# III. Environmental Requirements

PROJECT ENGINEERING DIVISION

## A. The Effect of Low Numbers of Microorganisms on Samples Assayed by the NASA Standard Procedure, *E. J. Sherry*

### 1. Introduction

The NASA standard procedure (NSP) (Ref. 1) used to assay a sample for viable bacteria makes two related assumptions. The first is that there is a large number of bacteria (100 or more) present in a sample so that extrapolated counts from serial dilutions are statistically meaningful. This is related to the second assumption, namely, that microorganisms are uniformly distributed in the recovery medium (i.e., each bacteria has an equal probability of being in any position of the medium). These assumptions allow one to interpret an assay procedure as an "occupancy problem[1]" and to extrapolate from the plate counts to the actual number of organisms present on the object sampled. Given large numbers of bacteria, the samples can even be broken into parts, assayed for different types of microorganisms, and the results stated with a certain confidence. However, when the number of organisms is low, a question arises as to the accuracy of the extrapolation procedure.

This condition, i.e., low numbers of microorganisms present per sample, was found in the samples taken during the *Mariner* Venus 67 sampling program (SPS 37-46, Vol. IV, pp. 48–55) and when samples were taken in a class 100 clean room.[2] If one then considers the assembly of a spacecraft in a class 100 clean tent (as is the case with the *Mariner* Mars 1969 spacecraft), one should expect low numbers of organisms per sample. This article discusses the accuracy of the extrapolation with two different assay and recovery techniques when the assumption of the presence of large numbers of organisms is negated.

### 2. Techniques

Data from the capsule mechanical training model (CMTM) assemblies (SPS 37-52, Vol. I, pp. 31–34) directed by the sterilization assembly development laboratory (SADL) team were used to form a data base for the statistical analysis (SPS 37-50, Vol. III, pp. 71–74). In

[1]That is, the plates are rooms and the microorganisms are guests, each with an equal probability of choosing any room. A guest finding his room means that a microorganism is plated out and incubated in an environment that will be suitable for it to grow. A guest not finding his room will mean that either a microorganism is plated out and incubated in an unsuitable environment, or not plated out at all.

[2]Paik, W. W., *et al*, The Effects of Disrupted Laminar Air Flow on the Microbial Burden of Assembled Hardware, JPL Technical Report (in preparation).

particular, the results form the first five assemblies in the CMTM Program (more than 2,300 assays were used).[3] Of the five assemblies, three were in a high-bay area and two were in a laminar down-flow tent[4] (designated as 201, 202, 203, and T01, T02, respectively, in Tables 1 and 2).

[3]The author wishes to thank G. H. Redmann and D. M. Taylor for permission to use the CMTM data prior to its publication.

[4]The high bay area was a class 100 K room, and the tent a class 100 room, as defined by Federal Standard 209a.

**Table 1. Percentage of CMTM assays showing no viable microorganisms**

| Assembly | Total number of assays | | Assays showing zero count, % | |
|---|---|---|---|---|
| | NSP | SP | NSP | SP |
| **Coupon assay technique** | | | | |
| 201 | 83 | 342 | 65 | 59 |
| 202 | 83 | 334 | 83 | 72 |
| 203 | 95 | 365 | 79 | 79 |
| T01 | 89 | 357 | 73 | 63 |
| T02 | 91 | 362 | 79 | 81 |
| Average | 88 | 352 | 76 | 71 |
| **Swab assay technique** | | | | |
| 201 | — | — | — | — |
| 202 | 42 | 152 | 71 | 61 |
| 203 | 29 | 120 | 69 | 66 |
| T01 | 21 | 102 | 57 | 50 |
| T02 | 34 | 133 | 61 | 66 |
| Average | 32 | 127 | 64 | 60 |

**Table 2. Percentage of assays showing no aerobic heat-shocked spores**

| Assembly | Total number of assays | | Assays showing zero count, % | |
|---|---|---|---|---|
| | NSP | SP | NSP | SP |
| **Coupon assay technique** | | | | |
| 201 | 83 | 342 | 95 | 92 |
| 202 | 83 | 332 | 99 | 97 |
| 203 | 95 | 364 | 98 | 97 |
| T01 | 89 | 357 | 98 | 93 |
| T02 | 91 | 355 | 99 | 99 |
| Average | 88 | 350 | 98 | 96 |
| **Swab assay technique** | | | | |
| 201 | — | — | — | — |
| 202 | 42 | 151 | 95 | 87 |
| 203 | 29 | 120 | 97 | 92 |
| T01 | 21 | 103 | 76 | 83 |
| T02 | 34 | 134 | 91 | 92 |
| Average | 32 | 127 | 90 | 88 |

The samples, coupons (polished stainless steel, 1×2 in.) and swabs (cross stroking a 2×2-in. area), were taken at random, according to a predetermined sampling plan (Ref. 2), and assayed according to either the NSP or SADL procedure (SP) (Ref. 3). The basic difference between the two procedures is that the SP incubates the plates for only aerobic bacteria while the NSP incubates for both aerobic and anaerobic bacteria. Counts were obtained and recorded after 72 hr. Generally, 450 coupons and 150 swabs were used per assembly with 80% of all samples assayed according to the SP and 20% according to the NSP.

