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AN IMPROVED TECHNIQUE FOR MICROBIOLOGICAL SAMPLING OF SURFACES: AGAR SPRAY

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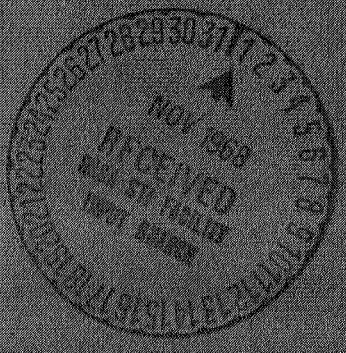

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AN IMPROVED TECHNIQUE FOR MICROBIOLOGICAL SAMPLING OF SURFACES: AGAR SPRAY *

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ABSTRACT

This report describes an improved technique for biological sampling of surfaces. While previous approaches have been limited to the removal of the microorganisms from the surfaces, the technique described herein deletes this often questionable operation and initiates growth and enumeration of the organisms in situ, i.e., without removing them from the surface. The method employs spraying molten (53°C) agar media directly onto the surface to be sampled and incubating the microorganisms thereon. A description of the spray apparatus with drawings is provided. Test results and advantages of the technique are presented.

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AN IMPROVED TECHNIQUE FOR MICROBIOLOGICAL SAMPLING OF SURFACES: AGAR SPRAY

Introduction

The International Planetary Quarantine Agreement of 1963 has resulted in increased activity and concern in being able to accurately determine the bioload of a given surface at a given time. Current techniques of assaying the bioload of a surface have been limited normally to a surface area per sample about the size of a petri dish.

One such method is Rodac* sampling which utilizes Rodac plates with solidified agar extending above the wall of the dish which are impressed against the surface being sampled. This action transfers organisms from the sample surface to the agar media where they are then incubated for enumeration. The disadvantages of this technique are: (1) the sampling surface must be smooth and flat to assure maximum transfer of the organisms from the surface to the agar media, (2) the association of the given colony on the media to the exact location on the surface is virtually impossible, and (3) the size of the sample surface is limited.

A second method is the swab-rinse utilizing sterile cotton swabs to swab the surface being assayed. Insonation of the swabs removes most of the organisms, which are then plated out in a nutrient agar for incubation and enumeration. The disadvantages to this technique are: (1) not all of the organisms are removed from the sampling surface nor from the swab, (2) not all of the surface is sampled, and (3) there are no means of associating a given colony to the exact location it occupied on the sample surface.

A third method employs the vacuum probe to remove microorganisms from the sample surface impinging them on a sterile membrane filter for subsequent incubation and enumeration. The disadvantages listed for the two methods described above are also applicable to a degree for this method.

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Analysis of the Problem

A complicated spacecraft and its individual components have a wide assortment of geometric shapes, material types, and surface irregularities. Presently there is no proven method available to reliably assay the bioload of this assortment of surfaces.

Some consideration has been given to monitoring the ambient air and applying this data with a knowledge of fine particle physics to predict the bioload on a spacecraft at any given time. However, the present state-of-the-art technology in the settling, impaction, and retention of fine particles on various surfaces under varying conditions is not sufficiently advanced to permit accurate prediction of bioloading in this manner. Experimentation is required to establish correlation between the environment and the viable particles being deposited on a given surface and to determine the optimum shapes for minimizing the settling and impaction of the microorganisms on a surface. To make these determinations with a high degree of accuracy and reliability it is necessary to be able to observe the deposition patterns of the microorganisms directly on the various surfaces of different configurations.

Considering the requirements for accurately assaying the bioload of a given surface at a given time and the techniques currently available, it can be seen that a need exists for, (1) a technique to grow microorganisms in situ to permit study and recording of the deposition patterns, and (2) a technique that permits enumerating the viable particles directly on surfaces of various shapes. The development of these techniques is the basis for the study defined in this report.

An ideal method of enumeration would be one by which the microorganisms are nurtured in-place on the surface where they are located for detection by visual means. To accomplish this, a proven method for accelerating growth is to overlay the surface with a nutrient agar medium. However, a method of application of the agar was needed that could be used for large and irregular surfaces with a minimum of displacement of the organisms on the surfaces. A spray technique was considered the most logical whereby the entire surfaces of an assembly could be coated with trypticase soy agar (TSA)* in a molten state and the assembly incubated. This technique meets the requirements that all of the microorganisms could be detected and the deposition pattern observed.

