Comparison of Real-Time Quantitative PCR with a Chairside Test for *Streptococcus Mutans Assessment*

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Objective: *To compare two methods for Streptococcus mutans detection and quantification in the human oral cavity: a chairside commercial test and a molecular-based real-time quantitative polymerase chain reaction (qPCR) method.*
Methods: *A total of 688 whole saliva samples were collected from 344 children aged 3 and 5 and their biological mothers. Caries status was examined using a World Health Organisation survey method. S. mutans levels were measured using the Dentocult SM Strip mutans test and scored as colony forming units per millilitre of saliva. Meanwhile, bacterial genomic DNA was extracted from the saliva, qPCR was performed with S. mutans species-specific primers, and absolute S. mutans DNA concentrations were obtained and scored as micrograms of DNA per millilitre of saliva. The two methods were compared for sensitivity, specificity, agreement and correlation with caries status.*
Results: *Significantly more participants tested positive for S. mutans by qPCR than in the chairside SM Strip test (82.4% vs 71.4%). When only the highest and lowest test scores were considered, the agreement between the two methods assessing S. mutans colonisation was 0.956. Children with high levels of S. mutans in their saliva were six to eight times more likely to develop dental caries at 5 years old.*
Conclusion: *The study provides new evidence supporting the use of the chairside SM Strip test or the qPCR assay for the detection and quantification of S. mutans colonisation in saliva as the analytical approach of choice for caries risk assessment in clinical and epidemiological studies.*
Key words: *colonisation, dental caries, qPCR, Streptococcus mutans*
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Dental caries, characterised by the irreversible destruction of the tooth, is a chronic condition that affects more than one-third of the global population[1]. Although the disease often progresses slowly, it can eventually become a serious chronic condition when left untreated, and it is the primary cause of tooth loss among young children. Today, untreated dental caries affects as much as 60% to 90% of school-aged children worldwide and negatively affects their growth, development, and quality of life because of the associated pain and discomfort[2]. Some affected individuals may even end up being hospitalised and thus face high treatment costs[3-5].

Although multiple elements such as socio-demographic status, diet, and oral hygiene cause this disease, the oral bacteria mutans streptococci (MS; in particular, *Streptococcus mutans*) are considered the major contributing factor initiating dental caries. The virulence properties of *S. mutans*, including its acidogenic (acid-producing) and aciduric (acid-tolerant) properties, and its ability to modulate caries-prone biofilm formation in human saliva and dental plaque have been well defined[6,7]. Because *S. mutans* is highly correlated with

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caries development and severity, colonisation has been used as a strong and practical indicator of the microbiological risk of dental caries and as an important biomarker for epidemiological studies of caries risk assessment[8-10].

Traditionally, culture-based methods have been the "gold standard" for detecting, measuring, and characterising *S. mutans* colonisation in the oral cavity; clinicians, cariologists, and oral microbiologists have used colony-forming units (CFUs) per millilitre as the measure of *S. mutans* colonisation[11]. However, the sample collection procedure, which must preserve bacterial cell viability, and the cultivation requirements have limited clinical and field applications. In 1989, Jensen and Bratthall developed a semi-culture-based dip-slide method using *mitis salivarius* broth medium plus bacitracin, which served as the beginning of a new chairside approach to estimate MS in saliva[12]. Currently, most commercial dip-strip methods are based on this technique, including the Dentocult SM Strip mutans test (Orion Diagnostica, Espoo, Finland). This approach has several advantages, including increased simplicity and availability relative to laboratory agar plates, ease of bacterial colony identification, and ease of result interpretation. Therefore, the strip mutans test is considered a more practical method for chairside application, and it has been widely used in clinical and epidemiological studies[13-15]. However, there are some concerns about methodological variability and the reproducibility of test results as bacterial growth depends on metabolic requirements that might be limited in test tubes; the results might underestimate *S. mutans* levels in saliva[16].

Alternatively, culture-based methods are rapidly being replaced by advanced molecular assays such as real-time polymerase chain reaction (qPCR) for *S. mutans* detection and quantification[17-19]. The culture-independent technique is sensitive in detecting bacterial DNA[20], provides a more accurate enumeration of *S. mutans* colonisation with high sensitivity and specificity[20,21], and is most commonly used in laboratory settings for caries risk assessment[22-24]. However, there are some concerns that the technique might overestimate *S. mutans* colonisation due to amplification of DNA from non-viable bacterial cells[25]. To date, both the culture-based chairside strip test and qPCR are commonly used to measure *S. mutans* colonisation in different populations. There are few reports available to compare the quantitative and qualitative test results obtained from the two approaches. Whether the SM Strip test underestimates or qPCR overestimates *S. mutans* levels in the oral cavity is unknown.

