Validation and inter-examiner agreement of mutans streptococci levels in plaque and saliva of 10-year-old children using simple chair-side tests

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Since there are few validation studies of chair-side tests of oral mutans streptococci, we compared a commercially available strip test with the conventional laboratory assay. Two plaque samples obtained from the mesial surfaces of the upper right and lower left permanent molars of sixty-five 10-year-old children (boys = 38, girls = 27) were cultured and incubated using chair-side site strip tests (Dentocult SM, Orion Diagnostica). Two plaque sampling tools, namely dental floss and micro-brush, were compared, and inter-examiner agreement between recordings of three examiners was assessed. Paraffin-stimulated saliva was then collected for laboratory and chair-side assays. The plaque and saliva chair-side tests correlated well with each other (Spearman rho, $r = 0.72$) and with the laboratory method, showing coefficients of 0.76 and 0.80 for saliva and plaque, respectively. Compared to the laboratory method, the sensitivity (Sn), specificity (Sp), accuracy (A), and kappa (K) values of the salivary and plaque chair-side tests were 0.63, 0.75 (Sn), 0.93, 0.90 (Sp), 0.82, 0.85 (A), and 0.58, 0.66 (K), respectively. Agreement between the two plaque sampling techniques was good (0.91). Inter-examiner agreement of plaque scores ranged between 0.65 and 0.86 when all density categories were analysed separately; when dichotomized into low and high categories, complete agreement was found. Agreement between the plaque and saliva chair-side tests and the laboratory salivary assay was good, and in terms of sensitivity, accuracy, and kappa values, the site strip plaque test surpassed the salivary chair-side test. □ *Cariogenic species; children; chair-side methods; mutans streptococci; strip test*

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The current chair-side methods for assessing salivary and plaque mutans streptococci (*ms*) levels have been described and validated against the traditional conventional laboratory technique in separate studies (1–3). The chair-side tests have since been used for many purposes, e.g. in assessing of caries risk (4, 5), in monitoring the effect of antimicrobial treatment in children (6, 7), and in clinical follow-up studies (8, 9). Chair-side methods have also been successfully applied to verify the transmission of *ms* from mother to child (10, 11).

Antimicrobial medication, mouthwash rinses, orthodontic appliances, erupting 3rd molars, and eating just before sampling are known to affect the *ms* level in the oral cavity (12–17). However, diurnal variation of *ms* levels and variation during 3 consecutive days is limited when assessed by chair-side tests (18, 19).

The use of paraffin chewing to detach *ms* cells from tooth surfaces for the chair-side assessment of salivary *ms* levels is well established. Methods of collecting plaque samples, however, have varied. Toothpicks, dental flosses, dental instruments, Q-tips, and micro-brushes have been used (4, 6, 7, 20), but these methods have not been compared.

We could not find any reports on inter-examiner variation between recordings of chair-side tests or on validation studies in which salivary and plaque chair-side test results of the same individuals were compared with those obtained by conventional laboratory techniques. Using chair-side tests we therefore studied the salivary and plaque *ms* levels of the same subjects, and compared the results with each other and with the conventional laboratory assay.

Materials and methods

Subjects

The children in this study were participants in the Special Turku Coronary Risk Factor Intervention Project (STRIP), which is a prospective, randomized long-term dietary study. The STRIP families were recruited at well-baby clinics between 1990 and 1992. The intervention children received individualized counselling aimed at reducing the intake of saturated fat and cholesterol as described (21, 22). The control children received general dietary recommendations as currently given at Finnish well-baby clinics and schools. At 10 years of age, 66 healthy children were selected from the participants of the main project and in accordance with the past 10-year history of sucrose consumption. The study children represented the lowest and the highest 5% ($n = 66$, 38 boys) of sucrose intake. Selecting subjects with extreme sugar intake was thought would provide a large variation

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Fig. 1. The inoculated plaque site strip and the salivary strip are attached to the cap of the culturing vial. Note that the inoculated sides of the strips face outwards to allow free access of the growth medium.

of oral conditions, therefore increasing the reliability and reproducibility of the present results. The sucrose intake results in relation to dental health are reported elsewhere (23). One invited child refused to comply with the salivary sampling.

The study was approved by the Ethics Committee of Turku University and the Turku University Central Hospital. Informed consent was obtained from all parents and children.