*Source – Baltimore Biological Laboratory

The agar overlay technique has been known and used for some time. A literature search reveals a number of references to agar overlay. A pour-type overlay was originally done by Hammer and Olson in 1930, and a dip-type overlay was pioneered by Barton, et al., in 1954. However, no reference was found to the use of any spray type of process for the agar overlay.

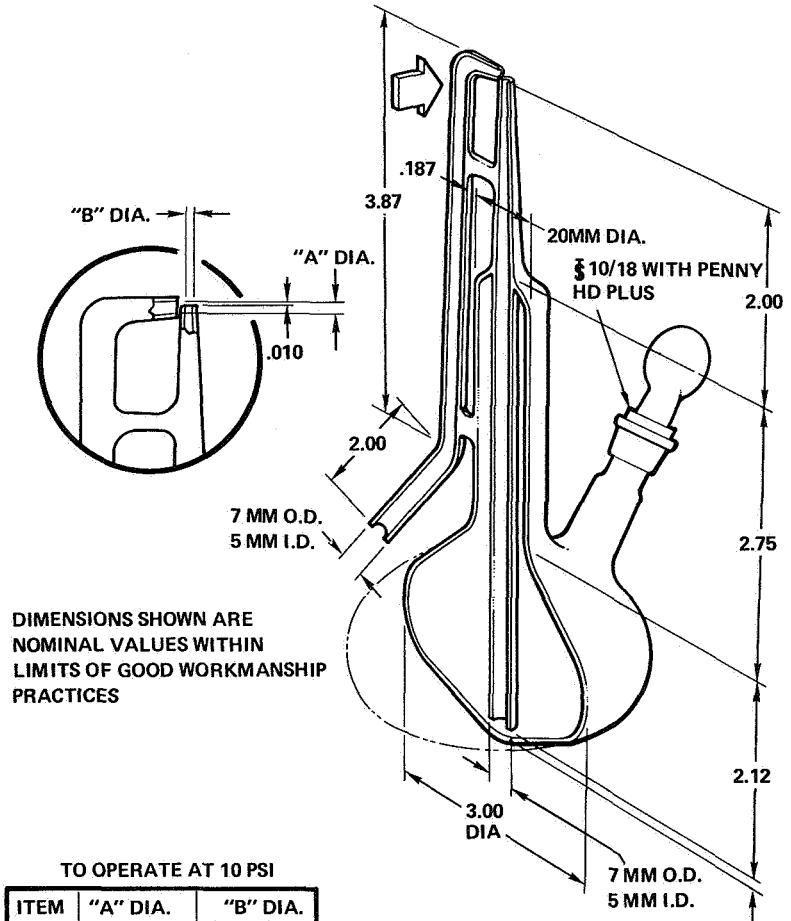
Agar Sprayer

The spray apparatus is an air-driven atomizer with a reservoir to hold a quantity of molten agar. Glass was used as the material for the sprayer to permit autoclaving the unit and to facilitate fabrication within the Sandia shops. Three sprayers were fabricated, with each having a different size air orifice and associated spray agar orifice. The three combinations of orifice sizes were considered necessary to determine what size would provide the optimum spray action. Figure 1 shows some of the pertinent details of the sprayer and the orifice sizes for the three units. In actual use it was found that there was no detectable difference in the atomizing action of the three units.

The units were designed to operate with 10 psi air pressure; however, it was noted during actual operation that air pressure as low as 5 psi provided satisfactory operation. Figure 2 shows one of the units in actual operation. The model units as fabricated may appear to have a relatively small agar reservoir; however, for the purpose of this study they were adequate. The reservoir could be designed for any reasonable size required for a specific operation. One factor which should be considered is the possibility of premature solidification of the agar in the reservoir. If this occurs, a heating mantel may be used to envelop the reservoir, to maintain the agar in a molten state. This problem did not present itself with the small units and in the manner in which they were used.

Surface Sampling Process

The experimental processes used in developing the spray technique involved sampling a variety of surfaces. Aluminum angle pieces 1-3/4 x 4 to 6 inches in length were used and some common laboratory items of various irregular shapes were selected. The majority of the sampling tests were performed by depositing spores on sterile surfaces within a clean environment (laminar air-flow room). The spores were Bacillus subtilis



TO OPERATE AT 10 PSI

ITEM	"A" DIA.	"B" DIA.
1	4 MM	2 MM
2	3 MM	1.5 MM
3	2 MM	1.0 MM

Figure 1. Agar Sprayer—Sectional View

var. niger suspended in 95% ethanol. The solution was aerosolized with a DeVilbiss* No. 40 nebulizer, and the spores were allowed to settle on the sample surfaces. Tests were also performed on some laboratory items which were contaminated with the naturally occurring microbial flora in the laboratory environment.

