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A comparison of S. mutans clinical assessment methods

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Abstract

The purpose of this investigation was to compare the tongue blade/Rodac plate assessment method, the Cariescreen® (APO Diagnostics Inc., Toronto, Ontario, Canada) method, and a modified Cariescreen method for S. mutans assessment. Ninety-one triple tests were done on 23 children 1 to 4 years of age, and the S. mutans counts were compared. When all 91 tests were compared, there was agreement 86.3% of the time between the tongue blade/Rodac plate assessment method and the modified Cariescreen method. When the Cariescreen method was compared to the tongue blade/Rodac® (Becton Dickins Labware, New Jersey) plate technique, there was agreement 72.2% of the time. Agreement tended to be at the low and high infection levels. When the Cariescreen and modified Cariescreen methods were compared with the tongue blade/Rodac method, most discrepancies were in the moderate range of S. mutans counts. Averages and standard deviations were 80.1 vs 32.1 for the modified Cariescreen method, and 86.9 vs 90.2 for the tongue blade/Rodac method. The linear product moment correlation coefficient was +.69. Overall, the modified Cariescreen method compared more favorably to the tongue blade/Rodac plate assessment than the Cariescreen method did.

Literature Review

The positive correlation between *Streptococcus mutans* counts and dental caries activity is well established. Nondentate infants have been shown to be *S. mutans* free. Catalanotto et al. (1975) found that some young children become infected with *S. mutans* as the dentition develops. Using a microbiological laboratory procedure, Alaluusua and Renkonen (1983) demonstrated that the early establishment of *S. mutans* indicated a high caries risk. These investigators found that children who harbor *S. mutans* in their plaque at age 2 years are likely to be more caries active and have significantly higher DMFS at age 4 years than children who harbor *S. mutans* later, or remain free from *S. mutans* infection. They concluded that the early establishment of *S. mutans* in the plaque of primary incisors indicates potentially early and extensive caries attack in the primary dentition. Kohler et al. (1984) also suggested that the time when *S. mutans* is detected is related to the development of caries. These investigators found that 77% of the children infected by *S. mutans* at 15 months had caries at age 3 years. Newbrun et al. (1984) demonstrated that children with high *S. mutans* counts had high DMFS increments over the previous four years. Thus, there appears to be increasing evidence that salivary *S. mutans* counts may be important in predicting future high caries levels in children.

Two problems are encountered when testing young children for *S. mutans* in the dental office. First, stimulated saliva samples are difficult to obtain from very young children. Second, the methodology used to estimate bacterial counts in many past studies has been complex and, consequently, inappropriate for use in a clinical setting.

In recent years, the possibility of using a clinically applicable method resulted from the work of Kohler and Bratthall (1979). They collected unstimulated saliva on a wooden spatula and inoculated a selective medium, *Mitis salivarius* with bacitracin agar (MSBA). The investigators found that this method of estimating *S. mutans* counts compared favorably to the results from more sophisticated microbiological techniques, such as those used by Westergren and Krasse (1978).

Investigators have continued to search for an ideal, easy-to-use technique for *S. mutans* assessment. Newbrun et al. (1984) used the MSBA and *Mitis salivarius* with bacitracin broth (MSBB) methods. Both methods used *Mitis salivarius* medium with bacitracin; the difference was that the MSBA method estimated colonies grown on agar, and the MSBB method estimated colonies grown in broth that adhered to glass. Although the MSBB method demonstrated *S. mutans* presence, the investigators noted that the test required stimulated whole saliva. They also found that the MSBA method for determining *S. mutans* correlated more highly with dental caries.

