Mathematical Estimation of the Level of Microbial Contamination on Spacecraft Surfaces by Volumetric Air Sampling

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Microbiological sampling methods presently used for enumeration of microorganisms on spacecraft surfaces require contact with easily damaged components. Estimation of viable particles on surfaces using air sampling methods in conjunction with a mathematical model would be desirable. Parameters necessary for the mathematical model are the effect of angled surfaces on viable particle collection and the number of viable cells per viable particle. Deposition of viable particles on angled surfaces closely followed a cosine function, and the number of viable cells per viable particle was consistent with a Poisson distribution. Other parameters considered by the mathematical model included deposition rate and fractional removal per unit time. A close nonlinear correlation between volumetric air sampling and airborne fallout on surfaces was established with all fallout data points falling within the 95% confidence limits as determined by the mathematical model.

Microbiological sampling of surfaces using the swab-rinse technique is being gradually replaced by more efficient methods of removing and estimating numbers of microorganisms deposited by handling or settling from aerosols. The vacuum probe adequately removes microbial contamination from sampled surfaces but does not give good recovery of fragile or easily desiccated microbial cells (5, 11). Surface samples using agar contact methods are adequate for estimating contamination levels on most environmental surfaces (6); however, in the case of spacecraft, the low level of surface contamination cannot be measured validly with these techniques. In addition, these methods do not distinguish between clumps of microorganisms (viable particles) and single cells so that resultant colony counts can be ambiguous. Enumeration of air-borne microorganisms deposited on surfaces by natural fallout has been accomplished by a method using environmentally exposed stainless steel strips (1 by 2 inches; ca. 2.5 by 5.1 cm) which are insonated to remove and break up viable particles prior to plating (7, 8). All of the abovementioned sampling methods require contact with the sampled surface. For spacecraft, certain surfaces, such as optical equipment, thermal blankets, solar panels, or electronic components, cannot be sampled by any contact or handling method. Consequently, the ability to estimate the level of microbial contamination on a spacecraft surface without having to actually sample the surface per se would be desirable. To this end, a set of mathematical models was designed (3, 10). Verification of the assumptions and predictions prescribed by one of the mathematical models required knowledge of the number of viable cells per viable particle and the deposition of viable particles on surfaces which were not horizontal. The objectives of this study were to: (i) determine if volumetric air sampling in conjunction with a mathematical model could be used instead of contact or rinse methods for estimating the numbers of microorganisms deposited on surfaces by natural fallout, (ii) to illustrate the estimation of the necessary parameters used by the mathematical model, and (iii) to test assumptions and predictions made by the mathematical model in a spacecraft assembly situation.

MATERIALS AND METHODS

Particle collection on angled surfaces. Stainless steel strips (SS strips; 1 by 2 inches) were cleaned by the method described in NASA Standard Procedures

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(4) and placed on aluminum trays, covered with aluminum foil, and sterilized in a dry heat oven at 175°C for 3 h (4). Trays, each containing 48 strips and modified to hold strips in a vertical position, were placed on the exposure platform located in the Manned Spacecraft Operations Building (MSOB) Class 100,000 clean room, and the aluminum foil covering was removed. Surface exposure angles were 0°, 30°, 60°, and 90° from the horizontal. After 1 week of exposure, six strips were randomly retrieved from each tray on three consecutive days of each week until all stripes were collected. Each strip was placed into a sterile 250-ml Erlenmeyer flask and returned to the laboratory, and 50 ml of sterile buffered Tween 80 solution (4) was added to each flask. The flasks were sonicated for 2 min, and the entire 50-ml solution was poured into petri plates (130 by 25 mm). Fifty milliliters of sterile, double-strength Trypticase soy agar (TSA, BBL) was added to each plate, mixed, and allowed to solidify. Plates were incubated at 35°C and plate counts were performed at 48 and 72 h.

