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Variables influencing Streptococcus mutans testing

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Abstract

When saliva is sampled to estimate S. mutans, variables may influence the results. The purpose of the present study was to assess the reliability of the tongue depressor for saliva sampling, and whether the sampling time during the day influences S. mutans counts.

The study population consisted of 27 children, between 24 and 66 months of age. Samples of unstimulated saliva were gathered on tongue blades four times during the day for each subject. Paired samples representing both sides of the tongue blades were inoculated onto elevated agar plates containing a selective medium, and anaerobically incubated at 37°C for 48 hr. After examining 76 paired samples of saliva, no significant differences in S. mutans counts were found between sides of the spatula, suggesting that the sampling technique was suitable. Analysis of variance showed significant differences within each subject over the four sampling times ($P < .01$). A significant difference was found between subjects' daily averages ($P < .05$).

Introduction

It is generally accepted that the early establishment of *S. mutans* in the oral cavity indicates potentially early and extensive caries attack in the primary dentition (Alaluusua and Renkonen 1983; Kohler et al. 1984).

Consequently, investigations have focused on developing methods for sampling and estimating *S. mutans* counts that are clinically applicable to young children. A method utilizing a tongue blade for sampling was suggested by Kohler and Bratthall (1979). Newbrun et al. (1984) found this sampling technique extremely easy to use and demonstrated that children with high *S. mutans* counts had high DMFS increments over the previous four years. They found that the Mitus salivarius Bacitracin agar (MSBA) method for determining *S. mutans* correlated highly with dental caries, and that unstimulated saliva could be sampled with a tongue blade.

The tongue blade technique and the MSBA method for analysis have been used by others, who also showed high correlations between *S. mutans* counts and dental caries activity in young children. Edelstein et al. (1987) found a positive relationship with 28 children. Weinberger and Wright (1989) obtained similar findings in a very young population having only primary teeth. Edelstein and Tinanoff (1989), using the same technique, found a highly significant correlation between dental caries and *S. mutans* infection in children less than 6 years old.

The effect of sampling time during the day was investigated by Birkhed et al. (1981) working with lactobacillus counts. They demonstrated that counts were four times higher upon awakening than during the daytime. Carlsson (1965) showed that early morning saliva samples contained higher levels of streptococci than day samples. The findings of Togelius et al. (1984) differed. Using the micropipette method, they tested 72 adults and found no statistically significant differences in *S. mutans* counts between morning and afternoon.

The investigation of Newbrun et al. (1984) demonstrated both false negative and false positive test results. In the study of Weinberger and Wright (1989) and Edelstein and Tinanoff (1989) several nonconforming test results were obtained; i.e. the results did not fit the established *S. mutans*—caries relationship. It is possible that some of the sampling was inaccurate or the test results were affected by sampling times. Before a longitudinal study is begun, some of the variables that could affect the results require further investigation.

Methods and Materials

Petri dishes (Rodac plates) were prepared with an elevated level of mitis salivarius agar (Gold et al. 1973). Bacitracin (E. Fougera and Co., Melville, NY) solution was made from two, 10-unit Bacitracin™ disks which were dissolved in 1 ml of water (Edelstein and Tinanoff 1989). Bacitracin was added to the surface of the medium on the day of the study, using a sterile cotton swab that previously had been imbedded in the Bacitracin

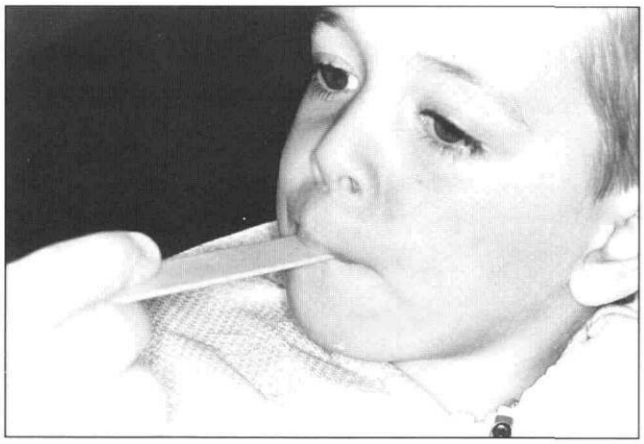

Fig 1. Saliva samples are obtained using a sterile tongue blade.

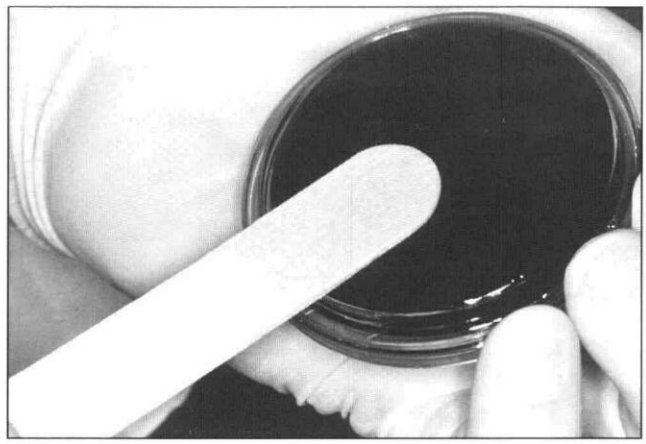

Fig 2. *S. mutans* growth (CFU) from both sides of the spatula.

solution. The plates were produced and refrigerated for storage (without Bacitracin) one week before the day of the study.

