Simplified sampling methods for estimating levels of lactobacilli in saliva in dental clinical practice

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The aim of the present study was to evaluate whether estimation of lactobacilli was possible with simplified saliva sampling methods. Dentocult® LB (Orion Diagnostica AB, Trosa, Sweden) was used to estimate the number of lactobacilli in saliva sampled by 3 different methods from 96 individuals: (i) Collecting and pouring stimulated saliva over a Dentocult® dip-slide; (ii) direct licking of the Dentocult® LB dip-slide; (iii) contaminating a wooden spatula with saliva and pressing against the Dentocult® dip-slide. The first method was in accordance with the manufacturer's instructions and selected as the 'gold standard'; the other 2 methods were compared with this result. The 2 simplified methods for estimating levels of lactobacilli in saliva showed good reliability and specificity. Sensitivity, defined as the ability to detect individuals with a high number of lactabacilli in saliva, was sufficient for the licking method (85%), but significantly reduced for the wooden spatula method (52%). □ *Cooperative behavior; dip-slide; methodology; saliva microbiology*

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In addition to clinical examination, microbiological analyses are valuable when planning individual treatment and prevention of dental caries (1). Studies have identified dietary habits, in particular the consumption of sugar between meals, as one of the most important factors in the etiology of dental caries (2–4). The number of oral lactobacilli is related to the carbohydrate intake (5–7). Grubb & Krasse (8) have shown that there is a good correspondence between lactobacilli in dental plaque and saliva. It is therefore of interest to estimate the number of lactobacilli in saliva in the prediction of dental caries, and to analyse the compliance of dietary counselling.

During recent decades, simple dip-slide techniques (9) for estimating the number of lactobacilli in saliva have become available; for example, CarioCheck®-LB (Hain Diagnostika), Dentocult® LB (Orion Diagnostica), and CRT Bacteria (Vivadent). The commonly used dip-slide techniques for estimating lactobacilli require collection of whole saliva, which may be difficult to obtain in certain patients, e.g. mentally retarded individuals, people with dementia, and young children. Different methods for estimating the number of lactobacilli in the oral cavity were described in the classic Vipeholm study (8). Köhler & Bratthall (10) described a method for estimating Streptococcus mutans in saliva by turning a wooden spatula around in the mouth. The spatula, contaminated by saliva, was pressed against a selective agar plate. After cultivation, the number of colonies was comparable to the conventional saliva samples. This method has been developed and is used in a dip-slide technique (Dentocult® SM, Orion Diagnostica, Trosa, Sweden) in the estimation of the number of mutans streptococci in saliva (11, 12).

The aim of this study was to investigate whether it was possible to estimate the number of lactobacilli in saliva in clinical conditions using simplified sampling methods.

Material and methods

Dentocult® LB (Orion Diagnostica AB, Trosa, Sweden) was used to estimate the number of lactobacilli in saliva. In accordance with the manufacturer's instructions, paraffin-stimulated saliva was collected and then poured over the Dentocult® LB dip-slide. This method was chosen as the 'gold standard'. Two simplified sampling methods were compared with this conventional method: (i) sampling of saliva was carried out by licking directly on each Dentocult® LB dip-slide (licking method), and (ii) an 18-mm wide wooden spatula was contaminated with the subject's saliva by being introduced into the mouth and turned around 10 times. Each side of the spatula was then pressed once against the Dentocult® LB dip-slide (spatula method).

