Intra- and inter-individual variation in salivary flow rate, buffer effect, lactobacilli, and mutans streptococci among 11- to 12-year-old schoolchildren

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Both intra- and inter-individual variation in salivary flow rate, buffer effect, and the levels of salivary mutans streptococci and lactobacilli were analyzed in 128 11-year-old children. The follow-up period was 9 months, with six saliva samplings done at regular intervals. Inter-individual variation was relatively large in paraffin-stimulated salivary flow rate: low (<1.0 ml/min) and high (≥2.0 ml/min) flow rates were measured in 18% and 13% of the children, respectively. Intraindividual variation during the follow-up period was found in 63% of the boys and in 73% of the girls. The buffer effect stayed stable in all samplings in 59% of the boys and in 42% of the girls. Buffer effect was significantly ($p < 0.001$) lower in girls than in boys. Mutans streptococci were analyzed by a chair-side method (Strip mutans test) and by cultivation on mitis-salivarius-bacitracin (MSB) agar plates. The results of the two methods correlated highly significantly ($r = 0.79$, $p < 0.001$). With the Strip mutans test no variation in test scores occurred in 49% of all subjects in all six samplings, whereas the respective percentage for MSB scores was only 19%. No variation in salivary lactobacilli occurred in only 18% of the subjects, and in 13% the intraindividual variation was as high as ≥3 logs. These results show that in young teenagers with a developing dentition, simultaneous changes in behavioral, hormonal, and dietary factors make single-point measurements of salivary factors too unreliable for caries-diagnostic or predictive purposes. □ *Diagnostic methods; physiologic variability; puberty; saliva*

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Salivary counts of mutans streptococci and lactobacilli, combined with the measurement of the salivary flow rate and buffer effect, are frequently used for diagnostic and predictive purposes in cariology (1–4). However, the variability of these tests on an individual level is not well documented. For the purpose of diagnosing and predicting caries, the intra- and inter-individual variation is particularly important at the time of tooth emergence, which allows new tooth surfaces to become colonized by cariogenic bacteria, such as mutans streptococci and lactobacilli (5). In permanent dentition, variations in bacterial counts at the time of eruption and maturation of second molars and premolars are of special interest. These early teen years are interesting also because at this age the diagnostic saliva tests may be of particular value in identifying patients at increased risk for dental caries (6, 7). However, among the confounding factors may be puberty-related changes in the levels of female sex steroid hormones, known to affect buffer effect (8) and counts of mutans streptococci (9).

Depending on the age group, time and method of saliva collection, and storage conditions, rather small (2, 10–12) or relatively large (13) intraindividual variations in the numbers of salivary mutans streptococci have been reported. Such variations in flow rate and buffer effect have been described recently by Söderling et al. (8). In this study we have analyzed both intra- and inter-individual variations in salivary flow rate, buffer effect, mutans streptococci, and lactobacilli among 128 young teenagers during a period of 9 months. We also compared the chair-side test (Strip mutans) and conventional agar plate method to quantitate salivary

mutans streptococci, since both methods are frequently used for caries-diagnostic and -predictive purposes.

Subjects and methods

Subjects and dental examinations

The study group comprised 128 schoolchildren (68 boys, 60 girls) who were 11 years old in 1990 when the study was initiated. All children lived in the city of Salo and its near surroundings. The amount of F^- in drinking water ranged from <0.3 to 1.0 ppm. Children with orthodontic appliances were excluded from the study group.

The data of base-line dental examinations and treatments in 1990 were collected from the records of the Salo Health Care Center, where these examinations had been done by in total six dentists. In the visual and tactile examination, only cavities extending to dentin were included in the decayed, missing, filled-teeth surfaces (DMFS) index. Radiographs were not taken. All diagnostic, preventive, and treatment measures were implemented in accordance with the guidelines and instructions of the National Board of Health in Finland. None of the children in this study population used chlorhexidine during the follow-up period. Because this study was not specifically designed to explore possible associations between clinical and salivary variables, no calibration of the clinical recordings by various dentists was done. Follow-up clinical caries recordings were made by the same dentists who did the base-line examinations 1 year (±1 month) earlier. DMFS values for boys and girls at the base-line examination are given in Table 1.

