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Microbiological Evaluation of the Vacuum Probe Surface Sampler

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The development of the vacuum probe, a new device for surface sampling, was recently reported. The original technique was slightly modified and a microbiological evaluation was conducted. The probe proved to be an effective sampling device, removing 98% and recovering 88% of surface contaminants resulting from the accumulation of airborne microorganisms. The probe was decidedly less effective in removing and recovering handling contamination than fallout contamination. There was also evidence that certain microorganisms could not survive prolonged exposure to airflow in the probe. However, the vacuum probe procedure recovered twice as many microorganisms as did the swab-rinse technique when compared directly.

The development of a new approach to microbiological sampling of surfaces was recently reported by Whitfield et al. (4). The key to this approach was an instrument described as a vacuum probe, used to remove microbial contamination from a surface and capture the microorganisms on a membrane filter. The filter was overlaid with a nutrient medium and incubated, and bacterial colonies were counted. The demonstrated ability of the vacuum probe to sample large, lightly contaminated surfaces in laminar-flow clean rooms suggested several useful applications. In particular, this approach and instrument offered an alternative method to the swab-rinse procedure currently used to assess microbial contamination on surfaces of spacecraft subject to planetary and lunar quarantine requirements (2).

A number of vacuum probes were obtained on loan from Sandia Laboratories, Albuquerque, N. M., for purposes of experimentation and evaluation. Instructions in the proper use of the probe were provided by the developers of the instrument. A series of experiments was designed and conducted to assess the vacuum probe technique in four areas of performance: (i) effectiveness in removing and recovering microbial fallout contamination from surfaces; (ii) effectiveness in removing microorganisms from surfaces contaminated by handling; (iii) effect of prolonged passage of air through the probe and membrane filter on the survival of microorganisms removed by the probe; and (iv) comparison with the swab-rinse procedure for recovery of naturally occurring microorganisms on surfaces.

MATERIALS AND METHODS

Modification of procedures. Preliminary tests with the original model of the probe indicated that a significant portion of viable particles removed from a surface was retained in the probe tip and cone and never reached the membrane filter. To permit recovery of these trapped microorganisms, the Teflon tip was shortened from its original length of several inches to approximately 0.5 inch (Fig. 1 and 2), allowing the entire cone and tip assembly to be submerged in a 400-ml beaker containing 250 ml of sterile rinse fluid [0.02% polyoxyethylene sorbitan monooleate (certified nontoxic Tween 80[2]; Hill Top Research, Inc., Miamiville, Ohio) in distilled water]. Insonation (3) (exposure to ultrasonic energy in a fluid) of the beaker removed the microorganisms from the cone and suspended them in the rinse fluid where assay was possible. It also was determined that many of the microorganisms captured on the filter occurred in clumps. After growth on the filter, each clump rather than each cell produced a countable colony resulting in an underestimation of the number of microorganisms present. The original procedure was modified to include insonation of the membrane filter in the beaker with the cone and tip assembly rather than overlaying the filter with a nutrient medium. This procedure removed and broke up clumps of microorganisms from the filter. These two modifications assured that virtually all microorganisms entering the probe would ultimately be suspended in the rinse fluid in consistently small colony-forming units.

Fallout contamination. To evaluate the effectiveness of the probe in removing and recovering microbial fallout contamination from surfaces, a series of experi-