**Viable cell per viable particle analysis.** Studies were conducted comparing the number of viable cells per viable particle on both membrane filters and SS strips. The SS strips, prepared as described above, were placed on aluminum sheets and exposed to the intramural environments of the Planetary Quarantine Laboratory and the MSOB clean room. After 1 week of exposure, 12 strips were collected. Six strips were placed on poured TSA plates and overlaid with molten TSA, and the other six strips were immersed in 50 ml of buffered Tween rinse solution in 250-ml Erlenmeyer flasks, sonicated for 2 min, and plated with 50 ml of double-strength TSA. Sterile membrane filter (MF) field monitors (Millipore Corp.) were placed on a six-place manifold, and air was drawn through the filter at a rate of 2 ft³/min (ca. 0.06 m³/min) (1/2 ft³/min [ca. 0.06 m³/min]) per filter) for a total of 60 ft³ (ca. 18 m³) of air sampled (10 ft³ [ca. 3 m³]) per membrane filter). Three 30-min air sampling runs using MF monitors were taken on the same day the SS strips were collected. Three of the filters from each sample run were plated directly on TSA, and three were treated as the SS strips. Insonated filters and strips were overlaid with TSA to ascertain complete removal of microorganisms from the surfaces. All plates were incubated at 35°C, and plate counts were performed after 24, 48, and 72 h.

To evaluate qualitative differences between the two sampling methods, approximately 10% of the microbial isolates were randomly picked from culture plates after incubation and identified (1).

**Volumetric and airborne fallout sampling.** Sampling of fallout contamination on surfaces was accomplished using SS strips as described in the viable cell per viable particle study, with six strips being retrieved at each sampling period. Treatment of the SS strip samples was the same as described for particle collection on angled surfaces. Air samples were collected using Reynier slit samplers with 0.5-h mechanical timers on days that SS strips were retrieved. Viable particles were impinged at a flow rate of 1 CFM, and petri plates (25 by 150 mm) containing approximately 85 ml of TSA were used. Plates were incubated at 35°C, and counts were performed after 24, 48, and 72 h.

**RESULTS AND DISCUSSION**

Four trays of 48 SS strips were exposed at each of the four angles. The results of the assays of these strips were statistically analyzed, and it was determined that the values from four trays at each angle could validly be pooled for further analyses. The data are presented in Table 1. The mean and median values observed at each angle were analyzed using a chi-square goodness-of-fit test for several theoretical relationships. It was found that both the mean and median levels of contamination at each angle were proportional to the projected horizontal area of the strip at that angle, being consistent with a cosine function. The goodness-of-fit was better for the median values than for the mean values. This suggests that, in general, the levels of contamination on surfaces inclined 30° and 60° from the horizontal would be 87% and 50%, respectively, of the level on a horizontal surface. Because some microorganisms were observed on vertical surfaces, a correction factor for these observed levels was applied.

Table 2 shows the results obtained in the study on the number of viable cells per viable particle. There were no significant differences between the two sampling methods in the MSOB area; but in the laboratory area, significant differences were evident, even though a high coefficient of variation was observed. Analysis of the distribution of particles in both areas using a chi-square test showed a fit consistent with a Poisson distribution. The viable cells per viable particle difference observed in the laboratory between the two sampling methods is probably due to the higher number of large particles settling out when filtration in the air handling system is not adequate.

The types of microorganisms isolated from the membrane filter and SS strips are shown in the following table.

| Angle of inclination to horizontal | Total no. microorganisms per strip |
|-----------------------------------|-----------------------------------|
| 0°                                | Mean | Median |
| 30°                               | 89   | 33     |
| 60°                               | 36   | 17     |
| 90°                               | 9    | 3      |

*192 strips were exposed at each inclination.
Table 3. A comparison of the percentage of nonsporeforming gram-positive rods (NSFGPR) from the direct-plated and insonated MF indicates that these microorganisms occur in clumps of from two to five cells, whereas most all the others occur in approximately a 1-to-1 ratio. The difference observed between the percentage of NSFGPR comparing insonated MF and SS strips may be due to desiccation of these microorganisms when exposed to the intramural environments over extended time periods. The same types of microorganisms are found in all sample treatments, allowing a statistically acceptable qualitative comparison of the volumetric and fallout data.