In a preliminary study, an investigation was carried out to evaluate whether an individual's counts of lactobacilli could be influenced by frequent saliva sampling. Five persons delivered saliva 5 times at intervals of 10 min in the following sequence: (a) a spatula was contaminated with the subject's saliva and pressed against the Dentocult® LB dip-slide; (b) saliva was sampled by licking directly on each Dentocult® LB dip-slide; (c) paraffin-

Table 1. Sequence of 5 samplings using 3 methods at 10-min intervals, and classified in accordance with the manufacturer's instructions

Subject	Spatula method	Licking method	Stimulated saliva	Licking method	Spatula method
AA	2	3	3	3	3
BB	2	2	2	2	2
CC	2	3	3	3	2
DD	2	2	2	3	2
EE	2	2	2	2	2

stimulated saliva was sampled in accordance with the manufacturer's instructions; (d) saliva was sampled by licking directly on each Dentocult® LB dip-slide; and (e) a spatula was contaminated with the subject's saliva and pressed against the Dentocult® LB dip-slide.

Ninety-six individuals between 15 and 75 years of age, all able to follow directions, participated in the first part of the study. All of them had well-maintained dentition without open carious lesions and were found to represent varying numbers of salivary lactobacilli. Each subject delivered saliva in accordance with the 3 sampling methods, at 10-min intervals for each sampling and in the following sequence: (a) sampling of paraffin-stimulated saliva in accordance with the manufacturer's instructions, (b) sampling of saliva by licking directly on each Dentocult® LB dip-slide, and (c) contamination of a spatula with the subject's saliva and the spatula pressed against the Dentocult® LB dip-slide.

In the second part of the study, 11 persons who had had $\geq$10,000 cfu/ml saliva in the first part were asked to deliver 3 new tests by licking on the substrate, and 3 tests by contaminating the spatula with saliva. The tests were collected on the same occasion with 10 min between each sampling. All tests were transferred to the bacteria substrate as described above.

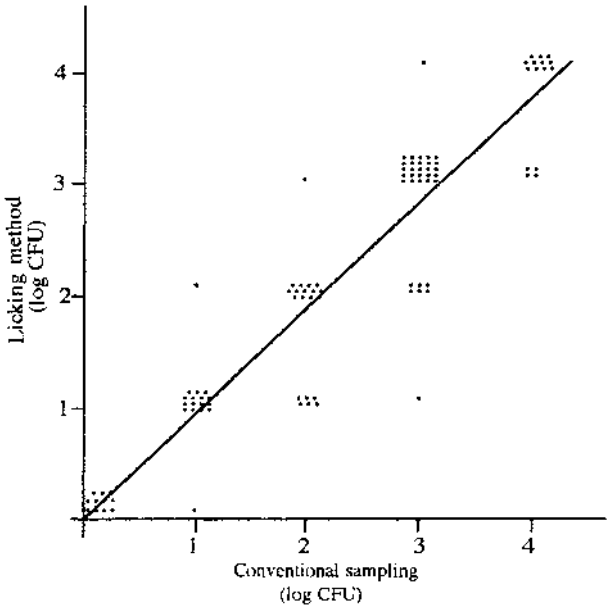

Fig. 1. Correlation between conventional sampling of lactobacilli and sampling by licking directly on the dip-slide ($n = 96$). Correlation coefficient = 0.929.

The cultivation was carried out at 37°C in an incubator for 4 d. The density of colonies was evaluated in accordance with the manufacturer's instructions. When no growth at all was detected the dip-slide was classified as 0. All tests were identified with a randomized number and evaluated by 2 examiners independently. When disagreement occurred, one of the examiners made the conclusive classification.

The relationship between the sampling methods was studied using a simple regression test, and their reliability analysed using the Friedman test, i.e. Stat View 4.01 (Abacus, Berkeley, CA, USA) on a Macintosh Performa 5400/160 with a 5% level of significance. The study was approved by the Ethics Committee, Faculty of Medicine, Uppsala University.

Results

The result of the preliminary study of 5 subjects is given in Table 1. The frequent saliva sampling or the succession of the sampling methods did not seem to influence the growth of lactobacilli on the dip-slide.

In the first part of the primary study, 8% of the slides differed in classification between the 2 examiners. In the second part, the examiners had different opinions in 9% of cases. The disparity between the observers was never more than one Dentocult class.

