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26 August 1959

New Technique for the Collection and Isolation of Airborne Microorganisms

Abstract. Pure cultures of airborne microorganisms are obtained by simple electrostatic attraction to small glass and plastic cylinders which are rolled over the surface of nutrient agar medium. Little equipment is required, and the necessary materials are inexpensive. Satisfactory qualitative sampling is possible under varying conditions of relative humidity.

The difficulty and expense involved in sampling microorganisms in the airborne state by conventional means has made the development of a simple inexpensive method desirable. Such a method has been developed by taking advantage of simple electrostatics. A small plastic rod, electrostatically charged by rubbing, is exposed to the air for a short time and then rolled over the surface of nutrient agar.

Table 1. Data from qualitative sampling for *Staphylococcus pyogenes* in a hospital. Volume of air sampled by liquid impinger, 25 lit. Period of exposure of charged glass rod, 30 seconds. Ratios, number of qualitative "positive" samples compared to number of samples taken. Temperature 75°F; relative humidity 56 percent.

Area sampled	Ratios for	
	Impinger technique*	Charged rod technique
Ward 1	2/4	6/8
Ward 2	4/4	4/8
Ward 3	2/4	1/4
Fracture room	2/4	5/8
Recovery room	2/4	6/8
Operating room	1/4	1/4
Linen room	0/2	2/4
Stores room	1/2	2/4
Physiotherapy	2/2	1/4
Laboratory	1/4	1/8
Bathroom	2/2	1/4
Main corridor	2/2	0/4
Total	21/38	30/68

* Impinger collections were assessed by the "drop plate" dilution method (*4*). One colony per plate (1/8 ml neat dilution) normally equals 3.2 organisms per liter (flow rate for Shipe impinger, 12.5 lit. per minute).

The materials (Fig. 1) required for simple electrostatic sampling are easily obtained at very low cost. In our experience 2-inch lengths of ¼-inch extruded plastic rod (dimpled at the ends) or tubing (inside diameter, ⅛ inch) or 7.5-mm glass capillary tubing have proved most satisfactory. In the laboratory, the rod or tube can be held for charging and exposure by any holder such as a hemostat, gut clamp, or pinch-type clothespin. A suitable rolling clip can be made by bending a length of No. 16 baling wire. For charging the rods, 2-inch squares of folded cotton cloth or gauze or short-pile fur are all that is required.

For routine field use a simple collection kit has been designed. This consists of a plastic tube mounted on a shaft to provide a handle and permit rolling; a charging sleeve: and a compartmented container for different media. These items are sealed within a package to keep the charging materials dry and the entire kit sterile.

The laboratory technique consists of grasping a dry, sterile rod or tube at one end with the clamp; with firm, wrap-around finger pressure, rubbing the rod five or six times with the charging material; holding the rod for the desired length of time (5 seconds to 2 minutes) in the atmosphere to be investigated; grasping the rod by means of the wire clip and rolling it back and forth over the flat smooth surface of the nutrient agar (preferably dried previously for 1 hour).

Relatively large numbers of organisms can be rolled onto the agar surface. With care in rolling, an even distribution of isolated colonies can be obtained. If it is anticipated that larger numbers of organisms will be collected, the rod is shaken in a small volume of diluent and the organisms are washed off the rod; dilution assessment by any of various methods follows.

This method of collecting and plating-out airborne microorganisms has been successful under varying conditions of temperature and relative humidity. Although electrostatic charges soon dissipate from glass at relative humidities above 70 percent, charges on plastics such as styrene, cellulose acetate butyrate, and acrylics provide sufficient attraction for successful collection at relative humidities ranging from 15 to 95 percent.

Collections of airborne organisms have been made in many atmospheres —in animal rooms, laboratories, vestibules, motor vehicles, elevators, and aerosol chambers (*1*), within dynamic aerosol equipment (*2*), and out-of-doors.