Data analysis. Earlier in this paper, the techniques used to collect data using fallout strips and Reynier samplers in the MSOB and planetary quarantine laboratory were discussed. These data are summarized as four data sets in Tables 4 and 5. As was mentioned above, six strips were removed from the environment at each sampling period. If we assume that there is negligible variation between fallout strips with regard to their collection efficiency and with regard to the microbial environments they inhabit, then each strip can be assumed to be a 100% sample of the same surface. This is, of course, only approximately true since variations do exist. The numbers of columns 4 of these tables are the means of the number of viable particles which have survived the assay procedures on the six strips which were removed from the environment after an exposure for the number of days given in column 3. Relying again on the assumption of the uniformity of the strips and assuming that for each data set \( t = 0 \) is the time when the strips entered the environment, we obtain a set of points for each data set of the form \( (t_i, y_i), i = 1, \ldots, m_r \). In this set of points \( t_i \) is the number of days relative to the zero for the data set, \( y_i \) is the measure (column 4) of the

| Area          | No. of days sampled | Mean | Coefficient of variation (%) | Statistical test \( T_{ss} \) | Significant difference |
|---------------|---------------------|------|-------------------------------|-----------------------------|------------------------|
| SS strips     | Membrane filters    | SS strips | Membrane filters | Theoretical | Calculated |                      |
| Laboratory    | 19                  | 7.67 | 3.42                         | 73                      | 94                   | 2.101 | 2.778 | Yes     |
| MSOB\(^b\)    | 12                  | 7.22 | 6.90                         | 100                     | 70                   | 2.201 | 0.120 | No      |

\(^a\) Average of 6 strips per day.  
\(^b\) Average of 9 MF per day.  
\(^c\) Manned Spacecraft Operations Building.

Table 3. Frequency of occurrence of types of microorganisms identified from direct-plated membrane filters, insonated membrane filters, and insonated stainless steel strips

| Types of microorganisms            | Laboratory area | MSOB\(^a\) |
|------------------------------------|-----------------|-------------|
|                                   | Membrane filters (%) | Membrane filters insonated (%) | SS strips insonated (%) | Membrane filters (%) | Membrane filters insonated (%) | SS strips insonated (%) |
| Nonsporeforming gram-positive rods | 24.1            | 44.1        | 17.3          | 16.5             | 71.0            | 46.0 |
| Gram-positive cocci                | 41.2            | 31.8        | 50.2          | 71.3             | 24.6            | 41.6 |
| Gram-negative rods                 | 0.7             | 0.2         | 0.3           | 0.0              | 0.7             | 0.7  |
| Gram-negative cocci                | 0.2             | 0.0         | 0.3           | 0.0              | 0.0             | 0.0  |
| Actinomycetes                      | 5.2             | 8.9         | 2.9           | 0.5              | 1.8             | 4.1  |
| Yeasts                             | 5.2             | 1.7         | 1.6           | 2.1              | 0.0             | 2.4  |
| Molds                              | 4.8             | 2.2         | 2.9           | 6.4              | 0.4             | 3.1  |
| *Bacillus* sp.                     | 18.6            | 11.1        | 24.5          | 3.2              | 1.5             | 2.1  |
| Total                              | 100.0           | 100.0       | 100.0         | 100.0            | 100.0           | 100.0 |
| No. identified                     | 420             | 406         | 376           | 188              | 276             | 291  |

\(^a\) Manned Spacecraft Operations Building.
expected number of viable particles on the fallout strip which has been exposed to the environment for \( t \) days, and \( N \) is the number of data points for the given data set. These points are indicated by triangles on Fig. 1 and 2 for the four data sets under consideration.

