Evaluation of Membrane Filter Field Monitors for Microbiological Air Sampling

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Due to area constraints encountered in assembly and testing areas of spacecraft, the membrane filter field monitor (MF) and the National Aeronautics and Space Administration-accepted Reyniers slit air sampler were compared for recovery of airborne microbial contamination. The intramural air in a microbiological laboratory area and a clean room environment used for the assembly and testing of the Apollo spacecraft was studied. A significantly higher number of microorganisms was recovered by the Reyniers sampler. A high degree of consistency between the two sampling methods was shown by a regression analysis, with a correlation coefficient of 0.93. The MF samplers detected 79% of the concentration measured by the Reyniers slit samplers. The types of microorganisms identified from both sampling methods were similar. Variables in the MF samplers, such as pore size, relative humidity, and flow rates, have been studied, but no effect was noted on recovery. The results show that the MF method could be used to estimate the number and types of microorganisms found in the air.

Engineering constraints have restricted the use of the Reyniers slit air sampler for microbiological sampling of the intramural air associated with spacecraft hardware. The large size of the sampler and the peripheral equipment needed prevent its use inside a spacecraft, i.e., Apollo Command Module, or in areas surrounding the spacecraft.

This study was conducted to test the feasibility of using membrane filter (MF) field monitors in lieu of the Reyniers air sampler (4) so that more sites in spacecraft associated environments could be sampled for airborne microbial contamination. The study was conducted over a 2-year period in two different types of environments, namely, (i) an uncontrolled laboratory area, and (ii) a class-100,000 (1) clean room in the Mannef Spacecraft Operations Building (MSOB), where Apollo spacecraft are assembled and tested.

MATERIALS AND METHODS

A manifold holding six MF field monitors (Fig. 1) was set up and run in parallel with two Reyniers slit air samplers at contiguous sites. Air was drawn through the manifold at a rate of 2 ft³/min (ca. 0.056 m³/min) for 30 min for a total of 60 ft³ (ca. 1.68 m³) of air sampled (10 ft³ [0.28 m³] per membrane filter). The two Reyniers slit samplers, each operated at an airflow rate of 1 ft³/min (ca. 0.028 m³/min) were run for 30 min, giving a total sample of 60 ft³. The membrane filters were aseptically removed from the field monitors within a laminar-flow clean bench and plated directly on Trypticase soy agar (TSA; BBL) plates. The TSA plates used in the Reyniers samplers and the plated membrane filters were incubated at 35 C and read at 24, 48, and 72 h.

RESULTS

Manipulation evaluation. The manifold was tested both physically and biologically for its ability to collect air samples. A calibrated flow meter (Fisher-Porter) was used to measure the air flow through each of the MF field monitors. The biological evaluation was determined by conducting a series of air sampling experiments and comparing the mean number of viable organisms.
particles collected on each of the MF at each of the six positions on the manifold. No significant differences were detected between the sampling positions on the manifold (Table 1). The mean number of viable particles per MF was 18.6, with a maximal deviation of ±2%.

Reyniers slit air sampler versus 0.45-μm membrane filters. The initial study with 0.45-μm MF field monitors was started in an uncontrolled laboratory area. Later, the study was moved to the MSOB to compare the two sampling methods under clean room environmental conditions. Table 2 shows the differences in the recovery of microorganisms from air between the two sampling procedures. In the laboratory, even though the Reyniers had a slightly higher mean recovery than the MF, the difference was not statistically significant. In the MSOB clean room, the recovery of microorganisms on the MF in this environment was about 60% of that on the Reyniers, a statistically significant difference.

Relative humidity and temperature were recorded throughout the experiments. In the laboratory and MSOB, the averages were 66%-76 F and 53%-72 F, respectively. The relative humidity in the clean room was 13% lower than that encountered in the laboratory. This lower relative humidity suggested that desiccation of microorganisms may have been the reason for the lower recovery rate on the membrane filter.

Isolates were identified throughout the study to determine whether a variation existed in the types of microorganisms recovered. Table 3 shows the percentage and types of microorganisms collected with each sampler. A greater percentage of the microorganisms in the Corynebacterium-Brevibacterium group was recovered by the Reyniers sampler than by the membrane field monitor. The lower relative humidity in the MSOB clean room had little effect on the survival of these non-sporforming gram-positive rods which are supposedly susceptible to drying. Recovery of these microorganisms on membrane filters showed a marked reduction in both sampling areas, indicating that the increased loss on membrane filters was probably due to desiccation and not necessarily to the effects of relative humidity. The remaining types of microorganisms recovered by both sampling methods were similar.