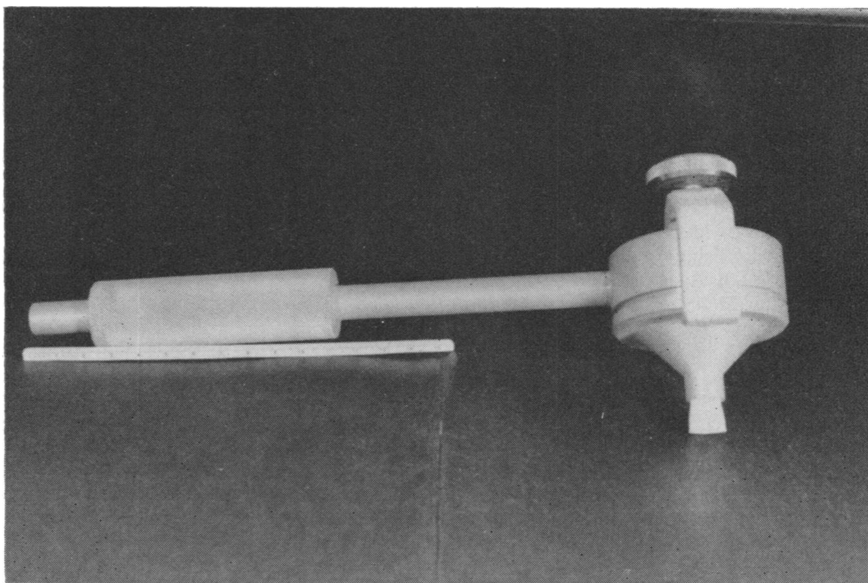

Fig. 1. *Assembled vacuum probe.*

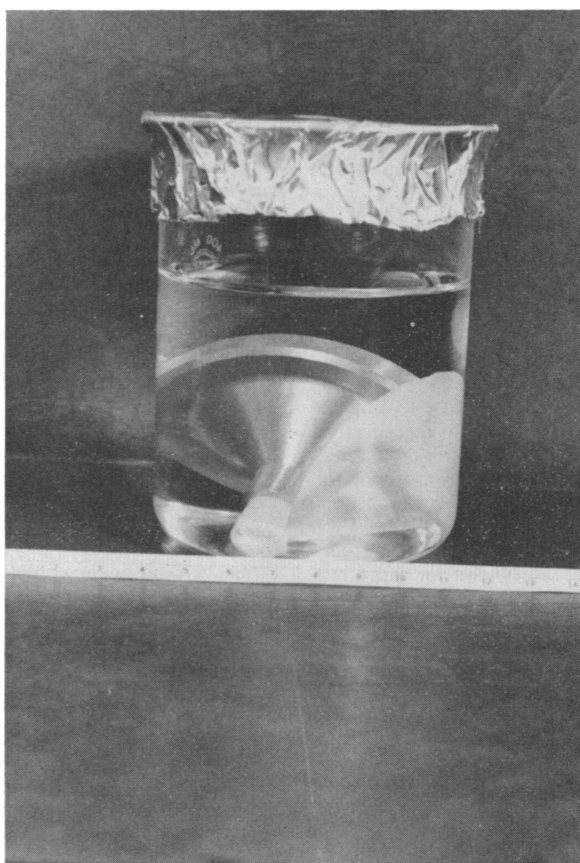

Fig. 2. *Cone and tip assembly immersed in rinse fluid.*

ments utilizing three types of surface contamination was conducted. The experiments consisted of exposing sterile stainless steel strips (1 by 2 in) to airborne microorganisms which subsequently accumulated on the surfaces. This type of contamination was termed "fallout contamination." The exposed strips were divided into two equal groups; one group served as a control to determine the mean number of microorganisms on each strip. These strips were assayed by a standard procedure (2) involving 2 min of insonation in sterile rinse fluid and subsequent plating of the fluid in Trypticase Soy Agar (TSA; BBL). Colony counts were made after 24, 48, and 72 hr of incubation at 32 C. Each strip in the remaining group was vacuumed by passing the probe tip over the entire contaminated surface three times. A sterile probe and filter were used for each strip. Preliminary tests had demonstrated that only certain types of membrane filters withstood insonation without disintegrating; therefore, Gelman Alpha 6 filters (Gelman Instrument Co., Ann Arbor, Mich.) were used in all experiments reported here. All vacuuming was done in a laminar-flow clean bench to avoid sampling of airborne contamination (1). The probe cone and tip, along with the filter, were insonated for 2 min in sterile rinse fluid, and portions were plated with TSA. Colony counts were made after 24, 48, and 72 hr of incubation at 32 C. To enumerate microorganisms not removed by the probe, the vacuumed strip was placed in a sterile petri plate and overlaid with molten (45 C) TSA. Counts of colonies growing on the strips were made after 24, 48, and 72 hr of incubation at 32 C.

