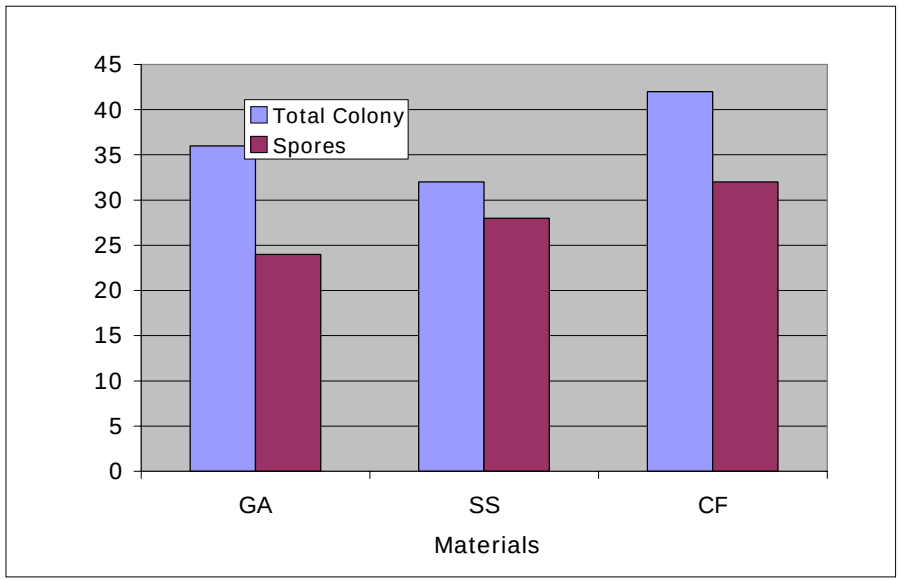


Spore distributions of the fallout coupon collection from various materials.

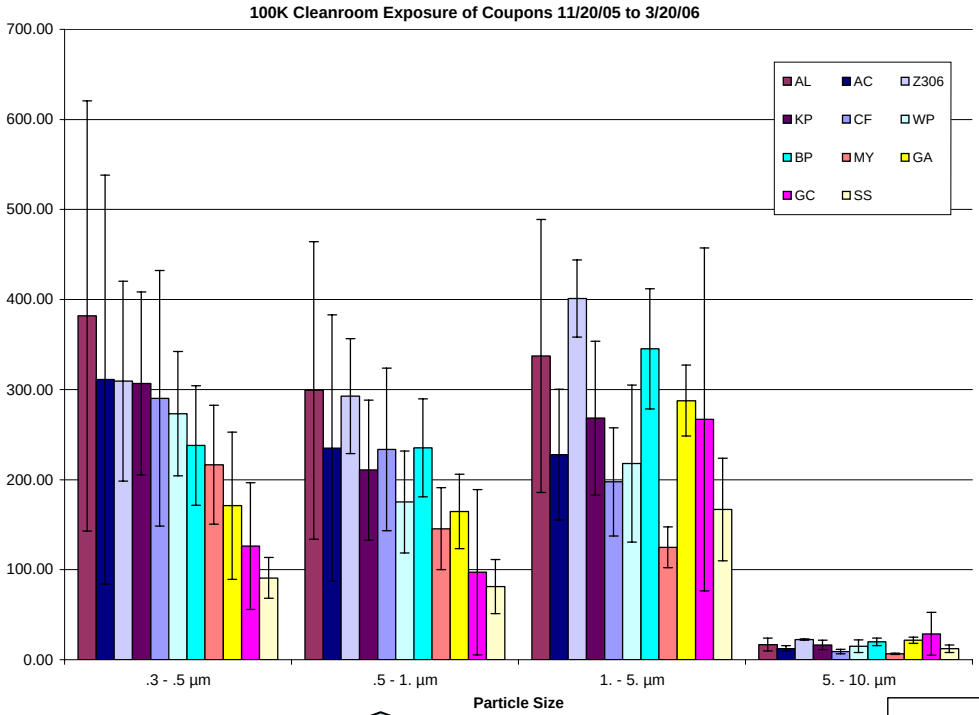
Three materials were studied.

