

A MODIFIED DIP-SLIDE TEST FOR MICROBIOLOGICAL RISK IN CARIES ASSESSMENT

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Abstract. This study was undertaken to evaluate the validity of a new modified dip-slide test kit for the estimation of salivary mutans streptococci, lactobacilli and *Candida* levels by comparison with the results obtained from conventional agar plate counts. Five milliliters of paraffin-stimulated saliva sample was collected from 118 6-12-year old children attending 4 primary schools in Thailand. Saliva was poured over a 3-compartment slide containing Mitis-Salivarius Bacitracin agar, Rogosa agar and Sabouraud dextrose agar. A slide was incubated in a 5%CO₂ incubator for 48 hours. The results of the modified test kit were compared with those from the conventional saliva sampling with dilution method. The salivary mutans streptococci, lactobacilli and *Candida* counts obtained from the conventional methods were significantly correlated ($p < 0.0001$) with the modified dip-slide estimates of these organisms. (Kendall Tau = 0.71, 0.58 and 0.76, respectively). The correlation between a modified dip-slide test kit and the conventional method indicates that this new test kit is suitable for the screening of salivary mutans streptococci, lactobacilli and *Candida* levels in patients. The test is simple and would be useful for the early selection of patients for dental examination. In addition, it would be a valuable educational aid for the motivation and dietary counseling among children.

INTRODUCTION

It is now well accepted that dental caries is an infectious and transmissible disease which is strongly modified by diet (Krasse, 1965). The various etiological factors involved in dental caries include cariogenic microorganisms, fermentable carbohydrates and susceptible tooth and host. Mutans streptococci (MS) are infectious organisms that colonize the teeth. They help form an intraoral biofilm commonly referred to as dental plaque through their ability to adhere to tooth structure by specific glucans. High salivary MS indicates that a strong cariogenic challenge to tooth is present (Klock and Krasse, 1979; Beighton *et al*, 1991). Lactobacilli, the other organisms that also contribute to dental caries because of their acidogenic and aciduric prop-

erties, have been shown to play a role in caries progression (Van Houte, 1980; Loesche, 1986). The level of lactobacilli is dependent on the number of cavitated lesion in the dentition. In addition, their presence can be considered a consequence of the circumstances that normally lead to dental caries. High lactobacilli count is also found in medically compromised patients or when the carbohydrates are consumed frequently (Larmas, 1992). The *Candida* species are the predominant yeasts found in the oral cavity. They have been shown to be more prevalent in the saliva of caries-active subjects than of caries-free one (Krasse, 1954; Russell *et al*, 1991). Moreover, a number of oral conditions are associated with elevated salivary yeast counts, such as the use of antibiotic medications or having low immune response. In school-age children, the presence of *Candida* has been shown to correlate with caries prevalence (Russell *et al*, 1991).

Several microbiological tests have been developed for the determination of microorganisms in medicine. Nevertheless, they have sel-

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dom been used to assess caries risk in dental practice due to the lack of suitable and reliable technique. Consequently, many improvement have been made to the conventional techniques, including the development of improved selective media and the use of modified laboratory techniques for performing viable count (Westergren and Krasse, 1978). Simple methods of sample collection have also been introduced, such as the use of tongue spatulas or plastic loops for salivary estimation (Kohler and Bratthall, 1979; Beighton, 1986). However, all these methods require the availability of microbiology laboratory facilities that are not convenient to use in the dental practice.

Recently, a commercial dip-slide test is available to use for quantifying salivary MS, lactobacilli and *Candida* at the chair-side or in field study. Although having advantages over the cumbersome and time consuming laboratory methods, they are still too expensive for widespread use in developing and under developed countries. A new modified dip-slide test (Fig 1) was developed in order to be cheaper and more convenient. Therefore, the present study was undertaken to evaluate the validity of this new modified dip-slide test for the estimation of salivary MS, lactobacilli and *Candida* levels by comparison with the results obtained from conventional agar plate counts.

MATERIALS AND METHODS

Subjects

The group studied consisted of 118 6-12-year-old children from 4 primary schools in Thailand.

Microbiological procedures

Stimulated whole saliva samples were obtained from all subjects by chewing a piece of paraffin for 5 minutes. For conventional technique, the saliva samples were serially tenfold diluted. Aliquots of 100 μ l were then spread onto duplicate selective plates of Mitis-Salivarius Bacitracin agar, Rogosa SL agar and Sabouraud dextrose agar for the enumeration of MS, lactobacilli and *Candida* species respectively. In the case of the modified dip-slide test, the remaining undiluted saliva was poured over the surface

of a 3-compartment dip-slide containing selective media for these three organisms. Excess saliva was removed by blotting the edge on absorbent paper and then the dip-slide was placed into a plastic tube. All of the agar plates and plastic tubes containing dip-slides were incubated at 37°C for 48-72 hours in a 5% CO₂ incubator. The colonies of MS, lactobacilli and *Candida* were counted under a stereomicroscope and checked by Gram staining. The dip-slides were examined directly in good illumination. The density of the growth of MS, lactobacilli and *Candida* were recorded and scored by comparison with a chart provided with the test.