The objective of this study was to compare two independent methods, the Dentocult SM Strip mutans test (a culture-dependent technique) and real-time qPCR (a culture-independent technique), with regard to the sensitivity, specificity, and accuracy of measuring *S. mutans* colonization in the oral cavities of caries-free and caries-active children, as well as their biological mothers. The results might help researchers and clinicians choose the most suitable approach to use for measuring *S. mutans* colonisation and predicting the risk for caries development.

Materials and methods

Participants

The current study recruited 344 children and their biological mothers. They were randomly selected from the Health Promotion Hospital in Chiang Mai, Thailand, in 2009, for a cross-sectional epidemiological caries study[26]. The children were 3 years old (n = 180) or 5 years old (n = 164); 188 of the participants were boys and 156 were girls. Participants of each sex were evenly distributed between the two age groups. All parents provided written informed consent before the study. The detailed study protocol was published previously[23,26]. This study received approval from the Ethical Committee of the Faculty of Dentistry, Chiang Mai University, Thailand (No. 12/2008). The study was also conducted according to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines.

Dental caries examination

Two standardised practitioners examined the caries status of all participants using the caries diagnostic criteria of the World Health Organization (WHO) Health Survey Methods for Field Studies[27]. The presence of caries was recorded as detectable cavitated lesions without radiograph assessment. Clinical oral health status was determined using the decayed, missing, and filled tooth surface (dmfs) index and the decayed, missing, and filled teeth (dmft) index for deciduous dentition in both the children and their mothers (DMFS/DMFT). The prevalence of caries (present vs. absent) and the number of teeth with caries for each participant were recorded.

Evaluation S. mutans via the strip test

The level of MS colonisation in the saliva was assessed using the Dentocult SM Strip mutans test (Orion Diag-

nostica, Finland) for all participants. Specifically, children and their mothers chewed a piece of paraffin wax for 1 min under the close supervision of a medical examiner. The strips were rotated on the tongue for 10 rounds, and excess saliva was removed according to the protocol by Jensen and Bratthall[12]. These test strips were immediately placed into a test tube containing a selective medium broth and bacitracin, incubated for 48 h at 37°C, and air-dried at room temperature. The levels of *S. mutans* colonies were examined and scored based on the presence of CFUs on the strips. Compared with the standard chart provided by the manufacturer, the following four categories were used: 0, 1, 2, and 3, which corresponded to $< 10^4$, 10^4-10^5, 10^5-10^6 and $> 10^6$ *S. mutans* CFU per ml of saliva, respectively[12]. Two investigators (PS and KP) independently recorded the scores for each participant. The inter-examiner agreement was 88%, which indicated a high degree of reliability between the investigators.

Evaluation of S. mutans via a qPCR assay

Additional whole saliva and supra-gingival plaque samples were collected after asking participants to brush their teeth using a sterile soft toothbrush (Soft P-20 Oral B, Cat. 33259737, Henry Schein, Melville, NY, USA) for 2 min. The toothbrush was immediately washed in a 50 mL test tube containing 10 mL of sterile phosphate-buffered saline (PBS). A total of 2 mL of the bacterial sample was transferred into an Oragene DNA collection container (Oragene, Ontario, Canada), sealed, mixed well, and processed according to the manufacturer's instructions. All of the bacterial samples were transferred to the microbiology laboratory at the Chiang Mai University Faculty of Dentistry within 4 h and stored at −20°C until further processing.

The bacterial genomic DNA samples were extracted using a modified protocol, as described by Li et al[28]. Briefly, 1 ml of saliva sample was centrifuged at 18,000 × g for 3 min. The pellet was washed, and bacterial DNA was extracted using a DNA purification kit (MasterPure; Epicenter, Madison, WI, USA). An additional 10 μL of Proteinase K stock solution (QIAGEN Inc, Valencia, CA, USA) at 100 mg/mL in TES buffer (10 mM Tris-HCl, pH 8, 1 mM EDTA, and 100 mM NaCl) and 2 μL of mutanolysin (Sigma-Aldrich, St. Louis, MO, USA) were added to the sample solution followed by a phenol/chloroform/isoamyl alcohol extraction procedure and isopropanol precipitation. DNA quality and concentration were measured with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA). The final concentration of each DNA sample was adjusted to 10 ng/μL for qPCR.

