An improved method for detecting mutans streptococci using a commercial kit

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Abstract: The improved detection of mutans streptococci (MS) in individuals was investigated using several modifications to a commercially available kit, Dentocult SM®. Significantly better detection of MS was achieved using plaque from the four approximal surfaces at two interdental spaces than with saliva (P < 0.001). Furthermore, the MS estimates for approximal surfaces at the same interdental space were similar (κ = 0.654) suggesting that differentiating the two surfaces does not improve the detection of MS, and that increasing the number of interdental spaces sampled is a more effective option. This study also evaluated a modification to the standard Dentocult SM® site strip method in which two strips were incubated per broth vial so that plaque from eight interdental spaces could be tested at the same time (new method). The results were compared to those obtained when one strip was incubated per broth vial (standard method). Although the MS estimates by the new and standard methods were comparable (κ = 0.721), the efficiency of MS detection was improved significantly by increasing the number of sites used for MS estimates (P = 0.01). In conclusion, MS detection at eight interdental spaces is recommended using the new Dentocult SM® method.


Key words: site strip; mutans streptococci; assessment of the oral environment.

Introduction

Mutans streptococci (MS) are thought to be the main etiological agents of dental caries in humans (1,2). The strong correlation between the presence of MS and the incidence of caries can be used to identify caries-susceptible individuals (3-5). Saliva and plaque samples are both used to measure MS infection in the oral cavity. From a theoretical perspective, plaque is more appropriate, because the surface of the tooth is the natural habitat of MS (6). However, several studies have found that salivary MS levels correlate better with caries than plaque levels when samples were taken from a limited number of sites (7). In addition, it is more difficult to sample and process plaque. Saliva, which is easy to sample and process, is preferred for clinical evaluation of MS, since it reflects the number of intraoral sites colonized (8).

In a recent attempt to predict the risk of caries on specific tooth surfaces, Bratthall et al. developed a simplified method for evaluating site-specific plaque MS levels, the Site Strip method (9), by modifying the previous version of Dentocult SM® Strip mutans kit, which was developed to evaluate salivary MS levels (10). Despite the clinical relevance of this site-specific method, few studies have evaluated its usefulness for screening individuals for caries risk (11).

In this study, the ability of Dentocult SM® to detect MS infection was evaluated using both saliva and dental plaque. The site-specific method using dental plaque was also modified to increase the efficiency of MS detection.

Materials and Methods

Subjects

Saliva and plaque samples were collected in 1999 from 156 preschool children (78 males and 78 females; aged
2-6-years) who attended two nursery schools in Tokyo, Japan. The parents of all children were asked for permission to examine their children’s teeth and to collect microbiological samples. In addition, dental plaque from 36 students (four males and 32 females) was examined for MS infection in 2001; this group ranged in age from 19-31-years, and included 12 dental students and 24 dental hygienist students from Tokyo.

Oral microbiological sampling and processing

The new version of Dentocult SM(R) Strip mutans, which also contains Site Strip, was used to evaluate MS levels in unstimulated saliva and plaque. To sample saliva, a strip was rotated 10 times on the surface of the tongue. Plaque was collected using a triangular wooden toothpick with two sampling sides, as described previously (12). The toothpick was inserted until it made firm contact with both approximal surfaces. It was then removed and one sampling side was placed on one of four separate pads on the plastic test strip.

When sampling plaque from children, site-specific plaque samples were collected from the four approximal surfaces at two specified interdental spaces (Table 1). The manufacturer’s instructions for Dentocult SM(R) recommend incubation of only a single strip per broth vial. To examine whether two strips could be incubated in one broth vial without reducing accuracy, the results on the facing approximal surfaces at the same interdental space were compared in adult patients using the one strip in one vial method (standard method) and the two strips in one vial method (new method). With the former method, plaque samples were collected from the four approximal surfaces at four specified interdental spaces (Table 1 and Fig. 1). In the latter method, another four specified interdental surfaces were evaluated in addition to the four approximal surfaces opposite those evaluated by the standard method (Table 1 and Fig. 1). If consistency between the standard and new methods was obtained, the new method would be recommended to increase the number of sites being evaluated.

Saliva and plaque strips were placed in the culture medium provided with the kit and incubated for 48 h at 37°C. After incubation, the strips were dried, examined under a microscope (×10), and scored from 0–3 using the manufacturer’s chart. One dentist, who was experienced with the method, determined all the scores. The highest plaque score among the surfaces tested for each subject was defined as the individual’s plaque MS level.