### 3. Analysis

The large percentage of zero counts found in the assays from each of the five assemblies made it necessary to use a nonparametric test (the Kolmogorov–Smirnov test) to attempt to compare data. Initially, all the samples of a particular type (swab or coupon) were compared by type and day taken. The test of the null hypothesis showed that each assay type was indistinguishable in a day-to-day comparison. Each assembly was tested in this way and yielded the same conclusion. With this information, all samples of the same type from one assembly were grouped and then tested assembly by assembly. Again, it was impossible to distinguish one assembly from another. These tests then allowed the grouping of data by types from all five assemblies to form an "average" assembly.

Table 1 presents the percentage of samples that showed absolutely no viable microorganisms[5] according to type of assay (coupons and swabs), procedure used (NSP or SP), assembly number, and average assembly. Analysis of the data presented in this table by the Kolmogorov–Smirnov test proved that there was a statistically significant difference ($\alpha = 0.1$) between the average number of zeros found by the NSP on coupons and the SP on swabs. Further, there was the same statistically significant difference between the average number of zeros found on coupons and swabs by either procedure.

Table 2 presents the percentage of samples that showed no heat-shocked aerobic spore growth.[6] These data were subjected to the same analysis as that presented in

[5]That is, after a 72-hr incubation period, there was no growth recorded on any of the plates for a given sample.

[6]That is, the plates incubated for aerobic heat-shocked spores show no growth after 72 hr. In approximately 20% of these cases, the other plates associated with the sample had vegative or anaerobic heat-shocked spore growth.

Table 1 and showed the same statistically significant differences.

Table 3 is typical of the data from all of the assemblies. It shows that the SP consistently exhibited a higher recovery percentage than the NSP. In addition, Table 1 shows that the SP gave, on the average, a 5% higher recovery percentage than the NSP; Table 2 shows that the SP is, on the average, 2% better. Unfortunately, the Kolmogorov–Smirnov test was unable to prove that the differences between NSP and SP were statistically significant.

**Table 3. Cumulative distributions for assembly 203 swab assays listed by assay procedure**

| No. organisms/assay | NSP (29 assays) | SP (120 assays) |
|---|---|---|
| 0 | 68.97 | 66.67 |
| 1 | 89.66 | 77.50 |
| 2 | 93.10 | 82.50 |
| 3 | 96.55 | 84.17 |
| 4 | 100.00 | 85.83 |
| 5 | 100.00 | 88.33 |
| 6 | 100.00 | 89.17 |
| 7 | 100.00 | 90.83 |
| 8 | 100.00 | 92.50 |
| 9 | 100.00 | 92.50 |
| 10 | 100.00 | 93.33 |
| 11 | 100.00 | 93.33 |
| 12 | 100.00 | 93.33 |
| 13 | 100.00 | 93.33 |
| 14 | 100.00 | 93.33 |
| 15 | 100.00 | 94.17 |
| 16 | 100.00 | 94.17 |
| 18 | 100.00 | 94.17 |
| 19 | 100.00 | 95.00 |
| 20–100 | 100.00 | 98.33 |
| 101–200 | 100.00 | 99.17 |
| 201–300 | 100.00 | 100.00 |
| >300 | 100.00 | 100.00 |

## 4. Conclusions

The first conclusion that appears from the analysis of the data in Tables 1 and 2 is that, using either procedure, the swabs give a statistically significant higher recovery percentage than coupons. This is contrary to expectation, since one expects swabbing to be a less efficient sampling procedure than the use of coupons. One possible explanation is that swab data were taken directly from the CMTM, while the coupons may have been given preferential treatment by the assembly personnel.

The second conclusion (although not statistically significant) is that the SP gives a higher recovery percentage than the NSP in the case of all microorganisms (Table 1) as well as in the case of only aerobic heat-shocked spores (Table 2). This is clearly shown in Table 3 where the SP consistently exhibits a higher recovery percentage. It is unfortunate that this observation cannot be stated with statistical significance at this time, but it must be noted that the Kolmogorov–Smirnov test would have been capable of rejecting the null hypothesis if the same percentage held for a larger sample population.

The last conclusion leads one to question the two assumptions that allow the NSP to be interpreted as an occupancy problem since the first assumption (large numbers of microorganisms per sample) is obviously violated (Table 3), and the analysis shows the second assumption (microorganisms uniformly distributed in the recovery medium) to be invalid. Thus, the use of the NSP would give a lower estimate of the total burden than the SP. That is, an extrapolation from the samples assayed by the SP would more accurately approximate the actual microbiol burden, because the second assumption is not as sensitive to a two-fold sample partition as it is to a four-fold partition. Carrying this logic one step further, it appears that one should use an assay procedure that plates out the entire sample and incubates for only one type of organism. In this way, since the relationship between types of organisms is fairly well established, if one obtained an accurate count of one type of microorganism (e.g., aerobic spores) one could, perhaps, more accurately extrapolate to the total actual burden. Finally, it must be stated that this is a problem relevant to not only spacecraft but to any sampling program where the number of organisms per sample is low.

## References


1. *NASA Standard Procedure for the Microbiological Examination of Space Hardware*, NASA Handbook 4340.1. National Aeronautics and Space Administration, Washington, Aug. 1967.
2. *Microbiological Sampling of Spacecraft Hardware*, SADL Operations Plan 3-120. Jet Propulsion Laboratory, Pasadena, Calif., Oct. 1968.
3. *Microbiological Laboratory Assay Methods*, SADL Procedure 3-211. Jet Propulsion Laboratory, Pasadena, Calif., Oct. 1968.