Salivary determinations

Paraffin-stimulated whole saliva was collected into a graduated tube for 5 min, during each visit at the same time of the day from the same individual. Before collection, each subject refrained from eating or drinking for 1 h, but toothbrushing was allowed in the morning. During the 9-month follow-up period five follow-up saliva samples were collected at regular intervals. All saliva analyses during and after each sampling were made by the same dentist (H. Tukia-Kulmala), experienced in doing and analyzing saliva tests, to prevent interindividual variations in the interpretation of the test results. The amount of saliva was measured to a precision of 0.1 ml, and the flow rate expressed in ml/min. The flow rate was grouped as low (≤1.0 ml/min), intermediate (1.1–2.0 ml/min), and high (>2.0 ml/min). Salivary buffer effect was assessed immediately after the collection using the Dentobuff strip method (14), in accordance with the manufacturer's instructions (Orion Diagnostica, Espoo, Finland). For statistical purposes, the buffer effect was classified as low with a final pH of saliva ≤4.0, intermediate with pH 4.5–5.5, and high with pH ≥ 6.0.

Mutans streptococci (MS) were quantitated immediately after saliva sampling by means of a commercial Dentocult SM Strip mutans test (15). The classification of test

Table 1. Clinical caries data and salivary flow rate and mutans streptococci of the study population at base line of the 9-month follow-up period

	Boys (n = 68)		Girls (n = 56)		
	Mean	SD	Mean	SD	Significance*
DMFS	0.63	1.43	0.70	1.74	NS
DS_{inc}†	0.16	0.41	0.37	0.87	NS
Flow rate (ml/min)	1.64	0.57	1.48	0.53	NS
Mutans streptococci‡	5.34	5.64	5.59	5.94	NS

* Student's t test; NS = not significant.
† New decayed surfaces (caries increment) during the 1-year follow-up period.
‡ Log colony-forming units/ml; mutans streptococci quantitated with MSB agar plates.

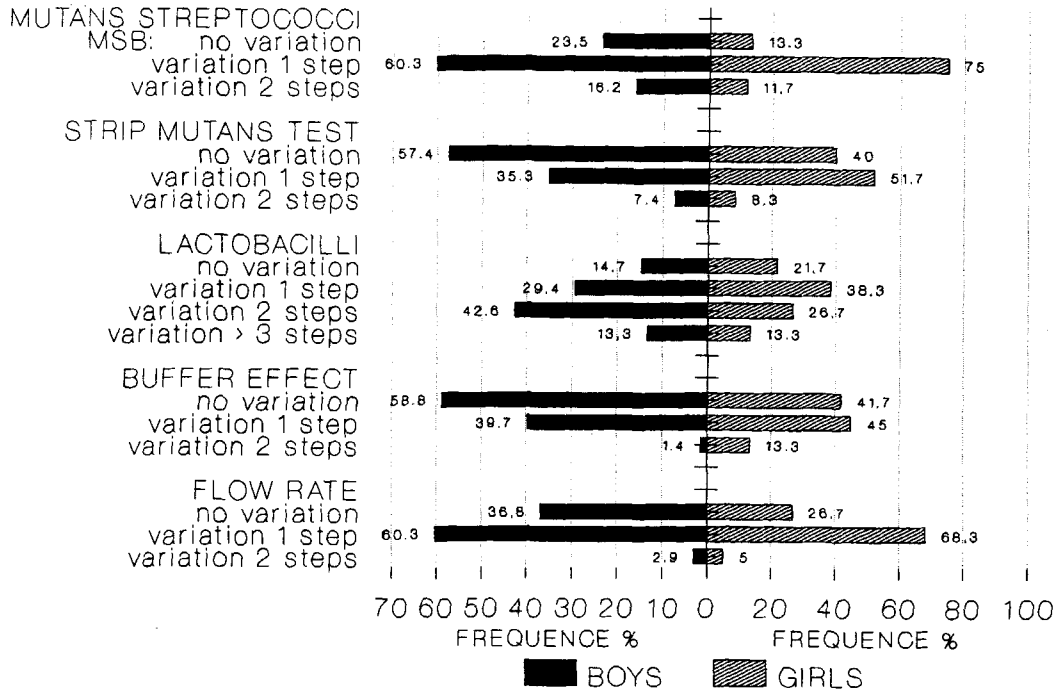

Fig. 1. Frequency distribution (in percentages) of intraindividual variations during the 9-month follow-up period (6 samples) in salivary mutans streptococci, lactobacilli, buffer effect, and stimulated flow rate in a group of 128 children. The steps are described in detail in the Methods section.