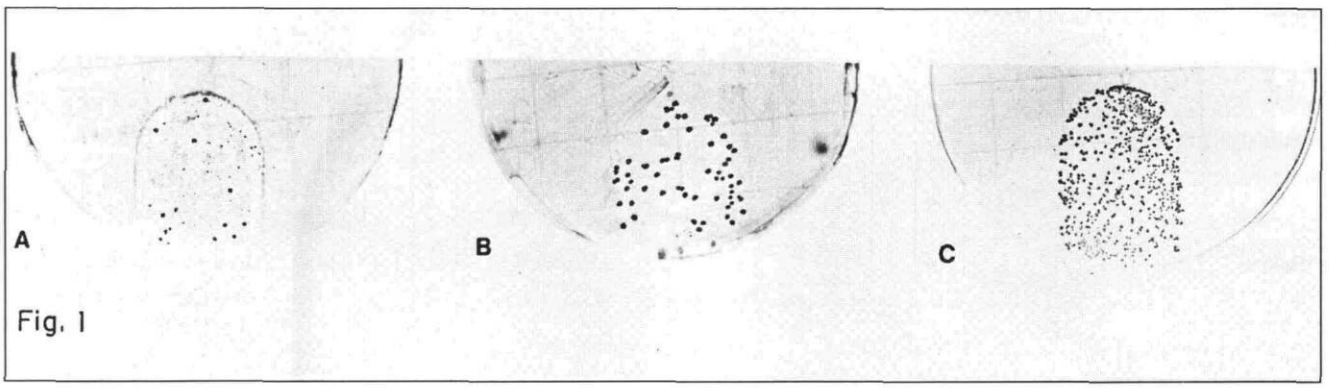

Fig 1. Colony growth of *S. mutans* in the Rodac plates. Tongue blade impressions are made in the media after saliva collection. *S. mutans* colonizations are (A) 1–20 CFU, (B) 21–100 CFU, (C) >100 CFU.

Using the MSBA method with a tongue blade, Edelstein et al. (1987) also found a positive relationship with 28 adults and children. Similar results were obtained subsequently by Weinberger and Wright (1989), and by Edelstein and Tinanoff (1989), in very young populations having only primary dentitions. The latter two reports noted that the ease of the tongue blade technique with very young patients makes the technique very attractive for clinical use. Weinberger and Wright (1989) also demonstrated that the tongue blade is a reliable method for sampling saliva. They examined 76 paired samples of saliva; no significant differences in *S. mutans* counts were found between sides of the spatula.

The media for the previously cited investigations were made in research laboratories, and had a limited shelf life of approximately four weeks. A commercially available product is now available with a shelf life of approximately six months. The product is based on the work of Jordan et al. (1987), who collected stimulated saliva in a vial containing buffered saline and bacitracin. The purpose of the present investigation was to compare *S. mutans* estimates from the tongue blade/Rodac plate technique, Cariescreen® (APO Diagnostics Inc., Toronto, Ontario, Canada, a commercially available dip slide method for sampling and estimating *S. mutans*), and a modified Cariescreen technique that used the tongue blade sampling and the commercial media for bacterial growth.

Materials and Methods

The population selected for the study consisted of 23 children with a mean age of 3 years, 1 month, ranging from 1 year, 11 months to 4 years, 3 months. All subjects attended a local nursery school in London, Ontario, Canada, a fluoridated community. Written consent in accordance with the Human Investigation Committee was obtained from parents before the start of the study. The saliva of 23 subjects was scheduled to be sampled four times during a single day. Since one of the children left school early, 91 triple saliva samples were taken. The three samples were for three *S. mutans* assessments that included the tongue blade/Rodac plate technique, the Cariescreen dip slide technique, and a modified Cariescreen dip slide technique. The descriptions of the methods follow.

The Tongue Blade/Rodac Plate Method (RS)

The laboratory method using researcher-made incubation plates has received considerable research and serves as the standard. When using the tongue blade technique of Kohler and Bratthall (1979), saliva was collected on a 1.8 mm wood, sterile, spatula (tongue depressor). The spatula was pressed against the tongue to gather a mixed saliva sample (Togelius et al. 1984). The blade then was pressed onto the surface of a Rodac plate that had been pre-prepared with an elevated level of *Mitis salivarius* agar (Difco Lab Ltd., Detroit, MI) with 20% sucrose. Bacitracin solution had been applied to the medium using a sterile cotton swab. The solution was prepared by adding two, 10-unit bacitracin disks to 1 ml sterile water (Tinanoff, personal communication 1987; Edelstein and Tinanoff 1989). The saliva collected on the tongue blade was placed onto the surface of the medium. Plates were sealed into plastic bags filled with expired air to enhance an anaerobic condition. They were incubated at 37° for 48 hr (Edelstein et al. 1987; Edelstein and Tinanoff 1989; Weinberger and Wright 1989).