Particle count distributions of the fallout coupon collection from various materials.

Again, most of particles are less than 5 um. The distribution also peaks in the 1 to 5 um range, though not as pronounced as in outdoor.



Cleanroom “A” Fallout Surface Sampling Data

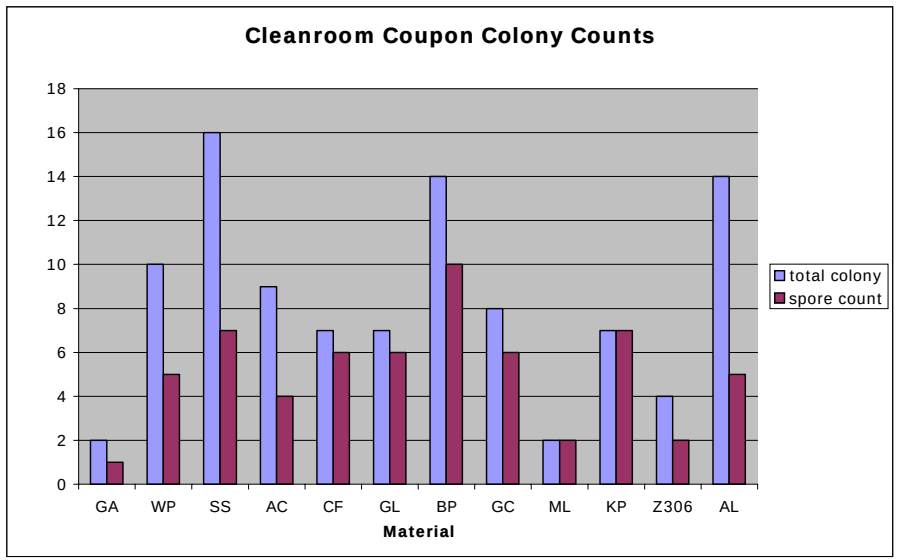


Spore distributions of the fallout coupon collection from various materials.

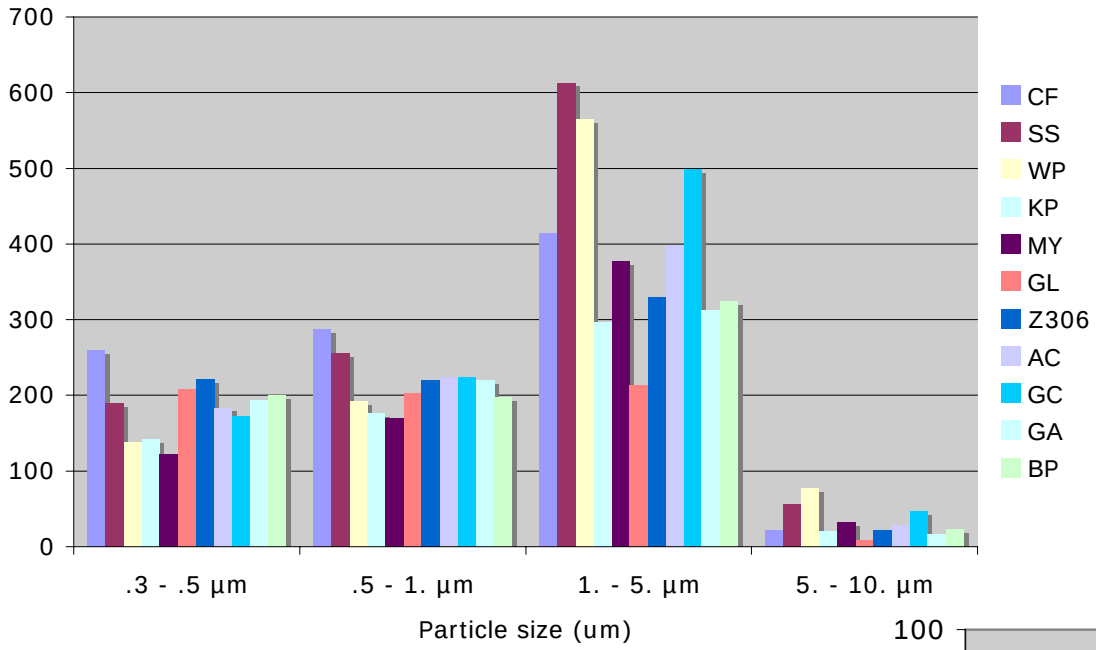
The spore count is low with poorer statistics (not shown).