Column 2 of Tables 4 and 5 indicates the concentration of viable particles in the environment for the data given in column 1. If we use the \( t = 0 \) as defined above for each data set, then we need to determine the airborne concentration of viable particles, \( C(t) \), for all values of \( t \). To do this we will assume \( C(t) \) is a step function. The concentration in column 2 for a particular day \( t_o \) of column 3 will be assumed to be the concentration of viable particles for times \( t \) in the range \( t_o - \frac{1}{2} \leq t < t_o + \frac{1}{2} \). To get this function for all other values of \( t \), we will use the mean concentration of viable particles calculated for each environment. For the MSOB we use all of the concentrations in Table 4. This leads to a mean of 0.99538 viable particles per \( \text{ft}^3 \) (ca. 30.5 \( \text{cm}^3 \)). In the laboratory data (Table 4), we will omit the 10.88 and the 0.84 from the calculation of the mean since they lie at the extremes of the distribution. This procedure leads to a value of 2.318 viable particles per \( \text{ft}^3 \) for the laboratory environment.

Due to restrictions on the handling of spacecraft surfaces it would be desirable if microbial concentrations on surfaces could be estimated from volumetric measurements. Roark (10) derived a model which could predict the number of microorganisms on a surface as a function of three factors. These factors are (i) the distribution of the number of microorganisms per particle, (ii) the deposition rate of particles onto the surface, and (iii) the fraction of particles removed from the surface per unit time.

The purpose of our analysis in this paper is to
nisms per particle which has a mean of one. Furthermore, the probability of a particle having more than one microorganism attached or having fewer than one attached will be zero. This reduces the model in reference 10 to the form used in reference 3. In a previous section, we discussed an experimental program to determine the distribution of microorganisms per particle. Since it is not important to our present discussions, we shall only observe that in analyzing this data it can be shown that the data is not inconsistent with the assumption that this distribution is Poisson. Such a model for this distribution was proposed in reference 9.

In order to analyze the deposition rate, let us assume that all viable particles on the fallout strips are deposited from the environment. The only effect that the concentration of viable particles in the environment has on the expected number on a surface should be reflected in the deposition rate $\lambda(t)$. Let us assume that:

$$
\lambda(t,\theta) = C(t)V(\theta,t) \alpha(\theta)
$$

where $\lambda(t,\theta)$ = deposition rate per square inch of surface at time $t$ due to the air flow in direction $\theta$ with respect to the surface, $V(\theta,t)$ = velocity (in inches/day) of air flow past the surface at time $t$ in direction $\theta$, and $\alpha(\theta)$ = collection efficiency of surface at angle $0$ to the direction of the air flow. Integrating equation 1 we obtain:

$$
\lambda(t) = \int_0^\pi \lambda(t,\theta)d\theta = C(t) \int_0^\pi V(\theta,t) \alpha(\theta)d\theta
$$

If we assume that the distribution of air flow past the surface is independent of time then we obtain the following expression:

$$
\lambda(t) = \eta C(t)
$$

where $\eta$ is constant. Thus the assumptions we have made imply that the deposition rate is directly proportional to the concentration of viable particles in the environment. This is the assumption made in other bioburden models (3).

The final factor, the removal fraction rate, will be assumed to be constant in time. This factor is intended to include not only physical removal but death as well. Obviously it would not be a constant over time and would also be very highly dependent upon the population of organisms involved. In our case, the constant value will be assumed for ease of our demonstration of the applicability of the model. This would be one area (as discussed in reference 9) which could require more sophisticated methods. For the purpose of this paper the assumption of $\mu$ being constant actually removes several degrees of freedom from the
model. Thus, if the model agrees reasonably well with observations under these assumptions, it would perform better if \( \mu \) was allowed to assume other functional forms.