Statistical analysis

The data were analyzed using the SPSS® software package. The degree of agreement between the MS scores for the approximal surfaces at the same interdental space was analyzed using kappa statistics. The Wilcoxon test was used to analyze the difference in saliva and plaque MS estimates for each preschool child. The chi-squared test was used to analyze the relationship between the efficiency of

![Fig. 1](image-url)
estimating MS for each individual and the number of interdental spaces examined.

**Results**

Detection of MS in saliva vs. plaque

In the 156 preschool children, the saliva and plaque MS scores were as follows: level 0, 59 and 49%; level 1, 15 and 11%; level 2, 15 and 9%; and level 3, 11 and 31%, respectively (Fig. 2). These levels suggest that plaque samples are superior to saliva for detecting MS. When the difference between MS estimates in saliva and plaque were compared for each individual using the Wilcoxon test, the results were found to be statistically significant (P < 0.001). MS was detected significantly more effectively in plaque from the four approximal surfaces at even two interdental spaces than in saliva.

Similarity of MS infection on approximal surfaces facing each other

The MS scores for the approximal surfaces at the same interdental space (54/55 and 74/75) are compared in Table 2, which shows that the MS estimates for the approximal surfaces were similar (κ = 0.654, P < 0.001). These results suggest that there was not a large difference in level of MS infection between approximal surfaces facing each other.

Standard Site Strip method vs. new Site Strip method

The plaque MS scores for the same interdental space determined using the standard and new Site Strip methods are compared in Table 3. The scores for the four approximal surfaces at the specified interdental space determined by the new method were in substantial agreement with those estimated by the standard method (κ = 0.721, P < 0.001) (Table 3).

The efficiency of detecting MS in plaque from adults was improved significantly (P = 0.01) by increasing the number of sites sampled as follows: one interdental space selected randomly from the results of the standard method, 43.9%; two interdental spaces (16/15, 36/35), 55.8%; four interdental spaces (standard method), 67.8%; and eight interdental spaces (new method), 79.7%.

Discussion

In a previous study, Köhler et al. reported that the earlier a child is colonized with MS, the greater the likelihood of them developing dental caries (13). Several studies have focused on the presence or absence of MS as a predictive indicator for identifying young children with a high caries risk (14,15). Early detection of MS is important to prevent caries. The consensus appears to be that plaque samples are superior to saliva for detecting MS in the oral cavity. However, collecting plaque from all the tooth surfaces is difficult and unrealistic when considering clinical convenience. Bratthall et al. recommended using plaque samples to evaluate site-specific plaque MS levels to predict caries risk on specific tooth surfaces (9).

Some studies, however, support the use of interdental plaque samples to represent the prevalence of MS in the whole dentition (16,17). In the current study, plaque collected from the four approximal surfaces at two interdental spaces was examined, and the technique was found to be significantly (P < 0.001) more efficient at detecting
MS than saliva sampling. In a previous study, however, salivary MS scores had a higher correlation with caries prevalence than plaque MS scores evaluated at five interdental spaces between the upper incisors (7), suggesting the limitations of estimating MS infection in individuals by evaluating dental plaque on a limited number of tooth surfaces.

The number of tooth surfaces that should be examined to quantitatively assess MS in order to predict an individual’s risk of caries has not been defined. It is well-known that MS has a localized pattern of colonization in the dentition and can vary in prevalence at different sites on the same tooth surface (18,19). Therefore, it seems obvious that improved MS estimates will be obtained by investigating plaque samples from a larger number of tooth surfaces. In this study, similar MS estimates were obtained for approximal surfaces facing each other (Table 2). This is consistent with the report of Lindquist et al., who found that 80% of approximal surfaces had the same MS infection score, defined by the number of colony forming units on MSB agar plates (20). This suggests that distinguishing between MS estimates for approximal surfaces is not useful for improving the efficiency of MS detection; increasing the number of interdental spaces evaluated is more important than increasing the number of approximal surfaces.

The Dentocult SM® Strip mutans kit contains one plaque strip, with four pads, per incubation vial. The vial is large enough to hold two strips, so if an additional strip were provided, the plaque from eight approximal surfaces at eight interdental spaces could be evaluated. However, the manufacturer’s instructions state that only one strip should be incubated per vial. In this study, two strips were incubated per broth vial to evaluate plaque from eight approximal surfaces at eight interdental spaces simultaneously (new method). When the results were compared with those obtained by incubating one strip per broth vial (standard method), substantial agreement between the two methods was observed (Table 3). Moreover, the efficiency of detecting MS was improved significantly by increasing the number of detection sites for MS estimates. Based on these results, it can be concluded that plaque samples, collected from four approximal surfaces at even two interdental spaces in the primary molars, were significantly better for detecting MS than saliva sampling. Furthermore, evaluating MS infection at eight interdental spaces using the new Dentocult SM® method yielded more accurate individual caries risk information.

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References