Collection, culturing, and evaluation of plaque chair-side samples

All samples were obtained in the morning between 0800 and 1200 h. The participants were asked not to eat, drink, or brush their teeth during the hour prior to sampling. Six of the children had orthodontic bands on the upper 1st molars. Care was taken that no antimicrobial medications or mouthwashes had been used for 2 weeks before sampling. Plaque samples were collected from the mesial surfaces of the upper right and lower left 1st permanent molars, as correlation between *ms* level of molar plaque samples and saliva has proved to be highest here (24). Waxed dental floss firmly attached to a floss-holder was used for sampling. The floss was taken gently through the contact point with even back-and-forth motions and along the proximal surface of the tooth to the gingival margin. Using the floss, the sample was spread on one of the four roughened sites of the strip (Dentocult® SM, Strip mutans, Orion Diagnostica, Espoo, Finland). The procedure was repeated on the same surface and the sample spread on the adjacent site of the site strip. Using a new dental floss, the remaining two sites of the strip were inoculated with

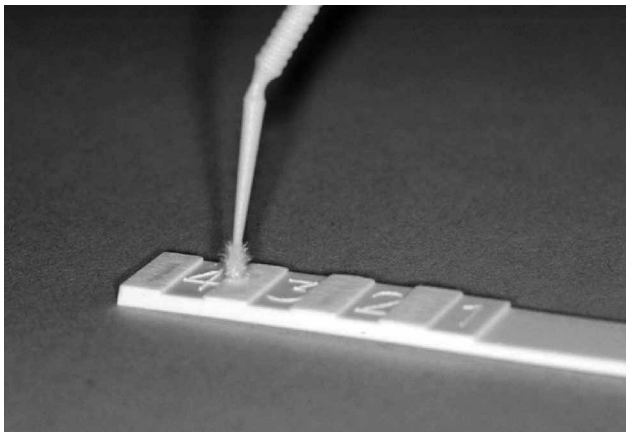

Fig. 2. Some of the plaque samples were collected using a microbrush (Microbrush® International Ltd. Clogherane, Dungarvan Co. Waterford, Ireland) and then spread on the site strip.

samples obtained from the lower molar, as described. The cultured strip was attached to the cap of the culturing vial (Fig. 1) and processed further as recommended by the manufacturer.

In 40 molars of 20 children, the level of plaque *ms* obtained by the floss method was compared with that collected using microbrushes (Microbrush® International Ltd., Clogherane, Dungarvan Co. Waterford, Ireland). Two consecutive samples were collected, one from the buccal margin of the upper right and another from the lingual margin of the lower left molar of the same child. The samples were spread on the site strip (Fig. 2) and incubated in selective Mitis salivarius bacitracin broth at 35–37°C. The manufacturer's current recommendation for minimum incubation time is 48 h, but the time can be prolonged to 96 h without any noticeable effect on the density of the colony forming units (CFU). We incubated the strips for 3 days, as the colonies became easier to evaluate along with the extended incubation. Immediately after incubation the strips were evaluated by comparing with the density chart provided by the manufacturer. The densities were evaluated by viewing the strips perpendicularly with the naked eye (Fig. 3). True colonies were distinguished from background staining by sideways inspection of the strips. Blue staining with spherical appearance and with an upward growing pattern was considered to be true bacterial growth. Four density categories, i.e. 0, 1, 2, and 3, were used for evaluating both saliva and plaque samples. According to the manufacturer, categories '0–1', '2', '3' correspond to $<10^5$,10^5–10^6, and $>10^6$ CFU/mL of saliva, respectively. To study inter-examiner agreement of site-strip test readings, three examiners (SK, ES, and KP) evaluated the incubated strips individually.

Collection, culturing, and evaluation of saliva samples

Chair-side saliva test. Paraffin-stimulated saliva was

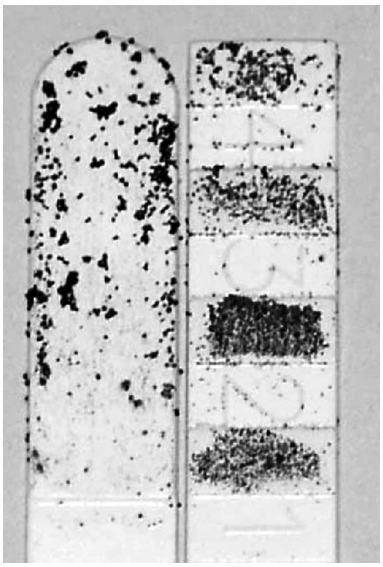

Fig. 3. After incubation, the strips were evaluated for density of colony forming units (CFU/mL) by comparing with the density chart provided by the manufacturer. The densities were evaluated by viewing the strips perpendicularly with the naked eye and, after drying for 2–3 days at room temperature, preserved for follow-up purposes.

collected as follows: the children were advised to chew on a piece of paraffin (1.0 g) for 1 min and to swallow the secreted saliva. The participants then continued to chew on the paraffin block at a constant pace for 5 min and spat the secreted saliva into graded test tubes. Aliquots of the saliva were transferred to a transport tube containing Tryptic Soy Broth with 10% glycerol w/v and stored at −70°C until used for the laboratory assay of *ms*. Earlier findings from our laboratory have shown that this procedure does not affect the viability of *ms* (25). After collecting the above sample, the roughened side of the strip designed for salivary mutans assay (Dentocult® SM, Strip mutans, Orion Diagnostica) was gently pressed against the tongue, turned around 10 times, and drawn out through lightly closed lips. The inoculated salivary strip was attached to the cap of the culturing vial already containing the site strip of the same subject (Fig. 1). Care was taken that the inoculated sides of the strips were always outwards to allow free access of the growth medium. The inoculated strips were incubated and evaluated as described above for the plaque samples.