If the surface under consideration is sterile at the time it is placed in the environment \((t = 0)\), then the expected number of viable particles on the surface is given by reference 10 as

\[
M(t) = e^{-\int_0^t \lambda(t)dt} \int_0^t \lambda(t)e^{-\int_0^t \lambda(t)dt}dt
\]

(3)

where \( M(t) \) is the expected number of organisms per square inch of surface at time \( t \) and \( \mu \) is the fraction of particles on the surface which is removed per unit time. Substituting equation 2 into 3 we obtain the following:

\[
M(t) = e^{-\mu t} \left[ \int_0^t \lambda(t)C(t)e^{\mu t}dt \right]
\]

(4)

Applying the model in the manner discussed in reference 10, we will determine \( \mu \) and \( \eta \) so that we minimize the following function:

\[
R(\mu, \eta) = \sum_{i=1}^{n}(y_i - M(t_i))^2
\]

The values \( \hat{\mu} \) and \( \hat{\eta} \) which we obtain are the maximum likelihood estimates of the true \( \mu \) and \( \eta \). In reference 10 it is also shown that the variance, \( V(t) \), of the density of viable particles on the surface at time \( t \) is related to the mean by the equation \( V(t) = M(t) \).

Thus the 95% confidence interval is given by:

\[
\left[ M(t) - 2.571 \sqrt{\frac{M(t)}{6}}, M(t) + 2.571 \sqrt{\frac{M(t)}{6}} \right]
\]

Figures 1 and 2 show a close nonlinear correlation between volumetric air sampling and airborne fallout on surfaces. All SS strip data points from both areas are encompassed by the 95% confidence limits determined by the mathematical model. The mean standard error of estimate for the MSOB and laboratory area were 2.243 and 2.2743, respectively, establishing an overall standard error estimate of 2.493. The removal fraction (\( \mu \)) is near for each of the sets. This produces the plateau effect observed by Favero et al (2). The deposition proportionality constant (\( \eta \)) is dependent upon the personnel activity in the area under study and the biological control exhibited by the air handling system. Thus it varies drastically from one area to another and from one period of time to another. The fluctuations are brought about by the fluctuation in the volumetric counts. This agrees with the observed data.

The above analysis indicates that the use of a mathematical model to predict surface loadings is reasonable under conditions commonly encountered when evaluating the microbial load on spacecraft surfaces. Application of a mathematical model is made possible for estimating total microorganisms on surfaces only by knowing the numbers of viable cells per viable particle, the effects of surface angles on particle depositions, and the airborne microbiological loadings in the vicinity of the surfaces. The application of the model illustrated in this paper is based on limiting assumptions. The model upon which this demonstration is based, however, allows for a generalization of this overall approach. In our case, alternate handling and cleaning of most spacecraft surfaces, for instance, renders the assumptions which we have made invalid, and thus the mathematical model cannot be used in the form presented here for direct assessment of total microbial loads.

Estimation of microbial load on the fragile surfaces using volumetric sampling along with swab-rinse sampling of noncritical surfaces allows a more complete evaluation of the total microbial load on a spacecraft. Other possible uses of this or similar mathematical models would be, for example, in quality control of pharmaceuticals, optical equipment, or sterile laboratory supplies, where handling is kept to a minimum and environmental conditions are carefully controlled.

In applying this or similar models to the estimation of microbial loads on a given surface in a specific environment, the following steps would need to be performed. (i) Test strips composed of the same materials as the surfaces to be used would need to be placed in the environment for a period of several days. Pieces of this test strip would be removed at various time intervals while volumetric samples were taken from the environment through the use of sampling equipment. (ii) Microbiological assessments would then be performed on the numbers of organisms and numbers of viable particles on the test strips as well as the numbers of viable particles in the areas immediately surrounding the surfaces. (iii) Using least-squares techniques, the parameters involved in the model such as deposition and removal rate constants would be estimated. (iv) The number of microorganisms per viable particle are determined using techniques outlined in this paper. (v) A test of the overall procedures should be performed, making use of the constants and volumetric samples to predict the
microbiological readings on test strips. The test strips could then be analyzed to determine the degree of agreement of the model and real world observations.

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