Twenty-seven children participated in the study. Informed consent was obtained for all subjects before the start of the study. Each child was scheduled for paired saliva samples at the following times: 1) within a half hr after breakfast, 2) mid-morning, having last eaten 1 to 1-1/2 hr previously, 3) before lunch with 1 hr elapsing since a mid-morning snack, and 4) following lunch and an afternoon nap, 1-1/2 hr since the last meal. Saliva samples were obtained using a 1.8 mm wood sterile spatula (tongue depressor). When saliva was sampled, about 3 cm of the spatula was introduced into the subject's mouth and both sides of the spatula were pressed against the tongue to gather a mixed sample (Togelius et al. 1984, Fig 1). To investigate the reliability of the sampling technique, each side of the tongue depressor was pressed onto the surface of a Rodac plate in opposite locations allowing for comparison of paired samples. Plates were sealed into plastic bags, and inflated with expired air; in this way, an enhanced $C0_2$ environment was obtained (Kohler and Bratthall 1979; Edelstein et al. 1987; Edelstein and Tinanoff 1989; Weinberger and Wright 1989). The plates were incubated at 37°C for 48 hr. Incubated plates were photographed on a light X-ray background to obtain a record of the bacterial colony growth.

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 forms a circle of approximately 1.5 cm^2 (Fig 2). The colonies were identified by their characteristic appearance according to the description of Jordan et al. (1968). Similar to previous studies, the *S. mutans* counts were divided into four ranges: 0, 1) 1-20, 2) 21-100, 3) >100 (Kohler and Bratthall 1979; Edelstein et al. 1987; Weinberger and Wright 1989). Analysis of variance was used to determine the reliability of the sampling technique and to assess the possibility of *S. mutans* levels varying during the day.

Results

The entire set of data for 27 subjects is provided in Table 1 (next page). The age, gender, number of teeth and each subject's daily low, high, and average scores of *S. mutans* is included. It is a nested data set because, although the subjects are independent from one another, the tests for the same subject during the day are all correlated. Note that 19 of the subjects were available at all time periods.

The *S. mutans* counts that were obtained during different time periods are offered in Table 1. These data demonstrate a large variation between subjects as the average counts ranged from 1.0 to 2.9. There is a statistically significant difference ($P < .05$) between the averages for subjects (Table 2). It also reveals that average test differences within subjects during the day were significant ($P < .01$).

Table 2 shows the nested analysis of variance for 19 subjects (next page). Note that data from 76 paired samples, representing the differences between sides of the tongue blade or tests within times was not significant. For the purpose of comparing times during the day, the average of these tests was used.

Table 3 identifies that two statistically significant time differences were mid morning vs. after lunch and before lunch vs. after lunch ($P < .01$, next page). In the mid morning-after lunch comparison, 13 subjects increased *S. mutans* counts after lunch, four were the same and five subjects' counts decreased. In the before lunch vs. after lunch comparison, nine subjects *S. mutans* counts increased, 10 were the same, and six decreased. There was a borderline difference between after-breakfast and mid-morning, which might be beyond chance if the sample had been larger. The number

Table 1. Data used for analysis of Strep Test

				Time of Day										
				After Breakfast		Mid Morning		Before Lunch		After Lunch				
Subject	*Age*	*Sex*	*TTH*	*#1*	*#2*	*#1*	*#2*	*#1*	*#2*	*#1*	*#2*	*Low*	*Average*	*High*
1	24	2	19	1	1	0	0	0	0	3	3	0	1.0	3
2	28	1	20	3	3	1	2	1	2	2	1	1	1.9	3
3	66	1	20	2	2	2	2	2	2	1	1	1	1.8	2
4	33	1	20	3	3	2	3	3	3	3	3	2	2.9	3
5	39	1	20	3	3	2	1	2	2	3	3	1	2.4	3
6	53	2	20	2	1	1	1	1	1	3	2	1	1.5	3
7	55	2	20	2	2	1	1	0	0	2	2	0	1.3	2
8	63	1	20	3	3	3	3	3	3	2	2	2	2.8	3
9	48	2	20	2	2	1	1	1	1	3	3	1	1.8	3
10	44	1	20	0	0	1	1	2	1	2	1	0	1.0	2
11	33	2	20	1	0	1	1	1	1	3	2	0	1.3	3
12	37	1	20	0	0	1	1	2	2	1	1	0	1.0	2
13	35	2	20	2	2	3	3	2	3	2	2	2	2.4	3
14	36	1	20	1	1	2	2	1	1	1	1	1	1.3	2
15	61	1	20	1	1	0	0	3	3	2	2	0	1.5	3
16	41	1	20	2	1	2	2	2	2	3	3	1	2.1	3
17	41	1	20	2	2	3	3	3	3	—	—			
18	42	2	20	3	3	2	2	2	2	2	2	2	2.3	3
19	43	1	20	3	0	2	2	1	2	1	1	0	1.5	3
20	53	2	20	2	1	1	1	2	2	2	2	1	1.6	2
21	24	2	16	1	1	1	1	—	—	1	1			
22	29	2	16	—	—	1	1	1	1	2	2			
23	25	1	12	—	—	1	1	2	2	2	2			
24	38	1	20	—	—	—	—	2	2	2	2			
25	39	1	20	—	—	—	—	3	3	3	3			
26	38	2	20	—	—	—	—	3	3	3	3			
27	47	1	20	—	—	—	—	1	1	3	3			