S. mutans colonisation was detected and qualified via qPCR with a set of species-specific primers. Chen et al[20] previously reported the primer sequences (forward primer Sm479F,5'-TCGCGAAAAAGATAAACAAACA-3', and reverse primer Sm479R, 5'-GCCCCTTCACAGTTGGTTAG-3'). A standard curve was established with a set of 10-fold serially diluted DNA samples (ranging from 10^7–10^1 fg/μL) from *S. mutans*. The UA159 (ATCC 700610) control strain was used as an external standard for absolute quantification. All qPCR cycles were performed using MyiQ2 (Bio-Rad, Hercules, CA, USA) with SYBR Green dye. Each 25 μL of the reaction mixture consisted of 1 × PCR Master Mix (QuantiTect SYBR Green PCR Kits, Qiagen, Valencia, CA, USA), 10 μM of each primer, and 10 ng of bacterial DNA samples. qPCR was performed under the following conditions: 15 min at 95°C; 44 cycles of 15 s at 94°C for denaturation, 30 s at 56°C for annealing, and 30 s at 72°C for extension; followed by a melting curve analysis of the PCR product. All qPCR cycles were performed in duplicate to eliminate variation between the same templates. The final analysis was based on the mean of the two reactions. Primer set specificity was detected via a melting curve analysis. The output data were analysed using MyiQ software (Bio-Rad iQ5 optical system software, version 2.1, Hercules, CA, USA). The *S. mutans* DNA value was used for statistical analysis. Moreover, the qPCR products were analysed via 1.5% agarose gel electrophoresis for 2 h to confirm the correct molecular size (479 bp) of the amplicons. The gel was stained with ethidium bromide (1 μg/mL) for 15 min and de-stained for 5 min. The gel images were photographed under a UV transilluminator and recorded with an AlphaImager 3300 imaging system (Alpha Innotech Corp, San Leandro, CA, USA).

Statistical analysis

The levels of *S. mutans* colonisation detected using the Dentocult SM Strip test were based on the CFUs scored from 0 to 3[12]. The qPCR results were scored based on DNA measurements in the following manner: 0, < 0.01 μg/mL; 1, 0.01-0.5 μg/mL; 2, 0.51-5.0 μg/mL; and 3, > 5.0 μg/mL. Both scoring systems were further summarised into two categories: the "low" tier (a score of 0 plus 1) and the "high" tier (a score of 2 plus 3). The WHO formula was used to calculate the sensitivity (Sn), specificity (Sp), and Kappa (κ) statistics for agreement[27]. Spearman's correlations, chi-square tests, and ANOVA were used, and odds ratios were calculated to compare the categorised chairside SM Strip test scores with the molecular-based qPCR values. All statistical

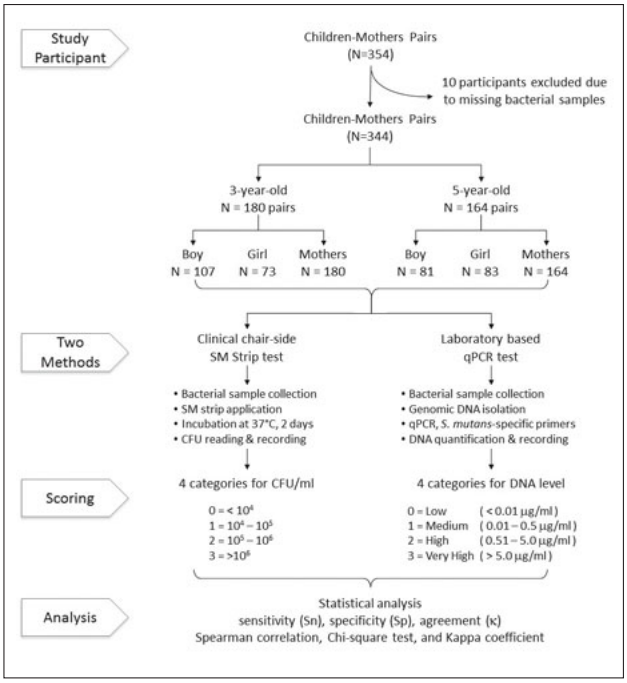

Fig 1 Schematic of the study design for the comparison of the chairside Dentocult SM Strip test and the laboratory-based qPCR assay among 344 children and their biological mothers.

evaluations were performed using SPSS, version 23.0 (IBM, Armonk, NY, USA). All tests were two-tailed, and *P* -values < 0.05 were considered significant.