scores 0–1, 2, and 3 corresponded to $<10^5$, $\geq 10^5$–$<10^6$, and $\geq 10^6$ colony-forming units (CFU)/ml, respectively (15). For laboratory analysis, 100 µl of whole saliva was immediately transferred into a tube containing 1 ml tryptic soy broth (Oxoid, Basingstoke, U.K.) supplemented with 20% glycerol. The samples were stored frozen (−20 °C) for 3–4 weeks and then thawed and mixed vigorously for 20 sec with sterile glass beads. For quantitation of MS the suspension was diluted 1:10 and 1:100, and 10 µl was plated in duplicate on mitis-salivarius agar supplemented with 15% sucrose and 0.2 U bacitracin per milliliter. The colonies were counted after 3 days' incubation in candle jars at 37 °C. The CFU/ml of $<10^5$ were considered low, $\geq 10^5$–$<10^6$ intermediate, and $\geq 10^6$ high levels of salivary MS. These steps were used for the analysis of the frequency distribution (Fig. 1 and Table 2), but the base-line levels (Tables 1 and 3) and the correlation between the two methods (Table 4) were calculated from individual CFU values.

Salivary lactobacilli were estimated immediately after saliva sampling with a dip-slide test, Dentocult-LB, as described by Larmas (16). In counts of lactobacilli the following classification was used: $<10^3$, 10^3, 10^4, 10^5, and $\geq 10^6$ CFU/ml (17).

Ten samples were excluded from microbiologic analyses because of a recent (less than 1 month) history of antibiotics, four of them in the first sample collection. In these 10 cases, the variations in salivary MS and lactobacilli were calculated from 5 different saliva samplings. All base-line values for the studied salivary variables are given in Tables 1 and 3.

Statistics

Spearman rank correlations coefficients, Student's *t* test, and the chi-square test were

Table 2. Mean frequency distribution, standard deviation (SD), and range in percentages of inter-individual variations in different salivary variables during the 9-month follow-up period (in total, 6 examinations) in a group of 128 children. The variables are described in detail in the Methods section

	Boys ($n = 68$)			Girls ($n = 60$)		
	Mean	SD	Range	Mean	SD	Range
Mutans streptococci						
MSB agar plates						
Low	57.9	13.5	(33.3–74.6)	42.3	11.6	(22.2–57.4)
Intermediate	32.8	7.6	(20.6–44.4)	41.0	5.3	(35.2–50.0)
High	9.3	6.6	(1.6–22.2)	16.7	11.4	(7.4–38.9)
Strip mutans test						
0–1	64.3	4.7	(57.1–69.8)	46.1	2.0	(43.6–49.1)
2	22.2	2.7	(17.5–25.4)	32.1	3.6	(27.3–38.2)
3	13.5	3.3	(7.9–17.5)	21.8	2.8	(18.2–25.4)
Lactobacilli						
$<10^3$	18.0	3.2	(15.6–25.0)	16.7	3.4	(12.5–21.4)
10^3	27.1	5.6	(18.7–34.4)	29.2	1.3	(21.4–30.4)
10^4	20.3	4.4	(12.5–26.6)	21.1	5.8	(12.5–30.4)
10^5	18.0	5.5	(9.4–26.6)	18.1	3.6	(14.3–23.2)
$\geq 10^6$	16.7	4.5	(10.9–21.9)	14.9	3.5	(8.9–19.6)
Buffer effect						
Low	0.5	0.7	(0.0–1.5)	5.3	3.2	(0.0–10.0)
Intermediate	19.6	6.2	(10.4–28.4)	38.9	6.7	(31.7–48.3)
High	79.9	5.6	(71.6–88.1)	55.8	6.1	(48.3–65.0)
Flow rate						
Low	20.1	2.5	(16.2–23.5)	25.4	8.1	(16.9–42.4)
Intermediate	58.6	3.6	(54.4–64.7)	59.3	4.3	(54.2–62.7)
High	21.3	5.7	(11.8–27.9)	15.3	6.2	(3.4–22.0)

used for statistical analyses. Significance was claimed at $p < 0.05$.

