Area-sampling Technique for Quantitative Pharyngeal Cultures

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An area-sampling device for obtaining quantitative samples of the oropharyngeal bacterial flora is described and illustrative data are presented.

Quantitative bacteriological techniques have provided more insight into host-parasite relationships than qualitative methods. Their application is limited by the availability of methods for obtaining reliable quantitative specimens. We were interested in relationships between aerobic bacteria in the human oropharynx and have devised a technique which affords reproducible quantitation of the oropharyngeal flora.

The end of the barrel of a 10-ml disposable plastic syringe is removed, and the barrel is filled with sterile 20% gelatin in water, capped, and refrigerated until use (Fig. 1). At the time of sampling, the gelatin is advanced with a plunger and pressed firmly against the posterior pharynx. Care must be exercised to avoid contact with the tongue or lateral structures. The distal 1 ml of gelatin is aseptically sliced off with a sterile tongue depressor, placed in 9.0 ml of sterile saline, and allowed to melt at 37 to 40 C. Serial 10-fold dilutions are made. Samples are placed on trypticase-soy-agar containing 5% sheep blood or on selective and differential media and spread with a flamed glass rod. Plates are incubated for 24 hr and colonies are counted. The syringe samples an area 1.77 cm². Results were expressed as colonies per square centimeter.

To determine reproducibility of the technique, recovery of marker organisms was studied. An 18-hr broth culture of a tetracycline-resistant Streptococcus salivarius was centrifuged, suspended in normal saline, and standardized to contain approximately $10^9$ organisms per ml. Approximately $10^9$ bacteria were deposited in the oropharynx of 11 subjects with a DeVilbis no. 127 atomizer. Oropharyngeal cultures were taken immediately and plated on blood-agar plates containing tetracycline at 20 μg per ml. Three series were done on different days.

Six consecutive daily cultures from six normal subjects showed considerable variation in total aerobic bacterial flora between different individuals, but the standard deviation between repeated cultures on the same individual was small (Table 1). Based on a mean recovery of $5.04 \log_{10}$ of the marker organism, the reproducibility of the technique had a 95% confidence level of ±0.51.

Quantitative sampling of the pharynx with swabs (3) or platinum loops (2) has been reported. The sampling problems, however, led to expressing the findings as a percentage of the total flora represented by a given species with little attention to the quantitation of the total flora (2-4).
Area sampling techniques such as the Rodac plate are commonly employed to obtain quantitative estimates of the bacterial contamination of surfaces (1). Litsky [cited by Walter (5)] described an agar-filled syringe technique in which the slice of agar obtained was directly cultured. This has the limitation of allowing quantitation of relatively few colonies, since agar does not melt at physiological temperatures and further dilutions cannot be made. Twenty per cent gelatin proved very satisfactory; it remains firm enough for sampling for about 30 min at room temperature after refrigeration (4°C). We usually obtain 5 to 10 samples with each syringe, cleaning the device with an alcohol sponge between applications. Our experience with over 1,000 such cultures indicates that it is rapid, inexpensive, and well accepted by most subjects.

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**LITERATURE CITED**


