Vacuum Probe: New Approach to the Microbiological Sampling of Surfaces

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The need for a device to sample large areas that are lightly contaminated with microorganisms motivated the development of the vacuum probe. The intended use of the instrument is to sample clean surfaces in laminar clean rooms, but the device could be used for sampling surfaces in other clean environments. Such a device was designed, fabricated, and tested at Sandia Laboratories, Albuquerque, N.M. In these tests, the vacuum probe removed a mean of 69% and assayed a mean of 67% of bacterial spores, approximately 1 μm in length, settled on smooth surfaces which were free from viscous films.

The recovery of microorganisms from surfaces has been studied by microbiologists since the early part of the century. During this period, five basic methods have evolved for the microbiological examination of surfaces: the agar overlay method, the agar contact method, the swab-rinse method, the rinse method, and the agar-dip method (14). Each method has individual advantages and disadvantages, but all were designed for sampling relatively large populations of microorganisms on small to moderately sized surfaces.

The planetary quarantine requirement that space hardware landing on planets designated as biological preserves be sterilized (8) has imposed a requirement for the sampling of large surface areas with small amounts of microbial contamination.

The settling-strip method (12) has been developed and used for estimating the viable contamination deposited on surfaces. With this method, sterile stainless-steel strips are placed in the same environment as the surface; after a determined period of environmental exposure, the strips are assayed for microbial contamination. The criticisms of this method are that it is an indirect method and that it is inaccurate when the level of microbial contamination is low.

The need for a device to sample large surface areas that are lightly contaminated with microorganisms motivated the development of the vacuum probe sampler (Fig. 1). The intended use of the instrument is to sample surfaces in laminar flow clean rooms.

For evaluation of the instrument, a severe case was chosen; i.e., aerosols of single Bacillus subtilis var. niger spores settled on surfaces. In practice, however, few single spores or bacteria can be expected to settle out onto surfaces from the atmosphere. Nobel et al. (11) indicated that the majority of airborne microorganisms in laboratory environments are carried on particles 4 to 20 μm in diameter. Since the removal efficiency for particles remains consistently higher for larger particles (3, 4), this evaluation was performed with the smallest viable particles to be expected in practice.

MATERIALS AND METHODS

Physical description of vacuum probe. The vacuum probe (Fig. 2) is an instrument that utilizes airflow through an orifice to remove particles from surfaces and a membrane filter to capture these particles. When the Teflon tip (Fig. 2 detail) contacts a surface, two orifices, one on the front side of the detail and one on the back, are formed. Since Teflon has a low coefficient of friction with most materials, the tip is readily moved back and forth across surfaces for sampling. The cone is a machined and anodized aluminum-magnesium alloy casting which serves as a mechanical mount for the tip and holds an "O"-ring to seal the membrane filter to the filter backing screen. A membrane filter 2-inches in diameter with a pore size of 0.45 μm or larger must be used or the filter limits the airflow excessively. A filter pore size should be selected which is consistent with the particle size to be sampled.

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The filter backing screen is a circular piece of 100 mesh stainless-steel screen clamped between an inner and outer ring. The filter backing screen is attached to the base with an autoclavable epoxy adhesive (Shell Chemical Co.; Epon 914). The base, which is also a machined and anodized aluminum-magnesium alloy casting, contains a plenum large enough to allow an even flow of air through the filter. The clamp holds the cone to the base, providing a rigid assembly and a good pressure seal. The anodized aluminum handle is sealed to the base with the same epoxy adhesive. The hollow handle serves to connect a vacuum system to the base during sampling.

Microbiological procedures. All experimental procedures were carried out in a class 100 laminar flow clean room (6). This particular clean room (2, 15) was well suited for such studies because of its size and airflow characteristics. Clean air flowing from one wall directly over the work surface to the floor allowed manipulations of contaminated test surfaces to be accomplished with minimal chance for additional contamination from personnel. The clean room was operated at all times, except during aerosolization and settling of particles. Just prior to aerosolization of bacterial spores, the clean room air circulation was turned off and spores were disseminated. After a settling period of 30 min, during which time the surfaces were contaminated with the test organisms, airflow was turned on and subsequent operations were performed in a clean air environment.

B. subtilis var. niger spores suspended in 95% ethyl alcohol were disseminated with a DeVilbis no. 40 nebulizer into a mixing fan which provided air circulation throughout the room. The spore suspensions used were cleaned by treatment in an ultrasonic bath and by differential centrifugation and were used in concentrations of $10^4$ to $10^5$ per ml. The fan was operated for 1 min following aerosolization; then the spores were allowed to settle. By microscopic observation, the the settled aerosol was seen to contain mostly single spores. By calculation, less than 1% of the spore-containing particles contained two spores and a negligible percentage contained more than two spores (7, 10). For purposes of the experiment, the spores were free from ethyl alcohol on contact with the surface because the ethyl alcohol evaporated in a very short time compared to the settle-out time of the particles (7).