Particle distributions of the fallout coupon collection from various materials.

Most of particles are less than 5 um. Because of the small number of particles collected, the statistical errors are relatively large.



Cleanroom “B” Fallout Surface Sampling Data



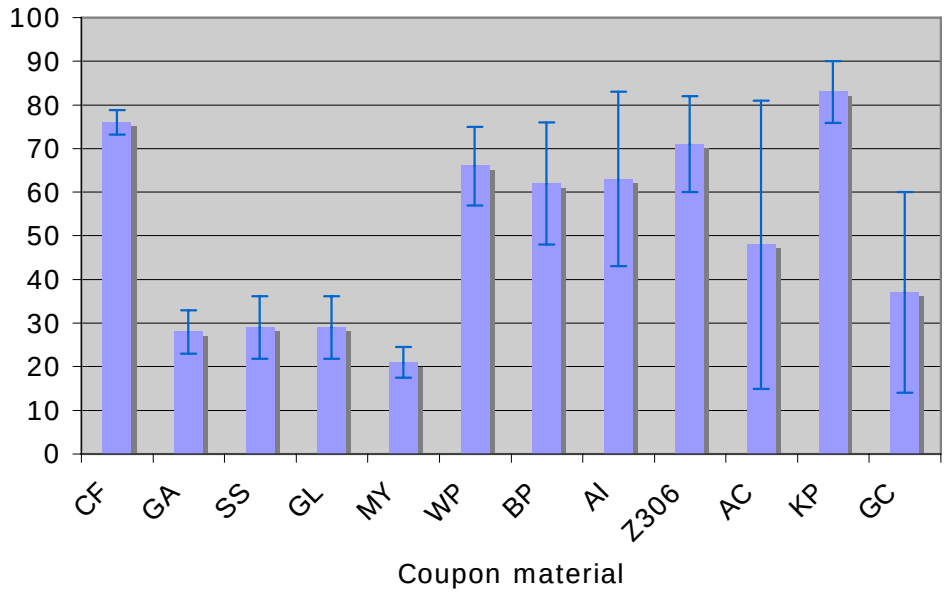
Colony count distributions of the fallout coupon collection from various materials.

Errors are from three identical coupons.