**MF pore size.** Two membrane filters, one of 0.45-μm pore size and one of 0.8-μm pore size, were compared for their ability to recover microorganisms from air. Two manifolds, one with 0.45-μm and the other with 0.8-μm MF field monitors, were placed 18 inches (ca. 45.72 cm) apart. Air samples were collected as described previously. The results showed that, although the mean recovery value for the 0.8-μm (0.91 viable particles per ft²) filter was greater than the value for the 0.45-μm (0.81 viable particles per ft²) filters, the difference was not statistically significant. Comparisons were made between MF having pore sizes of 0.8, 1.2, and 3.0 μm to determine optimal pore size for recovering microbial contaminants from air. Mean recovery was 1.32, 1.22, and 1.15 viable particles per ft².

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**Table 1. Biological evaluation of a membrane filter field monitor manifold**

| Position(s) on manifold | No. of samples | Mean no. of viable particles/sample |
|-------------------------|----------------|-----------------------------------|
| 1                       | 35             | 18.7                              |
| 2                       | 35             | 18.9                              |
| 3                       | 35             | 18.3                              |
| 4                       | 35             | 19.0                              |
| 5                       | 35             | 18.2                              |
| 6                       | 35             | 18.5                              |

**Table 2. Comparison of viable airborne particles collected by Reyniers air samplers and 0.45-μm MF field monitors**

| Location | No. of days sampled | Mean no. of viable particles per ft² of air |
|----------|---------------------|-------------------------------------------|
|          |                     | Reyniers sampler* | Membrane filters* |
| Laboratory | 20                 | 2.80                          | 2.55                          |
| MSOBd    | 13                 | 0.92                          | 0.58                          |

* Laboratory: T = 0.735; T0.05 = 2.093; difference is not significant. MSOB: T = 12.414; T0.05 = 2.179; difference is significant.
* Six samples per day.
* Nine filters per day.
* Manned Spacecraft Operations Building.

**Table 3. Comparison of types of microorganisms collected with the Reyniers samplers and the membrane filters**

| Microorganisms               | Organisms (%) collected at laboratory | Organisms (%) collected at MSOB |
|------------------------------|--------------------------------------|---------------------------------|
| Corynebacterium-Brevibacterium spp | 35.2                                  | 39.4                            | 16.5                            |
| Gram-positive cocci          | 42.2                                  | 41.2                            | 51.7                            | 71.3                            |
| Gram-negative rods           | 0.0                                   | 0.7                             | 0.4                             | 0.0                             |
| Gram-negative cocci          | 0.2                                   | 0.2                             | 0.0                             | 0.0                             |
| Actinomycetes                | 3.5                                   | 5.2                             | 0.0                             | 0.5                             |
| Yeasts                       | 4.9                                   | 5.2                             | 1.5                             | 2.1                             |
| Molds                       | 4.4                                   | 4.8                             | 4.3                             | 6.4                             |
| Bacillus spp.               | 9.6                                   | 18.6                            | 2.7                             | 3.2                             |

* Results of first year study.
respectively. Although no statistically significant differences in recovery among the various pore sizes were observed, the 0.8-μm MF recovered the highest number of microorganisms from air.

Reyniers slit air sampler versus 0.8-μm MF field monitors. One year after the first comparison between the Reyniers slit air sampler and the 0.45-μm MF field monitors were completed, the experiment was repeated with 0.8-μm MF. In the MSOB the results were similar to those found the previous year, but in the laboratory the difference between the two sampling methods was found to be statistically significant (Table 4). An explanation for the change may be attributed to the fact that initially the laboratory had few environmental controls and had been subjected to various environmental changes during the year. Approximately 10% of the total bacterial colonies were isolated and identified.

Distribution of the general types of microorganisms detected in the two areas by the two sampling techniques are shown in Table 5. It is evident that the distributions are similar and that the lower total count values measured with the MF samplers are not the results of failure to detect certain types of microorganisms.

**Regression analysis of data.** A total of 60 paired measurements were made in evaluating the qualitative and quantitative microbial recovery abilities of the Reyniers slit air sampler and the MF field monitors. The differences between the mean values obtained with the slit sampler and the mean values obtained with the membrane filter were tested statistically for each set of data, with the results being that the membrane filter usually recovered significantly fewer viable particles than did the slit sampler. To determine whether the difference between the two sampling methods was consistent and the MF values could be used to estimate the slit sampler values, the data were subjected to regression analysis. The data points, regression line, and equation for the regression line are presented in Fig. 2. The data demonstrated consistent relative agreement, as evidenced by the line passing very near the origin and a high correlation coefficient \( r = 0.93 \). It was found that, in general, the MF samplers detected 79% of the concentration of airborne viable particles measured by the slit samplers.