Results

In the beginning of the 9-month follow-up period DMFS indices or salivary variables did not differ statistically significantly between boys and girls, except for buffer effect, which was significantly higher ($p < 0.001$) among boys (Tables 1 and 3). The mean value for the flow rate was also higher among boys, but owing to the large interindividual variation the difference was not statistically significant. Interindividual variations were large also for cariogenic bacteria, especially for salivary MS (Table 1).

Correlations among salivary variables are shown in Table 4. First, the two methods to quantitate salivary MS—that is, the chair-side Strip mutans test and the laboratory cultivation with mitis-salivarius-bacitracin (MSB) agar plates—correlated positively ($r = 0.79$) and highly significantly ($p < 0.001$) with each other. Of the 124 samples in the base-line examination, 4 gave the lowest score ($<10^5$ CFU/ml) with MSB agar plates; in the Strip test these same samples gave the highest score. In contrast, none of the highest scores ($\geq 10^6$ CFU/ml) with MSB was associated with the lowest score in the Strip method.

Correlations between MS and lactobacilli were statistically significant both in children without earlier caries ($r = 0.35$, $p < 0.01$) and in those with earlier caries ($r = 0.50$, $p < 0.001$). In only one subject was the lactobacillus score very high ($>10^6$ CFU/ml) when the MS score was low ($<10^4$ CFU/ml, $n = 42$). All children with the highest MS count ($\geq 10^6$ CFU/ml, $n = 7$) also had at least 10^4 CFU/ml of lactobacilli. The number of MS correlated negatively with salivary

Table 3. Distribution of salivary lactobacilli, mutans streptococci (Strip mutans test) and buffer effect in different classes among the study population at base line of the 9-month follow-up period. The classes are described in detail in the Methods section

	Boys ($n = 68$)	Girls ($n = 56$)	Significance*
Lactobacilli			
$<10^3$	11	7	
10^3	19	14	
10^4	12	14	
10^5	13	9	
$\geq 10^6$	13	12	NS
Strip mutans test			
0–1	45	25	
2	15	19	
3	8	12	NS
Buffer effect			
Low	1	4	
Intermediate	7	17	
High	60	35	$p < 0.001$

* Chi-square test; NS = not significant.

flow rate ($r = -0.26$, $p < 0.01$), but a statistically significant positive correlation ($r = 0.51$–0.60, $p < 0.001$) was found between salivary buffer effect and flow rate, both among boys and girls (Table 4). Low buffer effect (final pH ≤ 4) was never found among subjects with the highest flow rate (>2.0 ml/min, $n = 17$), but high buffer effect (final pH ≥ 6) was measured in 10 subjects even though the salivary flow rate was low (<1.0 ml/min, $n = 23$).

The clinical caries indices, base-line DMFS, and 1-year caries increment (DS_{inc}), correlated positively and statistically significantly ($p < 0.001$, Spearman rank correlation) with base-line MS (CFU/ml) and lactobacilli, whereas an inverse relationship ($p < 0.001$) was found with base-line values for the flow rate and buffer effect (data not shown).

Intraindividual variations in salivary variables during the 9-month follow-up period are shown in Fig. 1. The overall variation of salivary MS counts was lower with the Strip method than with MSB cultivation (Fig. 1): no variation (that is, less than one step) in the six saliva samplings occurred in 49% of the strips but in only 19% of the MSB plates. Salivary lactobacilli varied intraindividually even more than MS. In

Table 4. Correlations between salivary levels of mutans streptococci assayed with mitis-salivarius-bacitracin (MSB) agar plates and the Strip mutans test, the level of lactobacilli, salivary flow rate, and buffer effect. The correlations are calculated from the base-line values (boys, $n = 68$; girls, $n = 56$)

	Mutans streptococci (Strip mutans test)	Lactobacilli	Flow rate
Mutans streptococci (MSB)	0.79 $p < 0.001$*	0.35† $p < 0.01$ 0.50‡ $p < 0.001$ 0.45§ $p < 0.001$	−0.26 $p < 0.01$
Buffer effect, boys			0.51 $p < 0.001$
Buffer effect, girls			0.60 $p < 0.001$

* Spearman rank correlation.
† Subjects without caries experience ($n = 62$).
‡ Subjects with caries experience ($n = 62$).
§ All subjects ($n = 124$).

13.3% of both boys and girls the variation was equal to or higher than three steps, corresponding to a change ≥3 logs in CFUs/ml (Fig. 1).