Growth of lactobacilli after licking directly on the dip-slide showed good correlation with the conventional method (correlation coefficient 0.929, $P = < 0.0001$). Three (3%) of the evaluated slides showed a higher Dentocult class and 18 (19%) a lower Dentocult class compared with the conventional method. One slide differed by more than one class (Fig. 1). The spatula method showed a lower correlation with the conventional method (correlation coefficient 0.865, $P = < 0.0001$). Two of the slides showed a larger number of lactobacilli compared to the 'gold standard'. In 45 slides (47%), a lower Dentocult class was registered compared to the method recommended by the manufacturer . Of these, 5 slides differed 2 classes (Fig. 2). In 11 subjects the conventional saliva sampling showed no growth of lactobacilli at all. Both simplified sampling methods registered the same result.

When the conventional sampling method showed

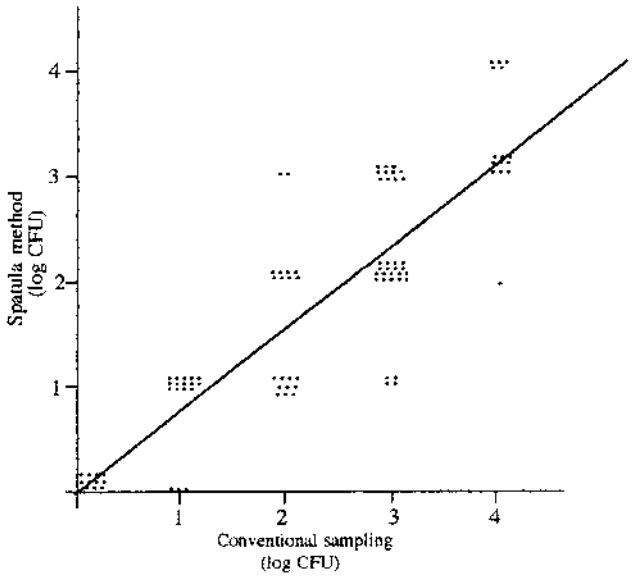

Fig. 2. Correlation between conventional sampling of lactobacilli and sampling by contaminating a wooden spatula ($n = 96$). Correlation coefficient = 0.865.

growth of lactobacilli, the licking method detected growth in all tests except one. The spatula method could not detect lactobacilli in 3 of the tests. Consequently, the sensitivities for the licking and spatula sampling methods were 99% and 96%, respectively. More valuable clinically is the ability to identify subjects with high values of lactobacilli ($\geq$100,000 cfu/ml saliva). The sensitivity of detecting individuals with a high number of lactobacilli (Dentocult classes 3 and 4) was 85% for the licking method and 52% for the spatula method. The specificities for identification of high values of lactobacilli for the licking and spatula methods were 98% and 96%, respectively.

In the second part of the study, the 3 tests sampled in the same way were compared for each individual. The correspondence was high for both the licking method ($P = 0.9341$, Table 2) and the spatula method ($P = 0.8150$, Table 3).

Table 2. Sequence of 3 saliva samplings by licking on the dip-slide at 10-min intervals, and classified in accordance with the manufacturer's instructions

Subject	First sampling	Second sampling	Third sampling
A	1	1	1
B	2	2	2
C	1	1	1
D	1	1	1
E	3	2	2
F	0	0	0
G	0	0	0
H	4	4	4
I	2	2	2
J	2	2	3
K	2	2	2

Table 3. Sequence of 3 saliva samplings by contaminating a wooden spatula at 10-min intervals, and classified in accordance with the manufacturer's instructions

Subject	First sampling	Second sampling	Third sampling
A	1	1	1
B	1	1	1
C	1	1	1
D	1	1	1
E	2	1	2
F	0	0	0
G	0	0	0
H	2	3	3
I	1	1	1
J	2	3	3
K	2	2	2

Discussion

With the simplified sampling methods investigated in this study, a lower number of individuals with lactobacilli in saliva was detected than with the sampling recommended by the manufacturer. For the samples taken from the same individual, however, both simplified sampling methods had a high reproducibility.