In one set of experiments, surface contamination consisted of naturally occurring airborne microorganisms which accumulated on test surfaces. A second set utilized fallout contamination from an artificially generated aerosol of 10-μm aluminum oxide particles seeded with spores of *Bacillus subtilis* var. *niger*. The third set employed fallout contamination from an artificially generated aerosol of soil particles which had passed a 125-μm screen and were contaminated with microorganisms occurring naturally in the soil.

Handling contamination. To evaluate the effectiveness of the probe in removing "handling contamination," 40 sterile stainless steel strips were contaminated by having one side of each strip touched by four finger tips from each hand of an individual. Each strip was touched by only one individual. The contaminated surface was vacuumed and an assay performed to determine the number of microorganisms removed. The strip was then subjected to the standard strip assay procedure to determine the number of microorganisms remaining on the surface. The number of microorganisms obtained by summing the results of these two assays was considered to be the total number on the strip. The number recovered by the probe, expressed as a percentage of the total number on the strip, represented the effectiveness of the probe as a sampling device.

Prolonged sampling. To explore possible differences between different bacterial types in their ability to survive exposure to airflow on a membrane filter, inocula of cells of *Staphylococcus aureus*, *Streptococcus faecium*, *Escherichia coli*, and *Pseudomonas alcaligenes* were prepared by growing each culture on TSA for 24 hr, harvesting, washing, insonating, and diluting the suspension in sterile, buffered, distilled water (2) to the desired concentration. Portions (1-ml) of each suspension were plated in quintuplicate with TSA. Each of 10 portions (1-ml) was dispersed in approximately 10 ml of sterile distilled water and drawn through membrane filters. The 10 filters were air-dried in a laminar-flow clean bench for 20 min, at which time five filters were placed in individual filter holders in a laminar-flow clean bench and air was drawn through these filters for 10 min at a velocity comparable to that in the vacuum probe. This simulated prolonged use of the probe without the deposition of additional microorganisms on the filter. At the end of this airflow exposure the five exposed and the five air-dried filters were placed on TSA and incubated along with the pour plates at 32 C for 48 hr. Colony counts were made and the mean value for each set of five plates was calculated. The experiment was repeated at least once for each organism.

A second experiment utilized 28 stainless steel strips contaminated with naturally occurring airborne microorganisms which had accumulated on the strips during a period of 1 week. Fourteen strips were vacuumed in the usual manner which required each probe to be run for less than 1 min before assaying. After each of the remaining 14 strips was vacuumed, the probe was allowed to run in a laminar flow clean bench for 10 min before assaying to simulate prolonged sampling.

Vacuum probe versus swab-rinse. To compare the vacuum probe and swab-rinse techniques for recovering microbial contamination from surfaces, 62 sampling sites on a variety of horizontal surfaces throughout the laboratory were selected. The vacuum probe was designed for use in clean environments where the level of microbial contamination in the air is negligible and does not contribute significantly to samples collected from surfaces. The sampling sites selected for this portion of the evaluation were not in a clean air environment and it was recognized that airborne contaminants would be collected along with the surface sample. However, preliminary experiments showed that the magnitude of this error was less than 1%. At each site, an area (4 inches^2) was swabbed in accordance with the swab-rinse procedure and a contiguous area (4 inches^2) was sampled with the vacuum probe. A ratio of the contamination level as detected by the probe to the contamination level detected by the swab was calculated for each site.