Results

The current study recruited 354 children and their biological mothers. Ten participants were excluded because of missing bacterial samples. The final analysis included 344 mother-child pairs with 688 valid SM Strip tests and 688 qPCR results obtained from each participant. The 344 mother-child pairs were divided into two groups according to the children's ages: 180 were assigned to the three-year-old group, and 164 were assigned to the group of 5-year-olds (Fig 1). No significant differences were found between boys and girls with regard to oral health, family socioeconomics, or behaviour variables. The distributions of participants in each SM Strip test and qPCR test categories are summarised in Table 1. *S. mutans* colonisation significantly differed between the two age groups. A significant proportion of children had higher *S. mutans* scores than their mothers, regardless of the method used (Fig 2). No differences were observed between boys and girls with regard to the *S. mutans* assessments.

The distribution of the caries status of the children (dmft/dmfs) and their mothers (DMFT/DMFS) with regard to *S. mutans* score is provided in Table 2. Higher caries scores in each category were significantly correlated with increased levels of *S. mutans* colonization. The results obtained from both methods demonstrated similar trends in the correlations between *S. mutans* levels and caries score; the correlation coefficients were higher among children (r = 0.601 and 0.591; $P < 0.001$) than their mothers (r = 0.343 and 0.335; $P < 0.001$; Fig 3).

The current study found that more than 82.4% of the participants tested positive for *S. mutans* according to qPCR compared with 66% of the mothers and 71.4% of the children using the SM Strip test. When qPCR was compared with the chairside SM Strip tests using the dichotomised two-tier scores, the overall sensitivity, specificity, and agreement values were 0.760, 0.717, and 0.477, respectively (Table 3A). The specificity

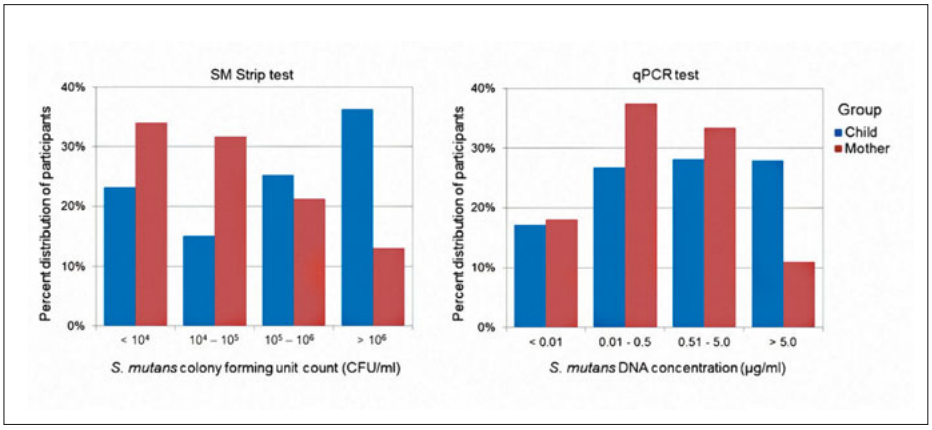

Fig 2 The distribution of the participants in each category. Our study found that significantly higher percentages of children were colonised with high levels of *S. mutans* than their mothers, based on the results of both the SM Strip test (r = -0.268, χ^2 = 66.001, $P < 0.001$) and qPCR (r = -0.147, χ^2 = 32.902, $P < 0.001$).