Bacterial growth counts on the plates were made by counting the number of *S. mutans* colonies on an area 1 cm from the tip of the tongue blade impression on the agar, which formed a circle of approximately 1.5 cm^2 (Fig 1). The colonies were identified by their characteristic appearance according to Coykendall (1977). Similar to previous studies the *S. mutans* counts were divided into four ranges: 0, 1–20, 21–100, and > 100

(Kohler et al. 1979; Edelstein et al. 1987; Weinberger et al. 1989). The appearance of the three ranges demonstrating *S. mutans* presence is shown in Fig 1.

The Cariescreen Dip Slide Method (CS)

The materials of the Cariescreen kit are shown in Fig 2. The following steps were recommended by the manufacturer. A bacitracin tablet was added to a vial containing buffered diluent and allowed to dissolve. A saliva sample was collected in the diluent vial. The slide was dipped into the diluent and left for approximately 10 sec before innoculation. The slide was replaced into a dip slide vial containing a carbon dioxide-generating tablet. Two drops of water were added to the tablet before the slide was replaced into the vial.

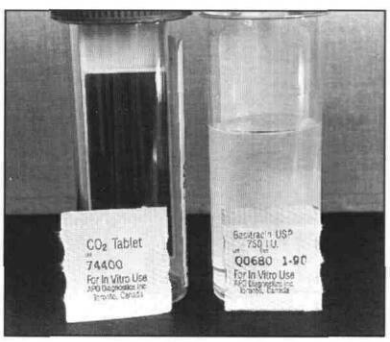

Fig 2. Part of the Cariescreen kit supplied by the manufacturer. Dip slide from A vial is placed into B vial after Bacitracin tablet is dissolved in diluent. After CO_2 tablet is dissolved in A vial with drops of water, the dip slide which has been inoculated with saliva sample is replaced in this vial.

Since gathering saliva from young children is difficult, it was decided to follow the manufacturer's instructions and use a cotton swab for saliva collection. The swab was rotated until moist on the dorsum of the tongue, and then rolled on the dip slide.

The vials were placed in an incubator for 48 hr at 37°C. Bacterial growth counts on the dip slides were estimated by visual assessment under a magnifying glass, and compared to a colony density chart provided by the manufacturer (Fig 3).

Modified Cariescreen Dip Slide Method (MCS)

Previous work by the authors indicated that there could be difficulty using the Cariescreen estimating chart for interpretation of results. Sometimes the colony growth may be spotty or spread unequally over the surface of the slide (Fig 4). Hence, the sampling method was altered to try to improve comparative results. The saliva was gathered using a tongue blade as in the RS technique and pressed on the dip slide as shown in Fig 5 (see next page). The dip slide also was mdoified by removing the remaining medium from one side. This allowed more light through the remaining medium and imrpoved viewing of the bacterial growth.

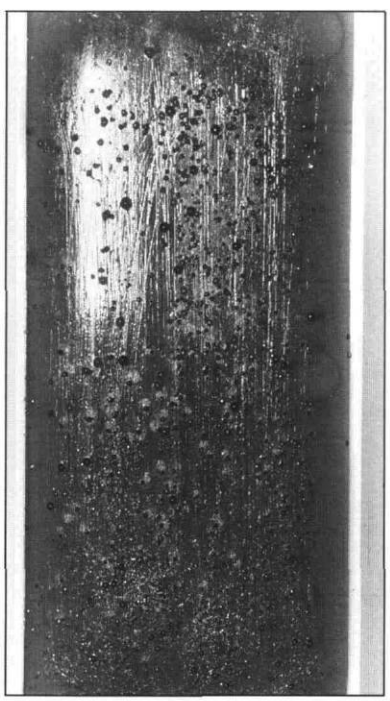

Fig 4. An example of an unequal colonization on the slide. The swab technique was used to gather saliva and inoculate the dip slide.