Statistical analysis

Microbial counts from the conventional technique were transferred to log 10 prior to analysis. Comparison between different methods for the estimation of salivary microorganisms was made using Kendall's rank correlation.

RESULTS

The modified dip-slide scores increased with the conventional salivary counts and significant correlation was noted between the two

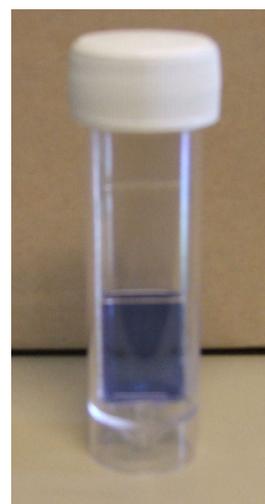


Fig 1—A modified dip-slide test set with plastic chamber containing a three-compartment plastic tray. Each compartment contains selective agar medium for lactobacilli, *Candida* and Mutans streptococci, respectively from the top to the bottom.

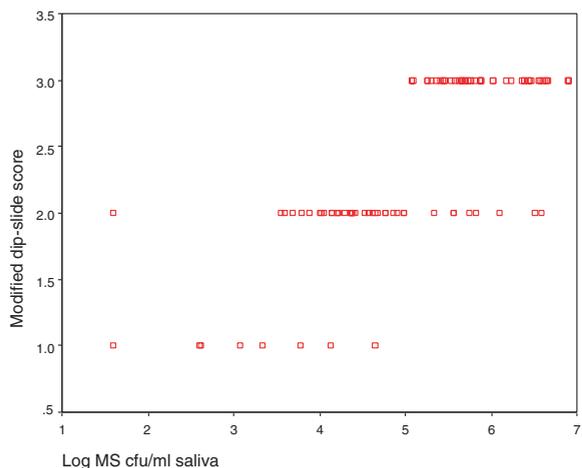


Fig 2—Relationship between the mean numbers of MS in saliva by conventional technique and modified dip-slide scores (Kendal Tau = 0.71, $p < 0.0001$).

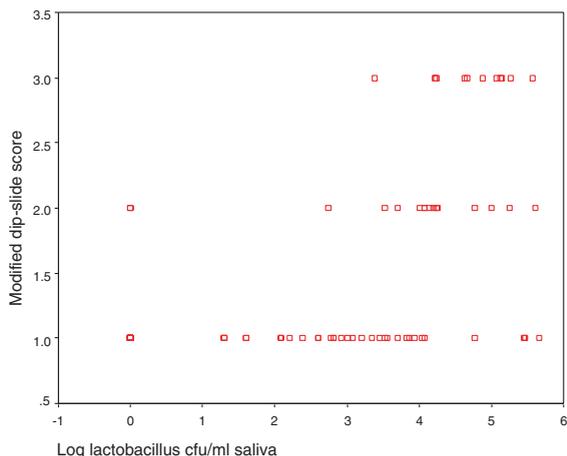


Fig 3—Relationship between the mean numbers of lactobacilli in saliva by conventional technique and modified dip-slide scores (Kendal Tau = 0.58, $p < 0.0001$).

methods. Figs 2, 3 and 4 show the relationship between the modified dip-slide and the conventional agar estimation of salivary MS, lactobacilli and *Candida*, respectively. In the case of MS, the correlation coefficient was 0.71 while the value for lactobacilli was 0.58 and for *Candida* was 0.76 (Kendall Tau, $p < 0.0001$). By grouping the modified dip-slide MS scores into low (score 1), medium (score 2) and high (score 3) categories, 68% of subjects with low score had low level of MS ($<10^3$ cfu/ml), 70% of subjects with moderate score had moderate level of MS (10^3 - 10^5 cfu/ml), and 100% of subjects with high score had high level of MS ($>10^5$ cfu/ml). Similarly when data from the salivary levels of lactobacilli were grouped into low (score 1), moderate (score 2) and high (score 3) categories, 73% of subjects with low score had low level of lactobacilli ($<10^3$ cfu/ml), 71% of subjects with moderate score had moderate level of lactobacilli (10^3 - 10^5 cfu/ml) and 68% of subjects with high score had high level of lactobacilli ($>10^5$ cfu/ml). In the case of the presence of *Candida* in saliva as yeast carriage, we categorized *Candida* scores into none (score 0), low (score 1) and high, (score 2). None of the subject with score 0 harbored *Candida*, 75% of subjects with low score had low level of *Candida* ($\leq 10^4$ cfu/ml) and 75% of subjects with high score had high