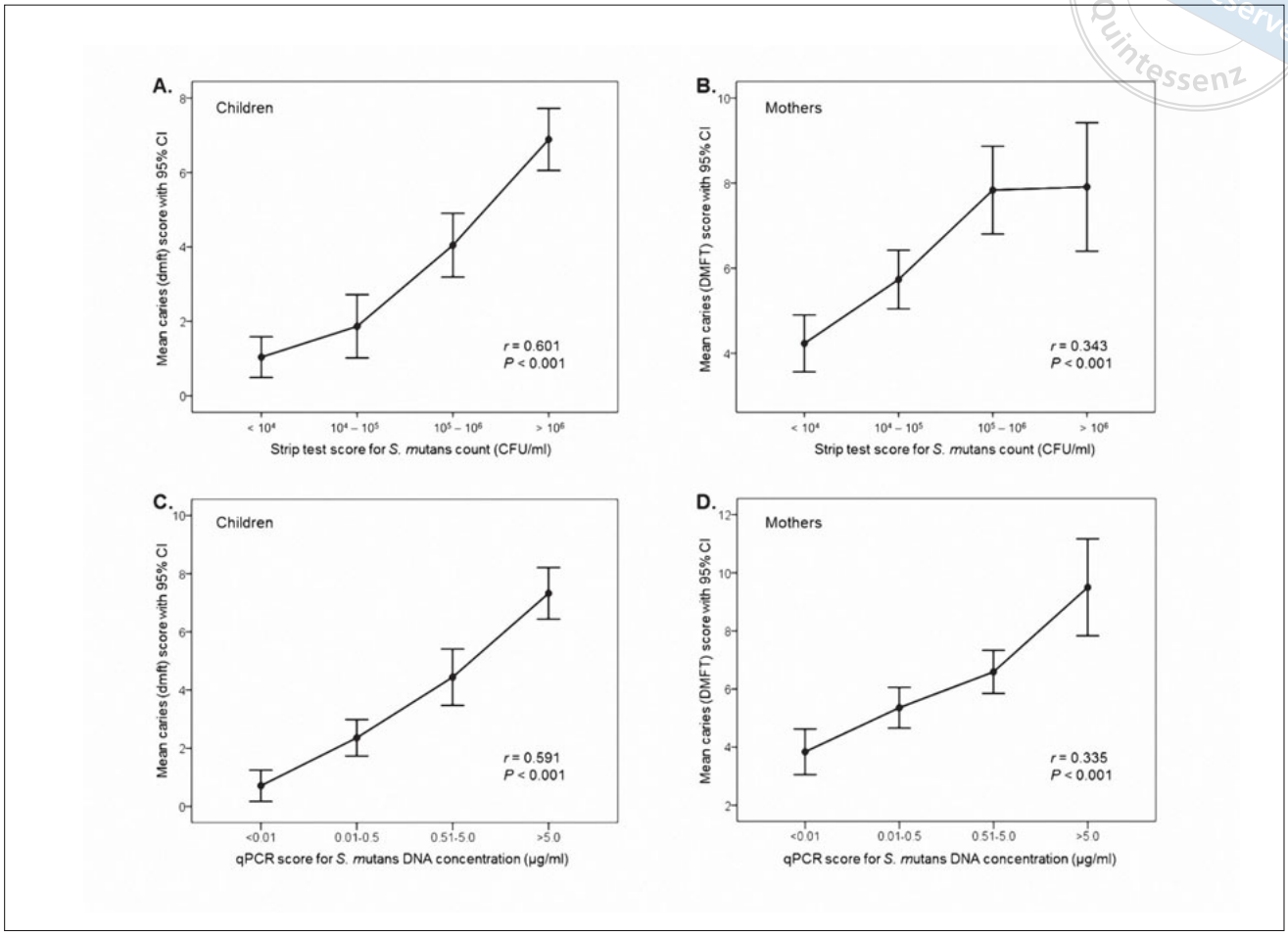

Fig 3 The distribution of the participants, children and their mothers in each category. The Dentocult SM Strip test results represented the following scores: 0 < 10^4 CFU/mL, 1 = 10^4-10^5 CFU/mL, 2 = 10^5-10^6 CFU/mL, and 3 > 10^6 CFU/mL. Similarly, the absolute DNA concentrations of *S. mutans* were obtained from the qPCR amplification and categorized into the following scores: 0 < 0.01 μg/mL, 1 = 0.01-0.5 μg/mL, 2 = 0.51-5.0 μg/mL, and 3 > 5.0 μg/mL. For both scoring systems, higher scores indicated higher counts of *S. mutans*. Although the overall agreement between the two methods was low, the test results were significantly correlated with each other. The agreement was greater among children, especially the 3-year-olds (K = 0.333, r = 0.727; $P < 0.001$), compared with their mothers (K = 0.230, r = 0.469; $P < 0.001$).