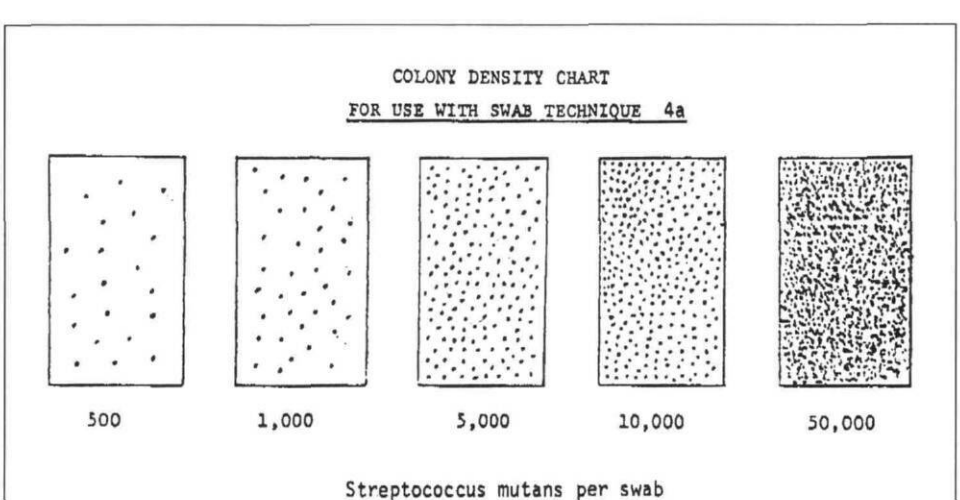

Fig 3. Colony density chart as provided by the manufacturer of Cariescreen.

Results

In the present investigation, the RS technique serves as the standard, and attempts were made to relate the results of the other techniques to it. Table 1 (see next page) compares the results of the CS technique to RS. Note that all CS counts of zero were estimated as 20 colonies or less with the RS. Both techniques indicated no risk or low risk of caries in all 54 tests. There is less agreement with CS cases showing 500 on the colony density chart. Sixteen of these cases were in

the no infection or low infection category, and five tests showed high susceptibility or the presence of caries. In the higher CS ranges, 13 of 16 (81.3%) were above the so-called low risk group on the RS.

Table 2 compares the MCS to the RS. Using this technique, 72 tests showed no risk or low risk of *S. mutans* infection. The RS pointed to a similar oral environment on 68 tests (94.4%). Of the remaining 19 tests, 14 (73.7%) using the MCS showed greater than 20 CFUs and also had higher than 20 CFUs on the RS. Four low MCS counts had 20 or more colonies using the RS technique, and five of the low RS counts were over 20 by the MCS method.

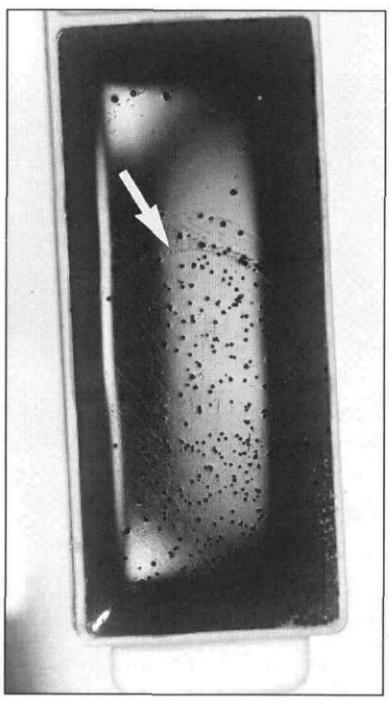

Fig 5. *S. mutans* colonies using the modified Cariescreen technique. A tongue blade was used to gather saliva and inoculate the dip slide. Arrow points to tip of tongue blade outline.

When all 91 tests were compared, there was agreement 86.3% of the time between RS and MCS. However, when the CS method was compared, only 66 (72.2%) of the tests agreed with the RS.

Averages and standard deviations were 30.1 vs 32.1 for the MCS system, and 86.9 vs 90.2 for the RS system. Hence, the counts were very much alike on the average. The linear product moment correlation coefficient of +.69, indicates there was good agreement between these systems.

Discussion

A previous study by Weinberger and Wright (1989) showed that children who harbored more than 20 CFUs of *S. mutans* were likely to have caries. If we consider the RS 0–20 colony forming units (CFU) as the no-risk or low-risk group, and greater than 100 CFUs as the very-high-risk or caries-evident group, then the group between 21 CFUs and 100 CFUs could be recognized as the potential high-risk group. Although white spot lesions are associated with high *S. mutans* counts (Van Houte et al. 1982), this might be manifesting clinically as very early decalcification.