The agar spray operation was performed in a clean environment (laminar air-flow bench) to preclude extraneous microorganisms from being impacted on the surface during this operation. Dry nitrogen was used as a pressurized gas to drive the sprayer; however,

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the building air supply could be used provided an in-line absolute filter is employed to remove any viable particles. The TSA used was prepared in accordance with standard procedures. The TSA was maintained at 53°C while in a water bath; however, during the transfer to the sprayer, while in the sprayer, and enroute from nozzle to surface, some cooling resulted. No attempt was made to measure these temperatures as the amount of cooling did not result in any detectable solidification of the TSA prior to deposition on the surface.

The spraying process was performed by holding the sprayer 8 to 12 inches from the surface and moving the sprayer parallel to the surface as required to deposit an even layer over the entire surface. This operation was repeated to build up a thickness of approximately 1/8-inch on the surface. Short intervals between each layer were allowed for solidification of the TSA to prevent flowing on the surface. Care was exercised in applying the initial layer of TSA to minimize dislodging or relocating organisms on the surface by the spray impaction. The initial layer of agar was a light spray coating, allowing about 10 seconds for solidification and adhesion prior to subsequent spray coats. Figure 2 shows the spraying operation.

To prevent excessive drying of the TSA overlay during incubation a clear sealant coat of Krylon* was sprayed over the TSA coated surface. Alternate methods that may be used to prevent dehydration of the TSA are to seal the TSA coated surface with a plastic film such as Saran Wrap* or to maintain a high humidity in the incubator. A test was also conducted to determine if increasing the agar-agar concentration of the TSA from 1.5 to 3.0 percent would reduce the drying rate. The results of this test were inconclusive as the TSA became granular, i.e., the agar-agar precipitated out of solution.

Tests were conducted to determine what effects a coating of Krylon (a vinyl plastic material) over the agar overlay may have on the growth of Bacillus subtilis var. niger. A number of 1 x 2-inch sterile stainless steel strips were exposed to the same cloud of Bacillus subtilis var. niger spores to provide several pieces with similar bioloading. The strips were then overlaid with about 1/8 inch of TSA. One group with only the agar overlay was incubated. The second group was spray coated with Krylon over the agar and then incubated. The enumeration of the colonies indicated no noticeable difference between the two groups.

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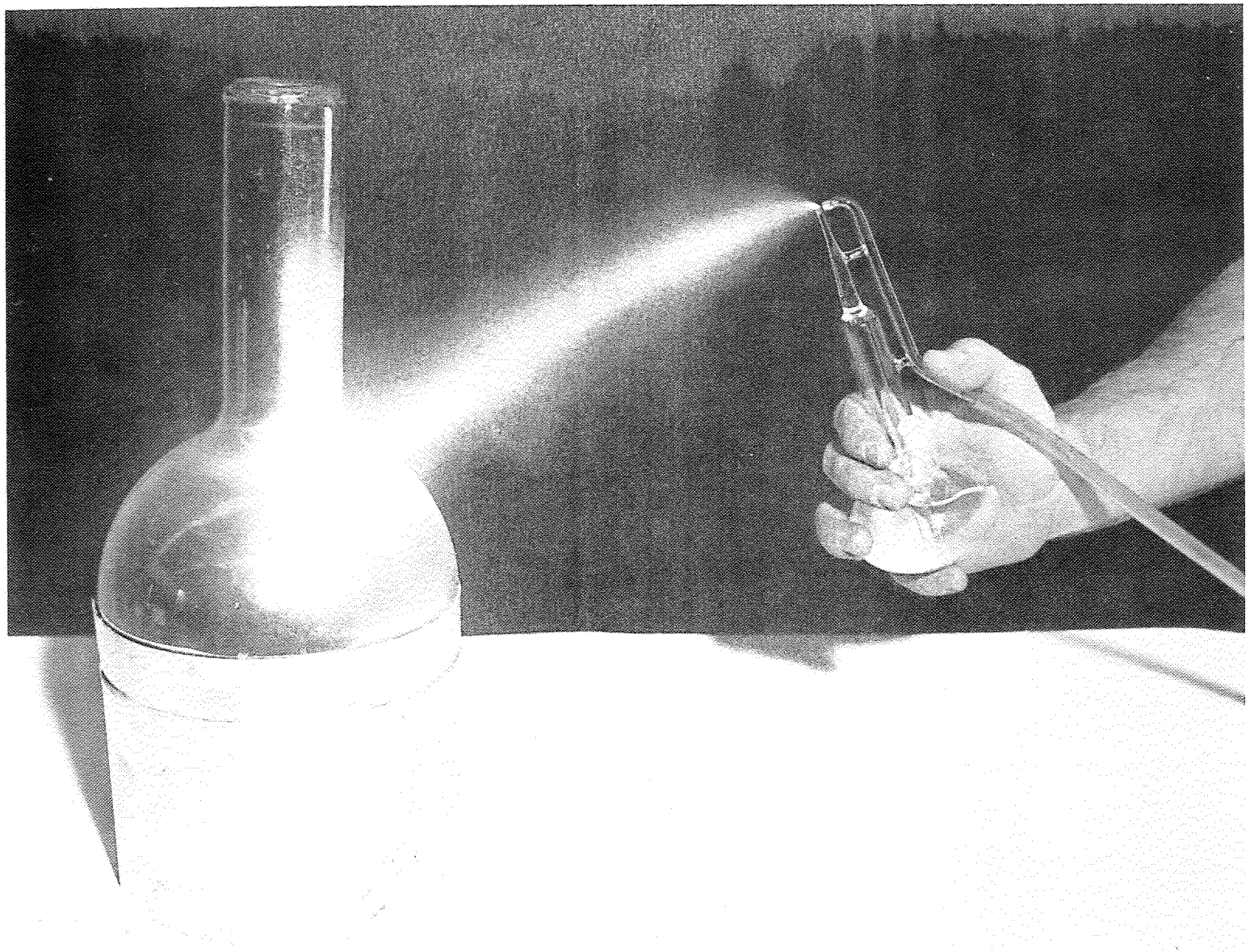