#1 and #2 refer to the tests done on the front and back of the spatula
— Subject not available

Table 2. Nested Analysis of Variance for 19 Subjects

Source	*DF*	*SS*	*MS*	*F*	*P*
Total	151	127.4737			
Subjects	18	48.7237	2.706872	2.31	<.05
Times within subjects	57	66.75	1.171053	7.42	<.01
Tests within times	76	12	.1578947	.26	N.S.
Tests within subjects	133	78.75	.5921053		

of pairs differs for each comparison because the full number of available pairs from Table 1 was used.

Discussion

Since the presence of *S. mutans* may be a good indicator of a patient's caries risk, studies have concentrated on a method of testing saliva in young children. For any method to be acceptable clinically, it has to have an appropriate sampling technique for young children, as well as a method for analyzing the sample in the clinical setting.

An aim of the present study was to assess the reliability of the tongue blade sampling technique. After sampling the saliva, both sides of the spatula were impressed on the MSBA medium and the *S. mutans* counts from both sides were compared. Since no statistically significant difference was found it indicated that the sampling technique has reliability. However, test results indicating a complete absence of *S. mutans* may be questionable in some cases. In the present investigation, six paired results were negative while at other times during the day the same subjects demonstrated a level of *S. mutans* infection. These findings could be the result of the sampling technique or the analyzing method. At two other test times, one side of the spatula was positive for *S. mutans* and the other side was negative. These results probably were caused by faulty sampling. Regardless of the cause, of the 76 paired samples (152 spatula impressions), it appeared that 5% of the samples were false negatives.

False negatives also were obtained in the investigations of Kohler and Bratthall (1979), Newbrun et al. (1984), and Weinberger and Wright (1989). In the study by Kohler and Bratthall (1979), it was found that the false negative cames represented 10,000 or less *S. mutans* per ml of saliva when the micropipette method was used.

The finding of variation in *S. mutans* counts during the day cannot be ignored. It has serious implications if these tests are to be used for prediction of dental caries activity in child patients. An initial concern is that the variation may be related to the type and time of the last meal. Differences in the mid morning–after lunch and before lunch–after lunch times were statistically significant ($P < .01$), and the difference between after breakfast and mid morning tended toward significance. Therefore, if food intake can influence *S. mutans* counts, a suggestion is that children should not eat, and drinking should be limited to water 1-1/2 to 2 hr before a caries activity test is performed.

TABLE 3. Tests of Average Paired Differences for Abridged Colony-Forming Scores

Sampling Times	*Pairs*	*Av Diff*	*SE*	*t*	*P*
Afterbreak vs. 2 Midmorning	21	0.333	0.199	1.673	NS
Afterbreak vs. 3 Bef-lunch	20	0.200	0.277	0.721	NS
Afterbreak vs. 4 Aft-lunch	20	−0.250	0.250	1.000	NS
Midmorning vs. 3 Bef-lunch	22	−0.182	0.193	0.940	NS
Midmorning vs. 4 Aft-lunch	22	−0.682	0.250	2.732	<.01
Before lunch vs. 4 Aft-lunch	25	−0.520	0.224	2.316	<.01

Another concern that is reflected by the data is the unpredictability of change in *S. mutans* counts. While the majority of children demonstrated an increase in counts after lunch as compared with before lunch, some counts did not change at all and other counts actually decreased. If *S. mutans* counts are to be of predictive value, the amount of *S. mutans* a child harbors at one point in time has to be comparable to that at another point in time (perhaps six months later). It is possible that the size of the increments in *S. mutans* may be a better predictor of impending dental caries than simply examining the count per se.

In the present study, the method of investigating colonies and the colony groupings (0; 1-20; 20-100; >100) were those used in previous studies (Kohler and Bratthall, 1979, Edelstein et al. 1987 and Weinberger and Wright 1989). It is possible that these groupings may be limiting, and grouping colonies on a wider range may be more meaningful. The investigators of the present study intend to repeat this investigation to explore the *S. mutans* count variability more fully.

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