Several studies have shown that the Dentocult® LB dip-slide technique handled in accordance with the manufacturer's instructions is comparable to cultivating lactobacilli in a microbiological laboratory (9, 13, 14).

It might be expected that saliva collected conventionally by chewing paraffin wax would include more bacteria from dental plaque than saliva collected passively. Köhler & Bratthall (10) reported a higher correspondence between sampling methods when paraffin wax chewing occurred first, before the spatula sampling, compared to the opposite sequence. In our study, however, no clear differences occurred when comparing samples of stimulated saliva with saliva samples collected by licking or by the spatula method. Nor did the sampling order influence the results. One explanation could be that lactobacilli are found not just on the tooth surface but also in considerable amounts on the tongue and oral mucosa (15). Thus the amount of lactobacilli released from the teeth during the stimulation of chewing may not be the determining factor in the estimation of lactobacilli in saliva samples. This hypothesis is supported by the observations of Crossner & Hagberg (13), who showed that tooth-brushing had little influence on lactobacilli counts. Grubb & Krasse (8) collected dental plaque after whole saliva was sampled and estimated the number of lactobacilli after cultivation. The counts of lactobacilli were compared with the counts after the inverted sequence of sampling and no differences could be detected. It is possible that for persons with open carious lesions a difference in lactobacilli counts will occur when saliva sampled with the stimulated method is compared to passively collected saliva samples.

Crossner & Hagberg (13) reported that after sampling of saliva twice from 10 dental students at 5-min intervals, no

differences in the counts of lactobacilli could be detected. In addition, the results in the different parts of this study indicate that the frequent sampling did not influence the lactobacilli counts.

Compared to the Birkhed et al. study (14), a high agreement in the dip-slide classification was observed between the two examiners. Birkhed et al. reported a higher agreement when cultivation was carried out at 37°C than at room temperature. An explanation of the higher values in this present study could be that all the dip-slides were cultivated in an incubator at 37°C. When different samples from the same patient are analysed, these should preferably be analysed by the same examiner.

Licking directly on the Dentocult® LB substrate had a higher correspondence with the number of lactobacilli in saliva determined by the 'gold standard' method compared with the spatula method. Köhler & Bratthall found (10) that when paraffin-stimulated saliva sampling was done before spatula sampling, Streptococcus mutans was detected by the spatula method, with one exception. When the spatula sampling was done before paraffin stimulation, Streptococcus mutans was detected with the spatula method in all but 6 cases (total 37 subjects). In our study, the licking method compared to the conventional sampling detected all individuals with growth of lactobacilli, except one. When the purpose of the saliva sampling is to identify individuals with a high number of lactobacilli, the licking method, detecting 85% of subjects, is useful. The spatula method could only detect 52% of the individuals with a high number of lactobacilli, so the usefulness of this method is therefore limited.

Despite the quantitative differences between the conventional and alternative methods in this study, the simplified methods, especially licking directly on the dip-slide, can be justified when examining individuals who cooperate poorly, i.e. people with mental retardation or dementia, or young children. Of the two simplified methods, the spatula method demands less cooperation. Thus, for individuals with extremely poor ability to cooperate, the spatula method could be a valuable tool.

An important examination in the clinical situation is comparing one individual's results from one time to another. This study shows that both of the simplified methods had a high reproducibility and could be useful as long as the sampling is done by one of the described methods. However, it is important to remember that for some of the persons examined a conventional sampling method would have detected a higher number of lactobacilli.

The two simplified methods for estimating levels of lactobacilli in saliva tested in this present study showed good reliability and specificity. If sensitivity was defined as the ability of the sampling method to detect any growth of lactobacilli, both methods had high values. If the definition was the ability to identify individuals with a high number of lactobacilli only, the licking method had adequate sensitivity.

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