value associated with children was greater than that associated with their mothers Table 3B vs. Table 3C). When only the highest and lowest scores of both tests were considered in the analysis, the overall sensitivity, specificity, and agreement values improved to 0.978, 0.977, and 0.956, respectively. Although the general agreement between the two scoring systems was low, the results were significantly correlated. The correlation was especially high among the children (Spearman's rho = 0.722; $P < 0.001$) in comparison with their mothers (Spearman's rho = 0.459; $P < 0.001$). This study also revealed that both tests produced comparable results for caries risk assessment. The overall odds ratio for participants to have dental caries with high levels of S. mutans colonisation (scores 2 and 3) was 2.5 for the SM Strip test and 2.4 for qPCR. A more than two-fold increase was evident when comparing the highest category with the 0 category, especially among children Tables 3A and 3B).

Finally, Table 4 summarises the major advantages and disadvantages of the two tests, taking into account factors such as the sensitivity, specificity, the cost per sample, procedural time, special equipment and personnel skill requirements to acquire qualitative and quantitative assessments of *S. mutans* colonisation in the oral cavity.

Table 1 Comparison of the two methods for *S. mutans* assessment.

Score category		Children (%)			Mother (%)
		3 years old N = 180	5 years old N = 164	Total N = 344	N = 344
Dentocult SM Strip test (CFU/mL)					
0	$< 10^4$	59 (32.8)**	21 (12.8)	80 (23.3)	117 (34.0)
1	10^4-10^5	20 (11.1)	32 (19.5)	52 (15.1)	109 (31.7)
2	10^5-10^6	35 (19.4)	52 (31.7)**	87 (25.3)	73 (21.2)
3	$> 10^6$	66 (36.7)	59 (36.0)	125 (36.3)**	45 (13.1)
qPCR test (DNA concentration, μg/mL)					
0	< 0.01	45 (25.0)**	14 (8.5)	59 (17.2)	62 (18.0)
1	0.01-0.5	40 (22.2)	52 (31.7)	92 (26.7)	129 (37.5)
2	0.51-5.0	52 (28.9)	45 (27.5)	97 (28.2)	115 (33.4)
3	> 5.0	43 (23.9)	53 (32.3)**	96 (27.9)**	38 (11.1)

Pearson's $\chi 2$ test; * $P < 0.05$; ** $P < 0.01$

Table 2 Correlations between the *S. mutans* assessment and the mean caries score for both children and their mothers.

Score category		Children (mean ± SD)						Mothers (mean ± SD)	
		3 years old N = 180		5 years old N = 164		Total N = 344		Total N = 344	
		dmft	dmfs	dmft	dmfs	dmft	dmfs	DMFT	DMFS
By SM Strip test (CFU/mL)									
0	$< 10^4$	0.3 ± 1.0	0.4 ± 1.5	3.0 ± 3.9	6.7 ± 14.2	1.4 ± 2.5	2.1 ± 7.8	4.2 ± 3.7	6.8 ± 7.7
1	10^4-10^5	1.8 ± 3.7	3.0 ± 8.0	1.9 ± 2.6	2.8 ± 4.1	1.8 ± 3.1	2.9 ± 5.9	5.7 ± 3.6	10.1 ± 9.0
2	10^5-10^6	2.8 ± 2.8	6.5 ± 8.9	4.9 ± 4.5	8.9 ± 11.5	4.1 ± 4.0	7.9 ± 10.5	7.8 ± 4.4	13.9 ± 13.7
3	$> 10^6$	5.5 ± 4.2	11.4 ± 11.4	8.5 ± 4.8	22.0 ± 18.8	6.9 ± 4.7	16.4 ± 16.2	7.9 ± 5.0	14.2 ± 12.5
Significance*		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
By qPCR (DNA concentration, μg/mL)									
0	< 0.1	0.2 ± 0.7	0.2 ± 0.8	2.4 ± 3.7	4.5 ± 8.8	0.7 ± 2.1	1.2 ± 4.6	3.8 ± 3.1	6.4 ± 7.5
1	0.1-0.9	1.6 ± 2.1	2.3 ± 3.4	3.0 ± 3.5	5.8 ± 10.6	2.4 ± 3.0	4.3 ± 8.4	5.4 ± 4.0	8.6 ± 8.7
2	1.0-5.0	3.4 ± 4.1	6.4 ± 9.6	5.6 ± 5.2	12.7 ± 16.4	4.4 ± 4.8	9.3 ± 13.5	6.6 ± 4.0	11.2 ± 9.4
3	> 5.0	6.2 ± 3.9	14.6 ± 12.0	8.3 ± 4.6	19.9 ± 18.0	7.3 ± 4.4	17.6 ± 15.7	9.5 ± 5.1	19.5 ± 17.2
Significance*		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$

*ANOVA.