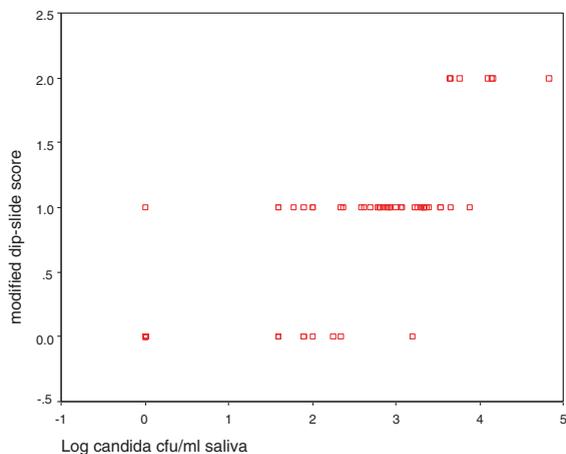


Fig 4—Relationship between the mean numbers of *Candida* in saliva by conventional technique and modified dip-slide scores (Kendal Tau = 0.76, $p < 0.0001$).

level of *Candida* ($>10^4$ cfu/ml).

DISCUSSION

The determination of salivary MS, lactobacilli and *Candida* has been shown to be useful for the assessment of individual patients or for the studies on population groups. Positive correlation has been demonstrated between these

organisms and caries increment and dietary sucrose (Van Houte, 1980; Zickert *et al*, 1982; Klock, 1984; Krasse, 1985; Steckslen-Blicks, 1987). The application of microbial tests to assess caries risk in children was demonstrated initially by Krasse (1985). In the first epidemiological surveys, tongue blades and agar plates were used, but these proved impractical for field-work and were replaced with liquid media into which the saliva-contaminated strips were inserted. At present, a convenient and rapid clinical test in the form of a chair-side modified dip-slide test kit has been introduced and supposed to be more cost-effective.

In this study, the demonstration of highly significant ($p < 0.0001$) positive correlation between a new modified dip-slide test and the conventional agar plate counts for MS, lactobacilli and *Candida* (Kendall-Tau=0.71, 0.58 and 0.76, respectively) was shown. Microbiological tests have been performed to identify high-risk children in a population and/or those persons who will develop dental caries or infection in the future, *ie* prediction purposes. Although an examiner with good clinical ability can predict a subject's caries risk, it is necessary for a considerable number of lesions to have already occurred or be developing. As prevention is preferable to treatment, it is better to identify the high-need population earlier, prior to the development of carious lesions. The cost/efficacy ratio of preventive treatment is also more favorable as children at greatest risk can be targeted (Alanen *et al*, 1994). It is sensible to try to identify the high-risk group before caries attack. Besides being helpful in identifying groups at high caries risk, microbiological tests can be used to assess the effectiveness of caries preventive treatment (Steckslen-Blicks, 1985; Bowden, 1997), and also whenever an extensive restoration treatment is being planned (Krasse, 1984; Suddick, and Dodds 1997).

In some studies, children with salivary MS of $>10^5$ cfu/ml saliva and lactobacilli $>10^4$ cfu/ml saliva have been considered at risk of developing dental caries (Klock and Krasse, 1977) while other study showed MS level of 2.5×10^5 cfu/ml as indicative of risk (Zickert *et al*, 1982). In this modified dip-slide test, the MS score of 3

corresponded with MS counts above the threshold value of 10^5 cfu/ml. This revealed children who may be potentially at high risk of developing caries. In the case of lactobacilli, the results showed a good correlation between the modified dip-slide and conventional counts. Most of the dip-slide readings of 3 appeared to correspond with conventional counts greater than 10^5 cfu/ml. As for the presence of *Candida*, the density of *Candida* on a modified dip-slide test was categorized into 3 levels. Yeast carriage was considered at scores of 1 and 2. However, in view of the range of values obtained in this study, particularly with scores of 0 and 1 in the test for *Candida*, false negative and false positive results will occur.

From our results, score of 3 with both MS and lactobacilli would appear to be indicative of high levels of potentially cariogenic bacteria and therefore suggests high risk. The *Candida* scores of 1 and 2 indicated *Candida* carriage and risk for yeast infection. In addition, their presence can be considered a caries risk status. However, the determination of caries risk categories should be carried out with caution when individual patients are considered. Other clinical factors, such as diet, past and present caries experience and oral cleanliness should be taken into account in addition to the microbiological tests. This modified dip-slide test provides a suitable and simple method for the screening of salivary MS, lactobacilli and *Candida* levels, and does not require special laboratory facilities nor special training in microbiology. It is useful for the early selection of patients for dental examination and would be a valuable educational aid for the motivation and dietary counseling among children.

In summary, the correlation between the new modified dip-slide test and the conventional technique indicates that this test can be used for the estimation of salivary mutans streptococci, lactobacilli and *Candida* levels in patients.

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