Table 1. Crosstabulation of tongue blade-Rodac plate standard (RS) and the dip slide (CS) technique results

CS	*Rodac Standard (RS)*				
	0	*1–20*	*21–100*	*>100*	*Total*
0	42	12	—	—	54
500	5	11	—	5	21
1000	—	1	1	1	3
5000	2	—	1	3	6
10,000 and over	—	—	1	6	7
Total	49	24	3	15	91

Table 2. Crosstabulation of tongue blade-Rodac standard (RS) and the modified Cariescreen dip slide technique (MCS) results

MCS	*Rodac Standard (RS)*				
	0	*1-20*	*21-100*	*>100*	*Total*
0	34	4	—	1	39
1-20	11	19	—	3	33
21-100	3	—	1	3	7
>100	1	1	2	8	12
Total	49	24	3	15	91

The Cariescreen colony density chart lends itself to similar categorization. Less than 500 could correspond to 0 to 20 on the RS, greater than 1000 seems to agree with greater than 100 CFUs, and 500 to 1000 may indicate potentially high risk corresponding to the 21 to 100 CFUs on the RS. However, at present, the exact meaning of the colony density chart is not understood fully. Further research is required to relate the observations of colony growth to dental caries status. When using the CS method, the manufacturer's instructions are to "collect a saliva sample in the diluent vial supplied and dip the slide (agar) into the diluent." Collecting saliva from very young children in this way is difficult. Instead, it was decided to use a cotton swab for saliva collection, an alternative method that was suggested by the manufacturer.

There is an inherent average score difference between the tongue blade and the swab technique, because the amount of saliva picked up from the mouth in a swab differs from that adhering to a wooden blade. When using the CS method, distribution of CFUs may be affected while applying the swab to the agar, as shown in Fig 4. In the present study, an unequal colonization sometimes was observed, making it extremely difficult to match the observations to the colony density chart.

As an alternative procedure, the tongue blade technique of saliva collection (Togelius et al. 1984) and the colony counting described by Kohler and Bratthall

(1979), were used with the Cariescreen medium. Counts of the RS and MCS were shown to be very similar. Hence, it is suggested that this might be the method of choice for sampling saliva of young children to make *S. mutans* estimates. The RS method has been studied widely (Kohler and Bratthall 1979; Edelstein et al. 1987; Weinberger and Wright 1989; Weinberger and Wright 1990) and, although its clinical potential has been recognized, it has not been available commercially (Edelstein and Tinanoff 1989). The modified Cariescreen method seems to offer some potential for use in young children.

Conclusions

Based upon the conditions of the present investigation, it appears that:

1. When the Cariescreen method is modified by using a tongue blade for saliva collection, the *S. mutans* counts resemble those obtained by the tongue blade/Rodac plate technique
2. When the Cariescreen method was used as directed by the manufacturer, the results were difficult to compare to the tongue blade/Rodac plate technique
3. Further research is needed to establish the reliability of the Cariescreen method of determining the *S. mutans* counts and relating them to oral environments.

The authors would like to thank APO Diagnostics Inc., Toronto, Ontario, Canada for the supply of SM-Cariescreen® for this study.

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High-fat diets may increase caries risk

High-fat diets not only can lead to cholesterol problems but also may increase chances of developing dental cavities, according to a recent study.

Dental researchers at the University of Medicine and Dentistry of New Jersey (UMDNJ) and the University of Washington School of Dentistry in Seattle have found that patients on high-fat diets have higher levels of lipids — fatty substances — in their saliva than those on low-fat diets. In turn, they also have more cavities.

The researchers analyzed the saliva of 50 patients — 25 on high-fat diets, and 25 on low-fat diets. High-fat diets included more than 35% fat content, while low-fat diets contained between 18 and 22% fat.

Patients on high-fat diets were found to contain 78 mg of lipids per 100 ml of saliva, whereas those on low-fat diets had only 4 or less lipids per 100 ml of saliva.

The dental researchers also are trying to develop a simple saliva test to reliably measure cholesterol levels as an alternative to blood sampling.