Table 3A Comparison of the qPCR test with the chairside SM Strip test with regard to sensitivity (Sn), specificity (Sp), agreement (K), and caries risk assessment among all participants (N = 688).

SM Strip test scores	qPCR test scores					Statistical outcomes		Risk for Caries
	0	1	2	3	Sum	χ^2, r, Significance	K	χ^2, *P*-value
0	90	74	31	2	197	$\chi^2 = 319.413$ r = 0.620 $P < 0.001$	0.285	SM Strip test
1	20	76	54	11	161			65.270, $P < 0.001$
2	9	51	65	35	160			qPCR test
3	2	20	62	86	170			81.797, $P < 0.001$
Sum	121	221	212	134	688			

				χ^2, r, Sig.	Sn	Sp	K	OR (95% CI)
	Low (0+1)	High (2+3)		$\chi^2 = 156.792$ r = 0.477 $P < 0.001$	0.760	0.717	0.477	SM Strip test
Low (0+1)	260	98	358					2.5 (1.8, 3.5)
High (2+3)	82	248	330					qPCR test
	342	346	688					2.4 (1.8, 3.3)

				χ^2, r, Sig.	Sn	Sp	K	OR (95% CI)
	Lowest (0)	Highest (3)		$\chi^2 = 164.348$ r = 0.956 $P < 0.001$	0.978	0.977	0.956	SM Strip test
Lowest (0)	90	2	92					4.1 (2.4, 7.0)
Highest (3)	2	86	88					qPCR test
	92	88	180					6.1 (3.0, 12.3)

Discussion

S. mutans has been well defined as a key microbial etiological factor for dental caries development. The effective detection and quantification of *S. mutans* colonisation in the oral cavity are critical for monitoring and evaluating caries treatment and intervention. *S. mutans* evaluation has been included in caries risk assessment systems/guidelines[29,30]. Traditionally, clinicians, cariologists and oral microbiologists have used CFUs per millilitre to measure the extent of *S. mutans* colonisation in the oral cavity. The Dentocult SM Strip mutans test developed in the 1980s[12] (Orion Diagnostica) has been used as a chairside test to estimate *S. mutans* colonisation in saliva. However, the lack of convincing evidence of the feasibility and reliability of a chairside assay has become a major barrier to using this assessment in clinical settings. In this study, we compared the two commonly utilised methods, the SM Strip test and the qPCR-based assay, and showed that both tests were highly correlated with regard to the determination and assessment of *S. mutans* levels in the saliva and caries risk assessment, especially among children.

Previously, we reported, in the same cohort of children, that *S. mutans* colonisation level based on the SM Strip mutans test was significantly correlated with factors such as the child's age, mode of delivery, premature delivery, mother's *S. mutans* level, mother's prechewing feeding habits, and children's tooth brushing habits[23,26]. In this study, we observed greater variation in *S. mutans* levels and in the correlation with caries status among the mothers compared with their children. Dietary and oral hygiene habits combined with other risk factors associated with oral chronic inflammation

Table 3B Comparison of the qPCR test with the chairside SM Strip test with regard to sensitivity (Sn), specificity (Sp), agreement (K), and caries risk assessment among all children (N = 344).