RESULTS AND DISCUSSION

A summary of the results of the experiments with fallout contamination is presented in Table 1. Although no explanation can be offered for the differences in recovery rates for the three sets of experiments, all rates were considered relatively high compared with those achieved with other sampling procedures currently in use. The relatively good agreement between the coefficients of

TABLE 1. *Comparison of the vacuum-probe procedure with the standara strip-assay procedure in recovering microbial contamination from surfaces of stainless steel strips*

Type of contamination	No. of strips assayed by each method	Vacuum probe		Standard strip assay		Probe recovery rate[a]
		Mean no. of microorganisms recovered	Coefficient of variation	Mean no. of microorganisms recovered	Coefficient of variation	
			%		%	%
Naturally occurring microorganisms in air (fallout)	40	320	59	335	57	96
B. subtilis var. *niger* spores on aluminum oxide dust (simulated fallout)	106	283	20	325	18	87
Naturally occurring microorganisms on soil dust (simulated fallout)	23	82	62	102	51	80

[a] Mean number of microorganisms recovered with the vacuum probe procedure expressed as a percentage of the number recovered with the standard strip assay.

TABLE 2. *Survival of various types of bacterial populations exposed to filtration, drying, and airflow*

Microorganism	No. of tests	Per cent surviving filtration and drying	Per cent surviving airflow[a]
Streptococcus faecium	2	75	100
Staphylococcus aureus	3	67	84
Pseudomonas alcaligenes	2	22	0
Escherichia coli	2	13	0

[a] Mean bacterial count surviving each procedure expressed as a percentage of the population exposed to that procedure.

variation for the probe and the standard procedure in each set suggested that the probe procedure accurately reflected the inherent variation in the deposition of fallout contamination. The ability of the probe to remove fallout contamination from the stainless steel strips was consistently high in all experiments, averaging 98% with a standard deviation of 2%. The removal of handling contamination from stainless steel strips was both markedly lower and more variable, averaging 55% with a standard deviation of 27%. Because of the great variability inherent in the deposition of handling contamination, no attempt was made to assess the effectiveness of recovery of such contamination with the probe. It was evident, however, that the overall effectiveness of recovery could not exceed the 55% effectiveness of removal and therefore would not compare favorably with the values ranging from 80 to 96% as determined for fallout contamination.

It was apparent from the results of the experiments in which specific types of bacteria were exposed to simulated prolonged sampling with the vacuum probe that considerable differences existed in the ability to survive filtration, drying, and subsequent exposure to airflow. Table 2 presents these data by expressing the mean bacterial count surviving each procedure as a percentage of the population exposed to that procedure. *S. aureus* and *S. faecium* survived filtration and drying moderately well, and after drying survived exposure to airflow quite well. *E. coli* and *P. alcaligenes* survived filtration and drying poorly, and were unable to survive exposure to 10 min of airflow. This finding was not unexpected, since it has been reported that in the filtration of bacterial aerosols the viability of some organisms is detrimentally affected if the bacteria remain on the filter during long sampling periods (5). However, the experiment involving naturally occurring fallout contamination revealed no significant effect from prolonged exposure to airflow. Assays of the probes and filters resulted in the recovery of a mean of 327 microorganisms from each probe that had been run 1 min and 311 microorganisms from each probe that had been run 10 min. Taken together, the results of these two experiments suggest that, although exposure to airflow can indeed be detrimental to the viability of certain microorganisms in pure culture, it does not significantly affect the viability of others which survive the drying effect in the natural environment. The deleterious effect

of airflow on bacterial spores, for instance, would appear to be negligible.

The mean value of 62 ratios of the number of microorganisms recovered with the vacuum-probe technique to the number of microorganisms recovered with the swab-rinse procedure was 3.0:1, with a standard deviation of 2.5:1. This indicated that in sampling a small area the probe recovered three times as many microorganisms as the swab, but considerable variation was experienced. Overall, the probe recovered 30,448 microorganisms from the 248 square inches sampled, compared with 14,285 microorganisms recovered by using the swab. Consequently, when sampling a relatively large area exhibiting a variety of surface contamination levels, the vacuum probe procedure might be expected to recover twice as many microorganisms as the swab-rinse technique.

The results of this evaluation supported the findings reported by Whitfield et al. (4) and have been the basis for initiation of field testing of the vacuum probe on spacecraft hardware at Cape Kennedy. Although certain limitations of this technique have been recognized, experimental use of the vacuum probe in other areas of surface sampling would seem to be justified.

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