The vacuum probe was sterilized and then dried to remove any moisture. Sterile technique was followed in the installation of the membrane filter, 0.8 μm pore size, 2 inches in diameter. This pore size was suitable for filtering the spores. With the filter in place and the

FIG. 1. Assembled and disassembled vacuum probes.

FIG. 2. Cross section of vacuum probe showing airflow and a detail view of the Teflon tip.
TABLE 1. Vacuum probe sampling efficiencies for B. subtilis var. niger spores

| Surface  | Total no. of spores aerosolized | Unvacuumed half | Vacuumed half | Filter | Cone treated in ultrasonic bath | Per cent removal* | Assay  
|----------|-------------------------------|-----------------|---------------|-------|---------------------------------|-------------------|-------
|          |                               |                 |               |       |                                 |                   |       
| Aluminum | $3 \times 10^4$               | 89              | 5             | 51    | 8                              | 94                | 66    
|          |                               | 129             | 5             | 104   | 32                            | 96                | 105   
|          |                               | 149             | 13            | 76    | 27                            | 91                | 69    
|          |                               | (126)*          | (6)           | (68)  | (24)                          | (95)              | (89)  
| Stainless steel  | $3 \times 10^4$               | 1,550           | 76            | 430   | 368                           | 95                | 52    
|          |                               | 886             | 88            | 428   | 414                           | 90                | 95    
|          |                               | 1,300           | 123           | 600   | 298                           | 92                | 60    
|          |                               | 1,776           | 91            | 964   | 575                           | 95                | 87    
|          |                               | (1,428)*        | (94)          | (605) | (414)                         | (93)              | (71)  
| Stainless steel  | $3 \times 10^4$               | 1,400           | 154           | 393   | 207                           | 89                | 43    
|          |                               | 1,340           | 120           | 262   | 161                           | 91                | 32    
|          |                               | 1,600           | 145           | 516   | 414                           | 91                | 58    
|          |                               | 808             | 100           | 250   | 460                           | 88                | 88    
|          |                               | (1,287)*        | (130)         | (355) | (311)                         | (90)              | (52)  
| Plastic   | $3 \times 10^4$               | 17              | 5             | 7     | 9                             | 71                | 94    
|          |                               | 40              | 4             | 5     | 7                             | 90                | 30    
|          |                               | 34              | 5             | 2     | 9                             | 85                | 32    
|          |                               | 28              | 5             | 6     | 16                            | 82                | 79    
|          |                               | (30)*           | (5)           | (5)   | (10)                          | (84)              | (50)  
| Glass     | $3 \times 10^4$               | 828             | 130           | 245   | 138                           | 84                | 46    
|          |                               | 1,010           | 95            | 189   | 104                           | 91                | 29    
|          |                               | 800             | 155           | 231   | 161                           | 81                | 49    
|          |                               | (879)           | (127)         | (222) | (134)                         | (86)              | (41)  

* Per cent removal = (number on unvacuumed half of surface — number on vacuumed half of surface)/
  (number on unvacuumed half of surface) × 100%.

* Per cent total = (number of filter + number on cone)/(number on unvacuumed half of surface) × 100%.

* Per cent filter = (number of filter)/(number on unvacuumed half of surface) × 100%.

* Per cent cone = (number on cone and tip)/(number on unvacuumed half of surface) × 100%.

* The numbers in parentheses represent averages.

The probe was tested for efficiency of removal of spores from four different types of surfaces: (i) glass dishes, 4 by 8.5 inches (10.16 by 21.59 cm), with a surface roughness height of 2.0 × 10^-4 inches; (ii) disposable plastic petri dishes (20 by 150 mm) with a surface roughness height of 5.5 × 10^-4 inches; a 4-inch (10.16 cm) square at the center of the plate was sampled; (iii) aluminum plates (4 by 8.5 inches; 10.16 by 21.59 cm) with a surface roughness height of 14.5 × 10^-4 inches; (iv) stainless steel plates (4 by 8.5 inches; 10.16 by 21.59 cm) with a surface roughness height of 15.0 × 10^-4 inches. Except for the plastic dishes, half of each surface was vacuumed with the other half
serving as a control. Plastic dishes were vacuumed while adjacent dishes served as controls.

Surface contamination was assayed by overlaying the surface with sterile Trypticase Soy Agar (TSA; BBL), incubating at 32°C for 48 to 72 hr, and counting the colonies. To determine whether the agar overlay procedure would move particles on the test surfaces, the sampled half of each surface was first crosshatched with molten TSA with a Cornwall syringe. After the TSA in the crosshatched portion had solidified, the entire surface was overlaid with more TSA. This cross-hatching procedure prevented the possibility of washing viable particles from the sampled half to the control half of the test surface or vice versa. However, overlaying directly with agar had no noticeable effect on the spore distribution.

The metal surfaces were placed in sterile pans for vacuuming and for assay. After these pans were overlaid with TSA, they were left uncovered within the unoccupied, operating laminar flow clean room for approximately 1 hr to allow the surface of the TSA to dry. The pans were then covered with Saran Wrap and incubated at 32°C for 48 to 72 hr.