Under controlled conditions of temperature and humidity (temperature, 70°F; relative humidity, 53 percent), collections of washed cells of *Serratia marcescens* have been made from dilute, dynamic aerosols by means of positively charged styrene rods. From aerosols containing 84.5, 12.1, and 1.5 organisms per liter, respectively, as determined by the liquid impinger method (flow rate, 16 lit. per minute), satisfactory samples (20 to 1 colonies per rod) were collected; the ratios of the number of "positive" samples to the number of samples taken were 12/12, 9/12, and 2/12, respectively, for these three aerosols.

Table 1 shows recoveries under uncontrolled conditions in a hospital. Included for comparison are results obtained by a standard air-sampling method.

In experimental use of the method of sampling airborne microorganisms by an electrostatically charged rod, the findings are as follows. (i) The amount of rubbing seems to have very little effect on collection, provided the whole surface has been charged. (ii) The number of aerosolized microbiological particles collected depends upon the concentration of these particles in the atmosphere and the effective electrostatic charge on the sampler during the sampling period. (iii) Firm rolling, with gentle pressure, six or eight times across the agar surface in the petri dish will assure an even distribution of isolated colonies, and nearly all the organisms collected will be removed from the rod.

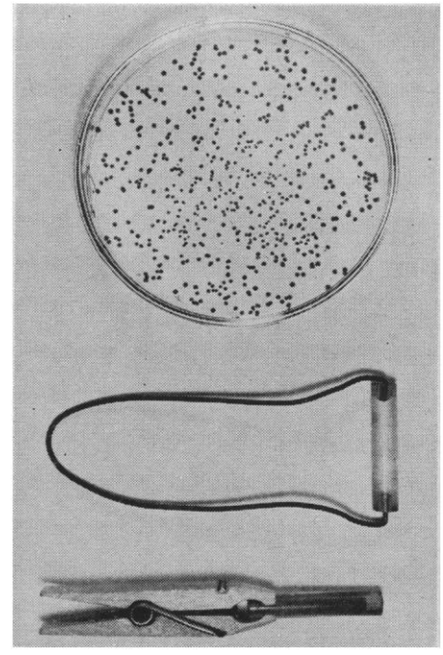

Fig. 1. Materials required for electrostatic rod sampling and a rolled plate showing isolated colonies of *Serratia marcescens*.

This method of sampling provides a means of qualitatively assessing the microbiological material suspended in the air in any situation. It should be especially useful in hospitals and in the field of agriculture (*3*).

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Incorporation of Tritiated Thymidine into Nuclei of Shoot Apical Meristems

Abstract. Tritiated thymidine enters readily into certain excised plant parts and into small aquatic plants. Attempts to introduce the radioisotope into shoot tips of seed plants via the roots have not proved satisfactory. The label readily enters the shoot if applied directly to immature leaves of a bud after the application of a wetting agent.

The general availability of tritiated thymidine has resulted in its use on a variety of organisms. Its use is designed to elucidate specific biochemical pathways of deoxyribonucleic acid synthesis which do not yield themselves readily to chemical analysis. Methods of application have varied considerably, as follows: small animals have been injected (*1*) or fed (*2*) with success; excised portions of tobacco pith incorporate the label (*3*); roots readily absorb thymidine (*4*); algae and aquatic cryptogams absorb the radioisotope with particular facility (*5*). Attempts to introduce and bring about incorporation of H^3-thymidine into the shoot systems of intact seed plants have not always been successful.

In a preliminary experiment, young (6 to 7 in. high) intact plants and excised shoots of *Chenopodium album* were placed in half-strength Hoagland's solution and H^3-thymidine (10 μc/ml). Shoot-tip samples were fixed at 3- and 5-day intervals. The material was fixed in FPA, processed in butyl alcohol, embedded in paraffin, and sectioned at 7 μ; the sections were then covered with autoradiographic stripping film AR.10 (Kodak). After exposure for 14 days the film was developed in DK19b (Kodak); the sections were stained with Harris' hematoxylin, and the slide was made permanent by mounting the sections and superposed film in Harleco resin. Examination of the autoradiographs did not reveal the presence of the label in the shoot apical regions, but nuclei of the root tips were labeled. Although longer periods of exposure to thymidine were not tried, the results of the preliminary tests indicated that the isotope becomes fixed in the meristematic root tips but does not move readily in the transpiration stream, as does P^{32} for example. The results indicated that other modes of movement and transport must be involved.