Intraindividual variation in salivary flow rate was slightly more common among girls than boys: the scores stayed the same in all samplings in 37% of the boys and in 27% of the girls (Fig. 1). The respective values for buffer effect were 59% and 42%. Of all the four salivary variables the buffer effect was clearly the most stable and lactobacilli the least stable over the 9-month follow-up period, both in boys and girls.

Discussion

The chair-side method (Strip mutans test) to quantitate salivary MS correlates highly significantly with the conventional culture technique with MSB agar plates, as shown also by Jensen & Bratthall (15). However, the intraindividual variation was notably lower with the chair-side test, even though a similar stepwise scale was used for both methods. The difference may be due to the freezing of the samples before the cultivation on MSB agar plates, whereas the Strip test is done from fresh samples. Because even with the Strip test 51% of the children had a variability of at least one step and, of these, 7.8% more than two steps, this result suggests that measurements of MS at this age are highly variable for an individual patient. The problem is even greater with the salivary lactobacillus count, which showed variation of ≥1 log in more than 80% of all individuals.

Minor (10, 12) but also significant (18) differences between morning, late morning, and afternoon samples of salivary MS have been reported. Using the Strip method, El-Nadeef & Bratthall (12) showed that short-term intraindividual variations in salivary MS during a 5-day period certainly occur, but pronounced discrepancies are extremely rare. Russel et al. (11) reported that salivary MS correlated significantly over three examinations during 1 year in a group of adolescents. In a 3-year longitudinal study, Alaluusua et al. (2) showed that 30% of children maintained the same MS score but 20% deviated more than two scores in a dip-slide test (not the Strip test) when done four times in 3 years.

As expected, the base-line levels of salivary MS and lactobacilli correlated positively with both past caries experience (DMFS index) and with 1-year caries increment (DS_{inc}). This accords with several previous studies (6, 19–25). The inverse relationship between the buffer effect or flow rate and the caries indices was also expected (11, 26, 27).

On a population level, a positive correlation between salivary MS and lactobacilli has been reported (2, 6, 19, 23). This was found also in our study group. The inverse relationship between salivary flow rate and the levels of MS has also been reported earlier (28, 29). Salivary flow rate among young teenagers is still increasing with age (8, 30), which may explain part of the intra-individual variability observed in our study. The same is true for buffer effect, but this behaves differently in girls and boys. With girls the buffer effect may even drop during early adolescence (8), obviously due to the effects of female sex steroids on salivary bicarbonate (31). In the present study, intra-individual variability of buffer effect was much greater in girls than in boys. The large variability in bacterial counts is obviously considerably influenced by gradually exfoliating primary teeth with concomitant emergence of new tooth surfaces at the age of 11–12 years. Our observations emphasize that, in line with the bacterial tests, measurements of buffer effect and flow rate are highly variable at this age. Therefore, only single-point measurements of salivary variables should not be used for caries-diagnostic or -predictive purposes for children with a developing dentition.