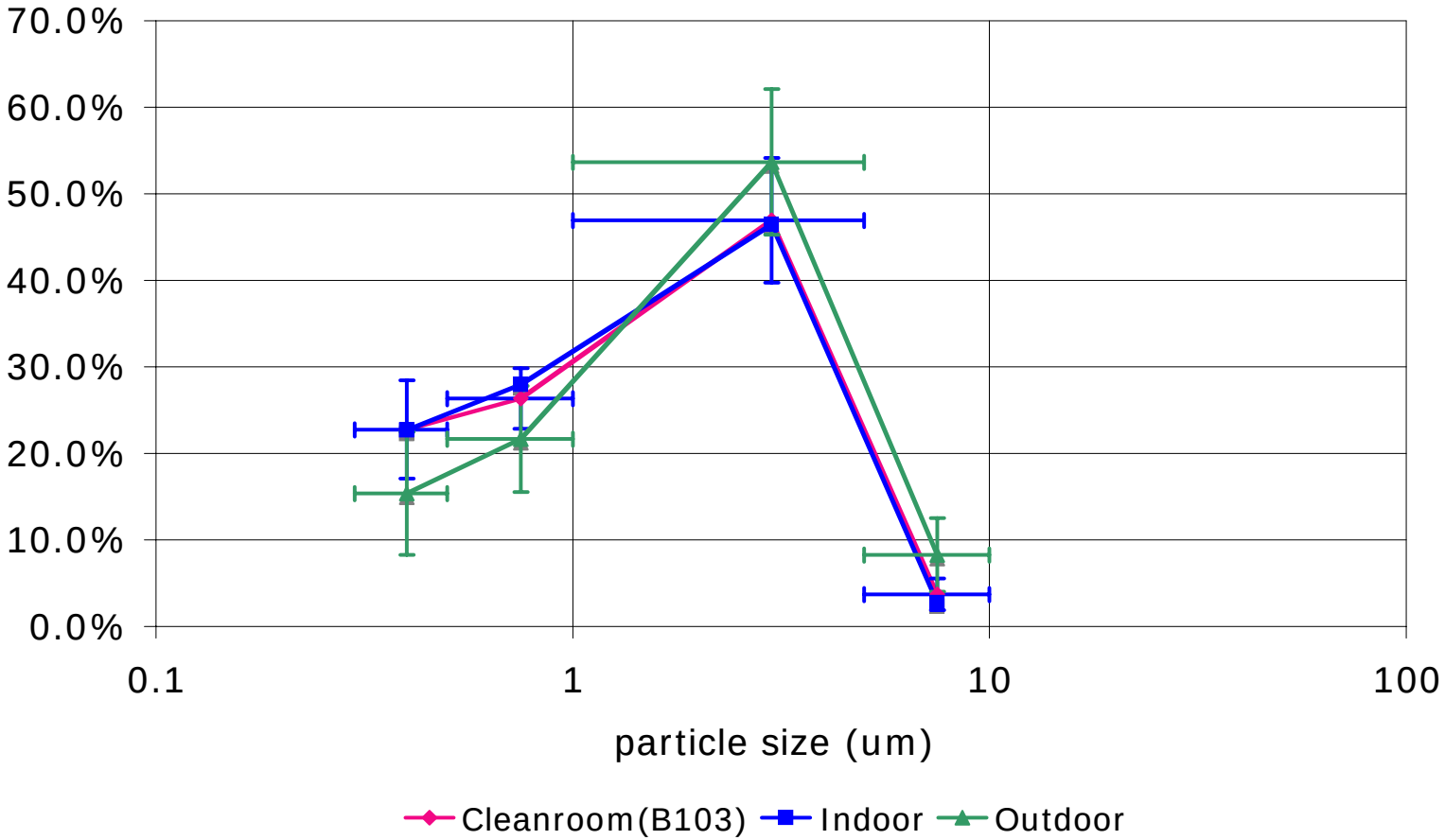
The collection time is about four months.

Particle count distributions of the fallout coupon collection from various materials.

The 1 to 5 um size bin peak is not pronounced as in the outdoor case. Material dependence is not strong.



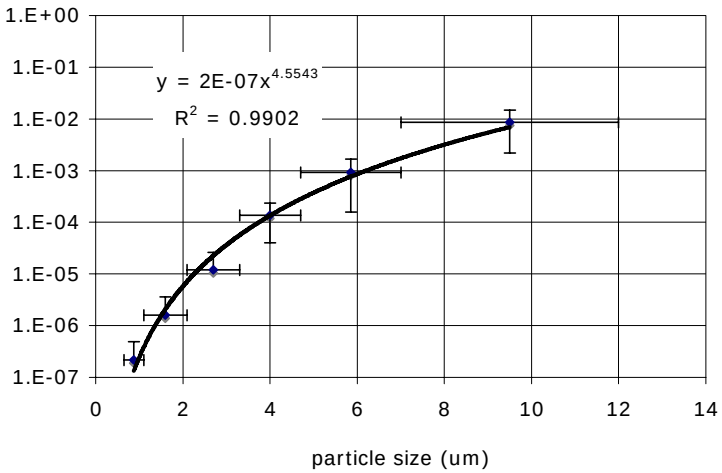
Particle Size Distribution From Coupon Collections