Plate culturing method. For microbiological analysis of saliva, the transport tubes were thawed and vortexed thoroughly for 1 min. After serial 10-fold dilutions, the bacteria were plated on Mitis salivarius agars containing bacitracin (26). The plates were incubated for 2 days in a 7% CO_2 atmosphere at 37°C. Growth of *ms* was identified on the basis of colony morphology, and counted using a stereomicroscope. Enumeration of *Streptococcus mutans* and *Streptococcus sobrinus* if present was performed as described (27). The total number of *ms* expressed as logarithmic units per mL of saliva was compared to the results of the chair-side tests.

Table 1. The World Health Organization formula (29) was modified and used to calculate the sensitivity (Sn), specificity (Sp), accuracy (A) and kappa (κ) scores of the chair-side tests

	Dichotomized plate culturing method		
Chair-side test	*ms*-high	*ms*-low	Total
ms-high	a	b	a + b
ms-low	c	d	c + d
Total	a + c	b + d	a + b + c + d

Sn = a/(a + c); Sp = d/(b + d); A = (a + d)/(a + b + c + d); κ = (Po−Pe)/(1−Pe), Po = (a + d)/(a + b + c + d); Pe = [[(a + b) × (a + c)] + [(c + d) × (b + d)]]/(a + b + c + d)^2

Validation analysis and statistical methods

Scores '0' and '1' of the chair-side test were considered low values for the salivary test, while scores '≥2' were considered high (28). Accordingly, the logarithmic value of 4.5, closer to Dentocult category 2 than 1, was used as a cut-point between '*ms*-high' and '*ms*-low' salivary values of the laboratory assay. As the CFU values were always 10 or 100 times higher in plaque than in saliva, and as the proportion of subjects with high salivary *ms* scores (n = 18) coincided best with those with high plaque *ms* scores (n = 22) when the cut-point was adjusted to '3', we used this density to distinguish between high and low *ms* values in plaque. If the density scores of the 'duplicate' plaque samples or those of the maxillary and mandibular samples were different (Fig. 3), the higher scores were always chosen as representing the plaque *ms* density of the subject.

Sensitivity (Sn), specificity (Sp), accuracy (A), and kappa (κ) scores were calculated from the formula (Table 1) by applying the World Health Organization guidelines (29). Sn = a/(a + c); Sp = d/(b + d); A = (a + d)/(a + b + c + d); κ = (Po-Pe)/(1–Pe), where Po is the proportion of observed agreement, i.e. (a + d)/(a + b + c + d), and where Pe is the proportion of agreement that could be expected by chance, i.e. (a + b) × (a + c) for *ms*-high subjects and (c + d) × (b + d) for *ms*-low subjects. Hence Pe is [[(a + b) × (a + c)] + [(c + d) × (b + d)]] / (a + b + c + d)^2. When there is total agreement, κ = 1. When there is total disagreement, i.e. a + d = 0, then κ = 0. A score of >0.8 indicates good agreement, 0.6–0.8 substantial agreement, and 0.4–0.6 moderate agreement.

Spearman correlation, chi-square, and kappa statistics were used to study correlation and agreement between the categorized chair-side density scores and logarithmic values. All tests were 2-sided and P values <0.05 were considered significant.

Table 2. Reliability of the dichotomized chair-side plaque and salivary tests as compared to the dichotomized plate culturing method, and to each other. Sensitivity, specificity, accuracy and kappa values were obtained from the formula described in Table 1

	Sensitivity	Specificity	Accuracy	Kappa
Chair-side saliva vs. chair-side plaque-*ms* scores	0.64	0.91	0.82	0.57
Chair-side saliva-*ms* scores vs. plate culturing method	0.63	0.93	0.82	0.58
Chair-side plaque-*ms* scores vs. plate culturing method	0.75	0.90	0.85	0.66
Dichotomized chair-side plaque-*ms* scores; dental floss vs. micro-brush	0.88	1.00	0.91	1.00

Results

The distributions of children with high and low salivary *ms* scores were 12 and 21 in the high sucrose intake group, and 6 and 26 in the low sucrose intake group, respectively. In other words, there were more children with high *ms* scores in the high sucrose intake group than in the low sucrose intake group, but the difference in the distribution was not significant ($P = 0.166$, chi-square).