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References

1. Crossner CG, Holm AK. Saliva tests in the prognosis of caries in children. Acta Odontol Scand 1976;35:135–9.

2. Alaluusua S, Kleemola-Kujala E, Grönroos L, Evälahti M. Salivary caries-related tests as predictors of future caries increment in teenagers. A three-year longitudinal study. Oral Microbiol Immunol 1990;5:77–81.
3. Klock B, Svanberg M, Petersson LG. Dental caries, mutans streptococci, lactobacilli, and saliva secretion rate in adults. Community Dent Oral Epidemiol 1990;18:249–52.
4. Russel JI, MacFarlane TW, Aitchison TC, et al. Caries prevalence and microbiological and salivary caries activity tests in Scottish adolescents. Community Dent Oral Epidemiol 1990;18:120–5.
5. Carlsson J, Grahnén H, Jonsson G. Lactobacilli and streptococci in the mouth of children. Caries Res 1975;9:333–9.
6. Zickert I, Emilson CG, Krasse B. *Streptococcus mutans*, lactobacilli and dental health in 13–14-year-old Swedish children. Community Dent Oral Epidemiol 1982;10:77–81.
7. Köhler B, Bjarnason S. Mutans streptococci, lactobacilli and caries prevalence in 11- and 12-year-old Icelandic children. Community Dent Oral Epidemiol 1987;15:332–5.
8. Söderling E, Pienihäkkinen K, Alanen M-L, et al. Salivary flow rate, buffer effect, sodium and amylase in adolescents: a longitudinal study. Scand J Dent Res 1992;100. In press.
9. Laine M, Tenovuo J, Lehtonen O-P. Pregnancy-related increase in salivary *Streptococcus mutans*, lactobacilli and IgA. In: Cimasoni G, Lehner T, editors. Borderland between caries and periodontal disease. Vol. III. Geneva: Editions Médecine et Hygiéne, 1986:193–200.
10. Togelius J, Kristoffersson K, Anderson H, Bratthall D. *Streptococcus mutans* in saliva: intraindividual variations and relation to the number of colonized sites. Acta Odontol Scand 1984;42:157–63.
11. Russel JI, MacFarlane TW, Aitchison TC, et al. Salivary levels of mutans streptococci, *Lactobacillus, Candida* and *Veillonella* species in a group of Scottish adolescents. Community Dent Oral Epidemiol 1990;18:17–21.
12. El-Nadeef MAI, Bratthall D. Intraindividual variations in counts of mutans streptococci measured by 'Strip mutans' method. Scand J Dent Res 1991;99:8–12.
13. Bentley C, Crawford JJ, Broderius CA. Analytical and physiological variability of salivary microbial counts. J Dent Res 1988;67:1409–13.
14. Ericson D, Bratthall D. Simplified method to estimate salivary buffer capacity. Scand J Dent Res 1989;97:405–7.
15. Jensen B, Bratthall D. A new method for estimation of mutans streptococci in human saliva. J Dent Res 1989;63:468–71.
16. Larmas M. A new dip-slide method for counting of salivary lactobacilli. Proc Finn Dent Soc 1975; 71:31–5.
17. Parvinen T, Larmas M. The relation of stimulated salivary flow rate and pH to lactobacillus and yeast concentrations in saliva. J Dent Res 1981;60:1929–35.
18. Weinberger SJ, Wright GZ. Variables influencing *Streptococcus mutans* testing. Pediatr Dent 1990; 12:312–5.
19. Klock B, Krasse B. Microbiological and salivary conditions in 9–12-year-old children. Scand J Dent Res 1977;85:56–63.
20. Klock B, Krasse B. Effect of caries-preventive measures in children with high numbers of *S. mutans* and lactobacilli. Scand J Dent Res 1978;86:221–30.
21. Crossner CG. Salivary *lactobacillus* counts in the prediction of caries activity. Community Dent Oral Epidemiol 1981;9:182–90.
22. Pienihäkkinen K, Scheinin A, Banoczy J. Screening of children through salivary lactobacilli and yeasts. Scand J Dent Res 1987;95:397–404.
23. Köhler B, Andréen I, Jonsson B, Hultqvist E. Effect of caries preventive measures on *Streptococcus mutans* and lactobacilli in selected mothers. Scand J Dent Res 1982;90:102–8.
24. Vanderas AP. Bacteriologic and nonbacteriologic criteria for identifying individuals at high risk of developing dental caries: a review. J Public Health Dent 1986;46:106–13.
25. Alaluusua S, Nyström M, Grönroos L, Peck L. Caries-related microbiological findings in a group of teenagers and their parents. Caries Res 1989;23:49–54.
26. Crossner CG, Holm AK. A descriptive and comparative study of oral health in 8-year-old Swedish children. Acta Odontol Scand 1975;33:135–42.
27. Agus HM, Schamschula RG. Lithium content, buffering capacity and flow rate of saliva and caries experience of Australian children. Caries Res 1983;17:139–44.
28. Gråhn E, Tenovuo J, Lehtonen O-P, et al. Antimicrobial systems of human whole saliva in relation to dental caries, cariogenic bacteria, and gingival inflammation in young adults. Acta Odontol Scand 1988;46:67–74.
29. Seppä L, Pöllänen L, Hausen H. *Streptococcus mutans* counts obtained by a dip-slide method in relation to caries frequency, sucrose intake and flow rate of saliva. Caries Res 1988;22:226–9.
30. Andersson R, Arvidsson E, Crossner C-G, et al. The flow rate, pH and buffer effect of mixed saliva in children. J Int Assoc Dent Child 1974;5:5–12.
31. Laine M, Tenovuo J, Lehtonen O-P, et al. Pregnancy-related changes in human whole saliva. Arch Oral Biol 1988;33:913–7.

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