Figure 2. Agar Sprayer in Operation

Removal of the agar from the sample surfaces was accomplished by peeling off the agar and then washing the surface in a detergent water, rinsing with tap water, with a final deionized water rinse. Variations in the cleaning process may be necessary depending on the particular surfaces and objects sampled.

Results

The colonies are readily visible on the overlaid surfaces after the incubation period. The colonies can be seen in the photographs of some of the sample surfaces. Figure 3 shows colonies on the flat side of an aluminum angle. Figure 4 indicates the spore deposition pattern on an irregular surface.

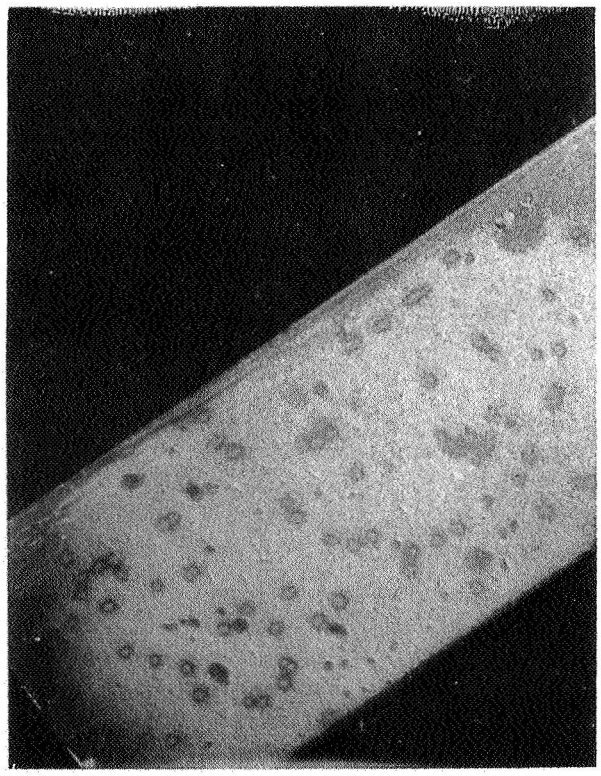

Figure 3. Bacillus Subtilis Var. Niger Colonies on a Flat Aluminum Surface

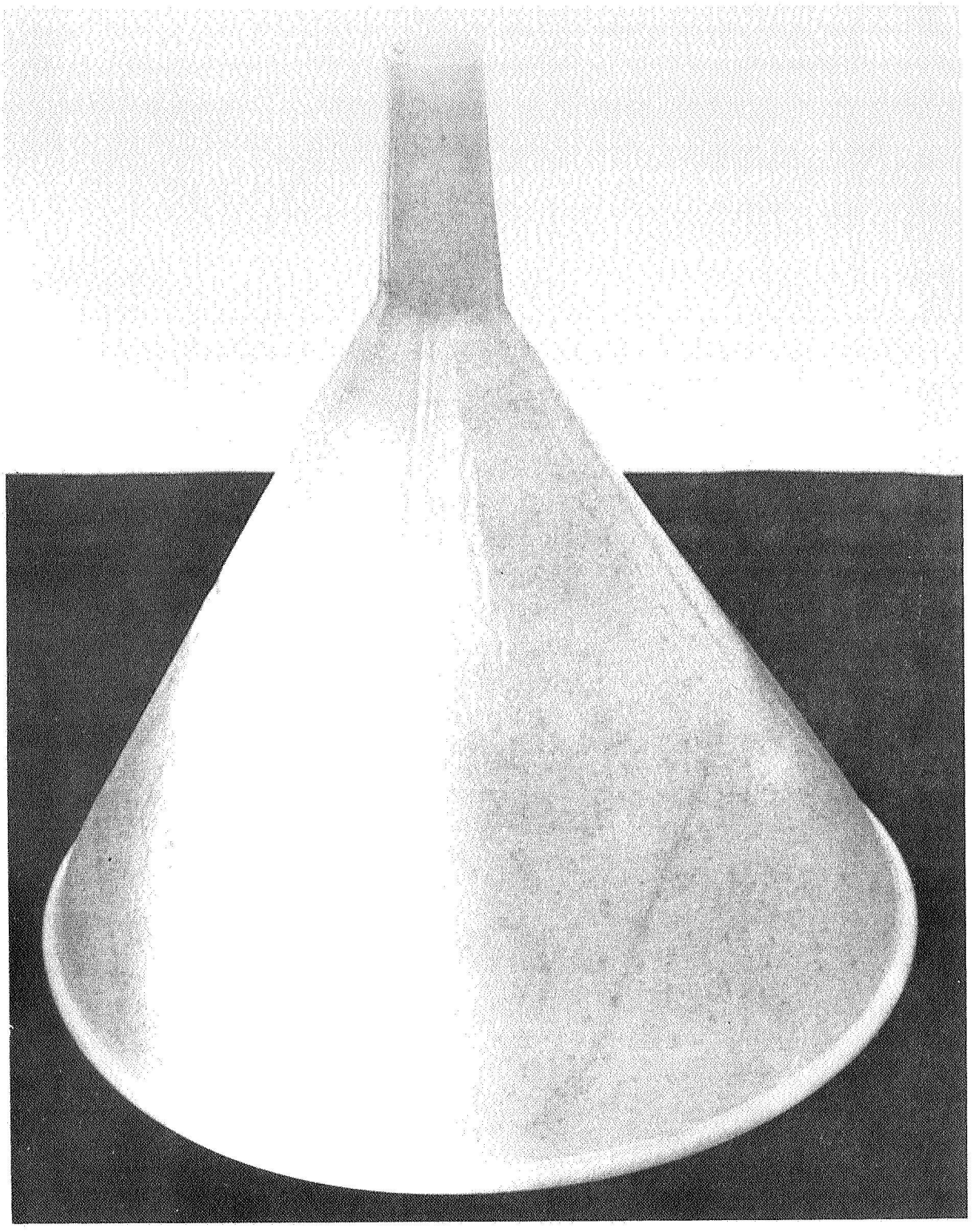

Figure 4. Bacillus Subtilis Var. Niger Colonies Indicating Spore Deposition Pattern on an Irregular Surface

Application and Limitations

This sampling process provides a means for evaluating the distribution patterns of viable particles on various types of surfaces and irregular shapes under diverse conditions. The enumeration of the bio-load of a particular area or the entire surface area of an object is possible. This process provides a valuable tool in readily obtaining reliable data in the study of fine particle physics, the evaluation of decontamination processes, and evaluating the environments to which a surface has been exposed.

The surface area or object to be sampled has no limitation as to size except as may be dictated by the clean environment facility in which the spray process is performed or the facility for the incubation. Some types of materials may not be compatible with the agar spray process. Some organisms require nutrients other than those supplied in the TSA or similar agars and would therefore not be detected. The removal of the agar and cleaning processes may present some limitations to this method.

Conclusion

The agar spray technique for biological sampling of surfaces provides a means for initiating growth and enumeration of organisms in place on the surfaces of interest. There are few limitations on the size or shape of the surfaces that may be sampled by this method. A spray apparatus was designed and fabricated for spraying the molten nutrient agar. The processes employed for spraying, incubation, and enumeration demonstrates the feasibility of this technique for obtaining reliable data in the study of fine particle physics, the evaluation of decontamination processes, and evaluating the environments to which a surface has been exposed.

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