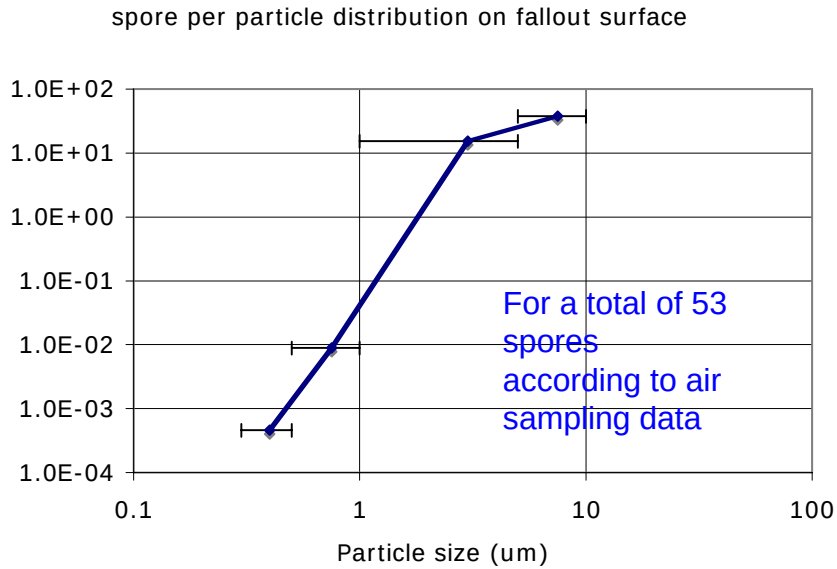


The horizontal error bars indicate the particle bin size range; the vertical error bars indicate the scattering of particle count among all different materials (twelve common spacecraft materials were used.)

The distribution curves show that the distributions in the cleanroom and indoor are nearly the same.

Spore Count Estimation From Particle Size Distributions





The function of spore per particle used in estimating the spore count for fallout coupons.

Note: this is a strong function of the particle size. Based on the particle size distribution, in fact, the dominant contribution is from the largest size bin, which has a large range and few particle count.

Spore count on the fallout coupons

Environment	Direct spore count (statistical error)	Estimated spore count
Outdoor	182(10)	26
Indoor	33(7)	4
Cleanroom	53(22)	1

Conclusions

- We have conducted a series of air sampling and fallout coupon collection experiments in order to understand better the spore and particle associations in air and on spacecraft surface. Since there is no instrument capable of direct measurements, correlation and interpolation methods are used.
- Air sampling in indoor and outdoor conditions show little qualitative difference, with similar particle size distribution and spore per particle distribution. Likewise, the particle size distributions of fallout collection are similar for indoor, outdoor, and cleanroom environment.
- Spore cluster as high as ten spores has been found in air sampling. Spore clustering is significant in spore transport modeling and spacecraft cleaning.
- Spore forming bacteria are associated mostly with larger particles. The number of spore per particle appears to be a strong function of the particle linear size. The scaling stronger than the particle volume may be attributed to the spore clustering on larger particles.
- Using the air sampling data, we have generated the spore on particle distribution on fallout collections. However, preliminary analysis shows that the estimate of the fallout coupon spore count from the air sampling distribution is less by an order of magnitude. Further studies needed to understand the discrepancy.
- No strong material dependence was observed. However. We have started studying vertically placed fallout coupons where material dependence is expected.

Acknowledgements

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