**DISCUSSION**

It is commonly assumed that the slit air sampler impacts airborne viable particles onto an agar surface without significantly altering the distribution of particle sizes and, consequently, clump sizes occurring naturally in the air. This assumption was questioned after extensive tests showed that levels detected by MF were consistently less than those detected by a slit sampler. One explanation was that large

### Table 4. Comparison of viable airborne particles collected by Reyniers air samplers and 0.8-μm MF field monitors

| Location     | No. of days sampled | Mean no. of viable particles per ft² of air | Reyniers sampler | Membrane filters |
|--------------|---------------------|--------------------------------------------|------------------|------------------|
| Laboratory   | 12                  | 2.43                                       | 1.24             |                  |
| MSOB         | 15                  | 0.60                                       | 0.36             |                  |

* Laboratory: \( T = 2.331; \ T_{0.05} = 2.074; \) difference is significant. MSOB: \( T = 5.083; \ T_{0.05} = 2.048; \) difference is significant.

* Six samples per day.

* Eighteen filters per day.

* Manned Spacecraft Operations Building.

### Table 5. Comparison of types of microorganisms collected with the Reyniers samplers and the membrane filters

| Microorganisms          | Organisms (%) collected at laboratory | Organisms (%) collected at MSOB |
|-------------------------|--------------------------------------|---------------------------------|
|                         | Reyniers | MF | Reyniers | MF |
| Corynebacterium-Brevibacterium spp. | 13.0      | 10.2 | 9.9      | 12.6 |
| Gram-positive cocci      | 52.3      | 58.5 | 78.5     | 75.7 |
| Actinomycetes            | 9.1       | 6.8  | 0.8      | 0.0  |
| Yeasts                   | 0.0       | 0.0  | 0.8      | 1.8  |
| Bacillus spp.            | 25.6      | 24.5 | 9.0      | 9.9  |

* Results of second year study.

![Fig. 2. Relationship between membrane filter and slit air sampler results with regression line and equation.](attachment:image)
particles passing through the sampler slit were fragmented into several smaller particles, resulting in an overestimation of the number of airborne viable particles. A Royco particle counting and sizing instrument was used to compare the particle size distribution of ambient air with the particle size distribution of ambient air which had passed through the slit of a Reynier sampler. These distributions (Table 6) show that air passed through a slit has a significantly higher percentage of particles in the 0.05- to 0.08-μm range and significantly lower percentages in the 3- to 6- and 6- to 25-μm ranges than ambient air. This tends to support the theory of large particles breaking into smaller particles as a result of passing through a slit.

Microorganisms that are found in the natural environment are relatively stable, with the organisms that are the most susceptible to environmental stresses having already been eliminated. Variables such as relative humidity, flow rate, and pore size had no significant effect on the recovery of microorganisms on MF; this was also noted in some of the original work done by Goetz (2).

Another factor that cannot be overlooked and needs further investigation is the physical loss of particles when the MF is being removed from the field monitors. It is known that air being forced through a cellulose-ester membrane creates a large electrostatic charge (2, 3). Observations made of the removal of MF from field monitors within a light chamber revealed the expelling of some particles from the surface of the MF upon removal from the field monitor. This may also account for the lower recovery of microorganisms from the MF than from the slit sampler.

The MF method is a very versatile means of air sampling. A field monitor using an aerosol adapter with a restricted orifice attached to a vacuum hose can be used for taking samples in restricted areas such as the interior of a spacecraft or in an operating room next to a surgical incision. A manifold may be used if an investigator is interested in a large number of samples or if he is interested in isolating certain types of microorganisms since each of the six MF from the manifold can be placed on different selective media.

It is apparent that the MF technique is applicable in the field of environmental microbiology because of its consistency in estimating the number of microorganisms in the air and its ability to detect accurately the types of microorganisms found in the environment. The small amount of equipment needed, the economy, and the ease of handling MF could make an air sampling apparatus available to every laboratory that needs to perform environmental microbiological sampling.

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| Particle size (μm) | Ambient air (%) | Ambient air through slit (%) | t        |
|-------------------|----------------|------------------------------|---------|
| 0.5–0.8           | 50.43          | 51.85                        | 3.8243* |
| 0.8–2.0           | 28.69          | 28.71                        | 0.1817  |
| 2.0–3.0           | 15.25          | 15.01                        | 1.0803  |
| 3.0–6.0           | 4.73           | 3.85                         | 5.7414* |
| 6.0–25            | 0.90           | 0.57                         | 7.4903* |

* Significant P < 0.001.