Evaluation of a Micromethod for Determination of Streptococcus mutans and Lactobacillus Infection

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Received for publication 27 May 1977

A micromethod was developed for quantitative estimation in saliva of Streptococcus mutans and lactobacilli. With a semiautomatic pipette, 25 µl of diluted saliva was spotted on the surface of an agar plate containing a selective medium. This volume gave a spot with a diameter of about 10 mm in which separate colonies could be counted. The results obtained with the spotting technique showed excellent agreement with those obtained with conventional agar plating. The method is convenient and results in a substantial saving of culture media.

Both Streptococcus mutans and lactobacilli seem to be closely associated with the development of caries (2, 8). The number of these microorganisms can be used to select children with a high caries risk. In the individual case, a microbiological examination provides an objective method for the assessment of the cooperation of the patient in a caries preventive program (5). However, in general dental practice, our microbiological knowledge is practically not used at all. One reason might be that simple methods for quantitative diagnosis have been lacking. For the lactobacillus count, a dip slide technique has recently been introduced (6), but such a technique is not yet available for S. mutans.

The present paper describes the use of a semiautomatic micropipette (1) for the quantitative estimation of S. mutans and lactobacilli in stimulated saliva. The results were compared with those obtained with the conventional agar plate methods.

MATERIALS AND METHODS

The micropipette takes up and delivers a fixed volume of liquid from a disposable sterile plastic tip (pipettes are available which deliver from 10 µl to 1 ml). Pipettes calibrated for different volumes were tested. For the purpose of this study, 25 µl proved to give a spot of suitable size.

One milliliter of saliva, collected by chewing a piece of paraffin wax, was transferred to VMG II transport medium (4).

After agitation for 30 s with a Whirlimixer (Fisons Scientific Apparatus, Loughborough, Leicestershire, England), the sample was diluted in phosphate buffer to 10⁻¹, 10⁻², and 10⁻³. From each of the dilutions, 25 µl was spotted in duplicate on one-third of the surface of an agar plate. By starting from the highest dilution, the same tip could be used for all three dilutions of the same saliva sample. For the determination of the number of S. mutans, a selective agar consisting of mitis salivarius agar (Difco B 298) with the addition of sucrose and bacitracin (so-called MSB medium) was used (3).

For the cultivation of lactobacilli, the dilutions were spotted on the dried surface of a Rogosa SL (Difco B 480) agar plate (7). The spots were allowed to dry and then a further portion of SL agar was poured over the surface. The agar surface must be dry; otherwise the drops will not remain localized. The plates were therefore dried in a sterile box before being spotted.

For comparison with the conventional agar plate methods used in our laboratory, 0.1 ml from the same saliva dilutions was inoculated on duplicate plates of MSB agar and spread over the surface by means of four to five glass beads. For the lactobacillus count, 1 ml from each dilution was inoculated in SL agar by means of the pour plate technique described by Rogosa et al. (7). All MSB agar plates were incubated at 37°C for 48 h in 95% N₂-5% CO₂, and the Rogosa agar plates were incubated aerobically at 37°C for 72 h.

The total number of typical colonies on the various plates was counted, and the results obtained with the two methods were compared by calculation of the correlation coefficients.

RESULTS

On the surface of MSB agar plates, the 25-µl volume gives a spot with a diameter of about 10 mm in which separate colonies of S. mutans can easily be counted at suitable dilutions.

The same is true for the growth of lactobacilli in Rogosa SL agar. Comparisons between the micropipette method and the conventional plating method are illustrated in Fig. 1 and 2. In Fig. 1 the abscissa shows, on a logarithmic scale, the number of S. mutans obtained with the conventional plating method, and the ordinate shows those obtained with the micropipette technique. Fig. 2 shows the analogous results for the lactobacillus count. Both diagrams illustrate a highly significant relationship between the two methods.
EVALUATION OF A MICROMETHOD

extend over the spot, thus making it difficult to count colonies of other species.

We have found that the micropipette method is very useful in large clinical studies where the purpose is to select persons with a high caries risk. Since it is of interest only to assess the infection of *S. mutans* and lactobacilli within certain rough limits and not to assess the exact number of bacteria in the saliva, one dilution is enough for plating. Consequently, samples from three patients can be spotted on one agar plate.

Following is the working procedure we have developed for routine preparation of saliva samples for culturing.

To a vial containing VMG transport medium (4), 1 ml of paraffin-stimulated saliva is added (the vial is filled to the top). At the laboratory, the whole content is transferred to a test tube containing an amount of phosphate buffer such that a $10^{-4}$ dilution of the saliva is obtained.

After agitation in a Whirlimixer, 100 $\mu l$ is transferred to 10 ml of phosphate buffer by means of a semiautomatic micropipette. This gives a final dilution of the saliva of $10^{-6}$. From this dilution, 25 $\mu l$ is spotted in duplicate on one-third of an agar plate. No growth means less than 40,000 bacteria per ml of saliva, whereas a growth of 25 colonies corresponds to approximately 1 million bacteria per ml.

The same technique can be used in the individual case for the assessment of dietary changes on the number of lactobacilli or for antimicrobial measures directed against *S. mutans*. In these instances, only dramatic changes of the number of microorganisms are of clinical interest.

ACKNOWLEDGMENT

This work was supported by the Swedish Medical Research Council (Project 4548).

LITERATURE CITED


DISCUSSION

The micropipette method is quick and simple and results in a substantial saving of culture media, anaerobic jars, and space in the incubator.

The results obtained with the spotting technique show excellent agreement with those obtained with conventional agar plating. The method of spreading the inoculum with glass beads has some advantages over spreading it with a bent glass rod, but the micropipette method is even more convenient. We therefore now use the micropipette method even for purposes other than the counting of *S. mutans* and lactobacilli. However, when plating saliva samples, the spotting technique is not suitable on agar plates which support the growth of very large colonies. For example, on ordinary Mitis salivarius agar, colonies of *S. salivarius* might

FIG. 1. Relationship between the number of *S. mutans* obtained with the conventional plating method (abscissa) and the micropipette method (ordinate) expressed on a logarithmic scale. Correlation coefficient = 0.90.

FIG. 2. Relationship between the number of lactobacilli obtained with the conventional plating method (abscissa) and the micropipette method (ordinate) expressed on a logarithmic scale. Correlation coefficient = 0.96.