The cone and membrane filter were removed with sterile techniques. Spores collected on the membrane filter were assayed by overlaying the filter with TSA in a sterile petri dish, incubating at 32°C for 48 to 72 hr, and counting the colonies which developed.

The entire probe tip and cone were treated in an ultrasonic bath (N. L. Peterson, personal communication). This treatment was performed for 8 min in sterile 0.1% Tween 80, and the treated fluid was plated with TSA and incubated at 32°C for 48 to 72 hr. Later experiments, performed by vacuuming 5-μm fluorescent particles and examining the interior of the vacuum probe with ultraviolet light, showed considerable particle deposition on the inside surface of the cone and on the Teflon tip. These experiments showed that the cone and tip would have to be treated in an ultrasonic bath to raise total assay percentages.

In testing for per cent assay as a function of per cent of critical flow (Fig. 3), an aerosol of $3 \times 10^4$ spores was settled on aluminum surfaces. The flow rate of air was varied with a flowmeter.

### RESULTS

Table 1 shows sampling efficiencies on four different types of surfaces exposed to different levels of contamination. Particle removal efficiencies were consistently in excess of 80%, with a mean removal efficiency of 89%. Removal efficiencies appeared to be independent of surface contamination densities studied. Total assay efficiencies were much more variable, but achieved a mean of 67% with a mean loss of 26% of those spores removed. Table 1, as well as the fluorescent particle studies, shows that there was considerable deposition of particles on the inside surface of the cone and on the tip.

Figure 3 shows the decrease of total assay efficiency when the vacuum probe was operated with subcritical airflow rates at the tip.

### DISCUSSION

As the test surfaces had levels of microbial contamination that varied between test surface and control surface, spore recovery of over 100% sometimes occurred. For the same reason, removal efficiencies of greater than 100% were encountered; consequently, percentages of over 100 should not be interpreted as incorrect data, but are within the bounds of statistical variation.

Spores or other particles measuring about 1 μm are difficult to disturb on a smooth surface because the boundary layer of air near the surface is difficult to move, and the small particles lie in the lower regions of this boundary. The force of adhesion between small particles and smooth surfaces is large compared to the normal aerodynamic forces encountered (3). The air entering the orifice when the tip touches a surface disturbs this boundary layer. The spores are dislodged and enter the moving airstream. The spores are then caught by a membrane filter.

A number of factors affect the removal and assay percentages in removing spores from different surfaces.

The most important criterion for high percentage removal and assay is that the two orifices in operation be at a critical flow rate. At critical flow, a shock wave is generated at the orifices. The air at the shock wave is very turbulent and tends to remove small particles effectively. The shock wave is generated when the ratio of the pressure inside the cone to atmospheric pressure...
is less than or equal to 0.528 (13). All calibrations should be made with the filter in place. These values can be measured directly with a mercury manometer having a cone with a pressure fitting in the side. Figure 3 shows that critical flow must be reached to achieve high removal and assay percentages.

One anticipated problem was that particles were being overlaid with Teflon from the tip during the sampling procedure. Studies with fluorescent powders 5 μm in diameter indicated that very few particles were overlaid with Teflon. The fluorescent particles were placed on an aluminum surface and vacuumed under ultraviolet light. Traces of the powder showed where the Teflon tip had passed, but the quantity was very small compared to the original loading. To further support these results, the experiment was repeated with a probe with a metal tip to eliminate Teflon overlay. Removal percentages did not change significantly.

Another factor affecting removal percentages is particle size. Experimentation showed that the larger particles were easier to remove, so B. subtilis var. niger spores approximately 1 μm in length were selected as the most difficult particle to remove (3, 4).

At the present time, very little work has been done here with spores overlaid with thin films such as oily handprints. Limited experimentation suggested that these particles were more difficult to remove (3, 4).

Electrostatic charges affected some experiments, especially those on glass and plastic surfaces, but the effects were not measured quantitatively. In some instances, very strong fields, strong enough to push the membrane filter from the bottom to the top of the petri dish in which it was lying before overlay, were generated on the filters and petri dishes. In general, aerosols generated by blasting particles with air have a relatively high density charge distribution (9); therefore, part of the variation in assay percentages is possibly a result of electrostatic forces interacting along with aerodynamic forces. The electrostatic forces may explain the lower assay percentages on plastic.

Three other effects of interest are relative humidity, surface roughness, and the natural adhesiveness between particular pairs of materials. Although all of this work was done at 45% relative humidity, Corn (3) reported that particle adhesiveness generally increases as humidity increases. The effect of surface roughness was poorly defined in our experiments; however, Corn (3) indicated that adhesion decreases as surface roughness increases. The surface roughness heights (1) of the surfaces used are given. Adhesive forces vary considerably for different pairs of materials (3).

The vacuum probe has been modified to provide a clean air sheath to allow the device to be used to sample surfaces in environments other than those provided by laminar flow rooms. A method is also being developed to impinge the particles directly onto agar nutrient rather than onto a filter which is then plated in agar nutrient.

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LITERATURE CITED