The chair-side floss plaque and saliva strip tests correlated well with each other (Spearman rho, $r = 0.72$) and with the plate culturing method, showing coefficients of 0.76 and 0.80 for saliva and plaque, respectively. When the chair-side tests were compared with each other, the sensitivity, specificity, accuracy, and kappa values were 0.64 (Sn), 0.91 (Sp), 0.82 (A), and 0.57 (K) (Table 2). When the chair-side saliva and plaque tests were compared with the plate culturing method, the values were 0.63, 0.75 (Sn), 0.93, 0.90 (Sp), 0.82, 0.85 (A), and 0.58, 0.66 (K), respectively (Table 2). Accuracy between the dental floss and micro-brush techniques was good (0.91), but 9% of the sites revealing low values with the brush technique were high with the floss technique (Table 2).

Inter-examiner agreement of chair-side plaque *ms* readings between three examiners was complete when the density categories were dichotomized into low and high categories, and varied from 0.65 to 0.86 when all density categories were kept apart.

When the chair-side plaque and saliva tests were compared with each other, 53 of the test results, i.e. 14 with *ms*-high values and 39 with *ms*-low values, were similar with both tests (Table 3). On the other hand, 8 false negatives and 4 false positives were obtained with the salivary chair-side test as compared to the plaque chair-side test, yielding 12 test results with disagreement (Table 3).

Table 3. Cross-tabulation of dichotomized chair-side saliva and chair-side plaque-*ms* scores

Dichotomized chair-side saliva *ms* scores	Dichotomized chair-side plaque *ms* scores		Total
	ms-high (3)	*ms*-low (0–2)	
ms-high (2–3)	14	4	18
ms-low (0–1)	8	39	47
Total	22	43	65

Chi-square; $P < 0.001$.

Discussion

Our results show that the sensitivity, specificity, accuracy, and kappa values of the salivary chair-side test were good, but that those of the plaque chair-side test were even better. In addition, the scores of the plaque test were the same irrespective of the surface and the instrument used for sampling. For toddlers and preschool children unable to comply with paraffin chewing, the plaque chair-side test is more practical than the tongue blade technique, which requires laboratory facilities (30). Yet, for schoolchildren and adults capable of chewing paraffin, the use of both plaque and saliva chair-side tests may be advisable. For the time being, there are more studies and normal values available for salivary than for plaque *ms* levels. Hence, assessment of both levels is recommended to ensure evaluation and enable comparisons with earlier studies.

We showed complete agreement between examiners when the results were dichotomized into positive and negative categories; even when all density categories were kept apart, the inter-examiner variation was relatively small. Currently, estimation of caries risk and planning of cost-effective prevention programs for children are, apart from past caries experience, based on the assessment of plaque and/or salivary *ms*. However, uniform interpretation of the test results needs repeated calibrations among examiners. Hence, special attention should be focused on calibration when new recorders first use these chair-side tests. For clinical purposes, it is sufficient if among toddlers mutans carriers can be differentiated from non-carriers (4) and if among young mothers, those with high mutans levels can be differentiated from those with low values (10, 25).

The use of a stereomicroscope to improve interpretation of strip test results may be a good adjunct for scientific studies (7). In clinical work, however, this is seldom possible, and yet the chair-side methods have been developed for use in health-care settings. Reliable results can be obtained even when evaluating density levels with the naked eye. If necessary, a magnifying glass, readily available in most dental settings for sharpening hand instruments, may be used to facilitate evaluation.

We showed good, but not complete, agreement between the chair-side tests of saliva and plaque. The reasons for our obtaining false results with the chair-side tests can be discussed. Such conditions may appear in cases with impaired chewing capacity. For example, children in their later mixed dentition phase may have several mobile primary molars close to exfoliation. This condition can restrict effective chewing and reduce detachment of plaque and *ms* from teeth during paraffin chewing. Another possibility for obtaining false-negative results with the site-strip test is that the proximal surfaces used here for collecting plaque samples may not have been colonized by *ms*, contrary to fissures. Such a time period may exist between exfoliation of 2nd primary molars and full eruption of 2nd permanent bicuspids. At this stage of dental development, fissures of permanent molars may form a more suitable habitat for *ms* than the mesial surface temporarily exposed to the flushing effect of salivary flow. Partially erupted teeth can elevate the level of salivary *ms* (16).

We believe that agreement between plaque and salivary test results should be further studied in different clinical conditions, such as in cases of increased tooth mobility, decreased chewing capacity, and erupting molars.

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