The application of H^3-thymidine "dropwise" to the terminal bud [preceded by the application of a drop of the wetting agent Tween-20 (0.1 percent)] resulted in foliar penetration and subsequent movement of the label to all young leaves, to the shoot apex, and to subjacent stem regions. One drop (approximately 0.05 ml of a solution containing 10 μc of the label per milliliter) was applied each day for 3 days. Whether movement, after initial penetration, was primarily through the phloem was not determined. To test the possible rapid movement in the phloem, the label was applied to fully expanded photosynthesizing leaves. After 3 days very little, if any, of the isotope could be detected in the autoradiographs of meristematic regions. It is possible that the thymidine molecule did not penetrate the mature outer walls of epidermal cells. The radioisotope was introduced also by injecting it into the stem a short distance below the shoot apex. This procedure resulted in the general distribution of the label into young leaves and the shoot apex. This method, however, does not appear to be as effective as that of tip application.

As may be noted in Fig. 1*A*, labeled nuclei are apparent (they appear totally black at this magnification) in the leaf to the left, in the developing pith, and to a lesser extent in the leaf at the right. Presumably the label was initially in direct contact with the leaf to the left. In Fig. 1*B* it may be noted that nuclei of cells of the shoot apex near the tip (on the right), as well as those in subjacent regions, have reduced silver grains over them. The two centers of activity on the upper right flank are well within the region of presumed mitotic inactivity, as described by some workers (*6*). It would not appear that these cells are inactive in deoxyribonucleic acid synthesis. If endomitotic reduplication and metabolic turnover are ruled out, the incorporation of thymidine into deoxyribonucleic acid is indicative of subsequent mitotic activity.

The utilization of H^3-thymidine, in conjunction with the use of P^{32} (*7*), should provide reliable information relative to sites of mitotic activity and aid in the elucidation of the growth of shoot apical meristems (*8*).

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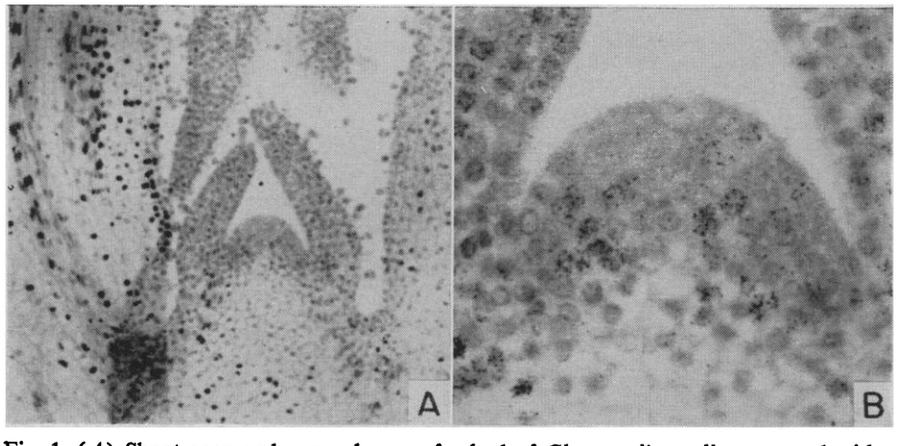

Fig. 1. (*A*) Shoot apex and young leaves of a bud of *Chenopodium album* treated with H^3-thymidine. Nuclei which incorporated the label are dark in appearance (× 125). (*B*) Details, shoot apex of same specimen (× 580).

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