SM Strip test scores	qPCR test scores					Statistical outcomes			Risk for caries
	0	1	2	3	Sum	χ^2, r, Significance		K	χ^2, *P*-value
0	51	19	9	1	80	$\chi^2 = 256.096$ r = 0.722 $P < 0.001$		0.377	SM Strip test
1	5	33	12	2	52				114.464, $P < 0.001$
2	2	30	31	24	87				qPCR test
3	1	10	45	69	125				97.300, $P < 0.001$
Sum	59	92	97	96	344				
					χ^2, r, Sig.	Sn	Sp	K	OR (95% CI)
	Low (0+1)		High (2+3)		$\chi^2 = 125.078$ r = 0.603 $P < 0.001$	0.715	0.876	0.599	SM Strip test
Low (0+1)	108		24	132					3.4 (2.4, 4.8)
High (2+3)	43		169	212					qPCR test
	151		193	344					2.9 (2.1, 4.1)
					χ^2, r, Sig.	Sn	Sp	K	OR (95% CI)
	Lowest (0)		Highest (3)		$\chi^2 = 113.959$ r = 0.966 $P < 0.001$	0.981	0.986	0.966	SM Strip test
Lowest (0)	51		1	52					6.0 (3.3, 10.6)
Highest (3)	1		69	70					qPCR test
	52		70	122					7.8 (3.7, 16.6)

could contribute to the increased variation in the mothers. Our hypothesis is supported by numerous other studies, which have also reported that an individual's oral hygiene conditions, e.g. smoking, dietary habits, periodontal status, and use of medications, can significantly affect *S. mutans* measurements using the SM Strip test[31-33].

Advances in molecular biology have led to the rapid replacement of culture-based methods with more sensitive and specific molecular-based assays, such as real-time qPCR, for *S. mutans* detection and quantification. We previously reported that the lowest detectable level using the standard PCR method was approximately 0.01 ng of *S. mutans* DNA in a reaction using the same *S. mutans* -specific primer[20]. The results from this study demonstrated a 10% increase in the percentage of participants who tested positive for *S. mutans* colonisation using qPCR compared with those who tested positive according to the SM Strip test, which corresponds to a 16% increase for the mothers. A child with a high level of *S. mutans* ($> 10^6$ CFU/ml or > 5.0 µg/ml DNA) in his or her saliva would be 6 to 8 times more likely to develop dental caries at the age of 5. Higher *S. mutans* scores were predictive of higher dmft/DMFT and dmfs/DMFS values for children/mothers. The age at initial *S. mutans* colonisation and the level of colonisation are significant predictors of caries development and disease severity. This finding provides consistent evidence supporting the etiological link between *S. mutans* colonisation, as a critical biomarker for a cariogenic

Table 3C Comparison of the qPCR test with the chairside SM Strip test with regard to sensitivity (Sn), specificity (Sp), agreement (K), and caries risk assessment among all mothers (N = 344)

SM Strip test scores	qPCR test scores					Statistical outcomes		Risk for caries
	0	1	2	3	Sum	χ^2, r, Significance	K	χ^2, *P*-value
0	39	55	22	1	117	$\chi^2 = 86.958$ r = 0.459 $P < 0.001$	0.230	SM Strip test
1	15	43	42	9	109			9.769, $P = 0.021$
2	7	21	34	11	73			qPCR test
3	1	10	17	17	45			10.931, $P = 0.012$
Sum	62	129	115	38	344			

				χ^2, r, Sig.	Sn	Sp	K	OR (95% CI)
	Low (0+1)	High (2+3)		$\chi^2 = 36.730$ r = 0.327 $P < 0.001$	0.796	0.516	0.319	SM Strip test
Low (0+1)	152	74	226					3.0 (1.0, 8.8)
High (2+3)	39	79	118					qPCR test
	191	153	344					2.3 (1.1, 5.1)

				χ^2, r, Sig.	Sn	Sp	K	OR (95% CI)
	Lowest (0)	Highest (3)		$\chi^2 = 49.032$ r = 0.919 $P < 0.001$	0.975	0.944	0.919	SM Strip test
Lowest (0)	39	1	40					2.5 (0.7, 9.5)
Highest (3)	1	17	18					qPCR test
	40	18	58					4.6 (0.7, 30.1)

environment in the human mouth, and an increased risk of caries development.

Molecular-based qPCR is an excellent method to measure the level of *S. mutans* colonisation in human saliva and might significantly improve the sensitivity and specificity of the results compared with culture-based methods, such as the SM Strip test. Despite the low agreement between these two techniques, the study showed almost perfect results regarding the sensitivity and specificity of both methods when the highest and lowest scores were used. The odds ratios for caries risk in the children groups were increased more than two-fold. Apparently, there was a discrepancy between the highest and the lowest scores, especially with regard to the maternal samples. The importance of delineating these scores depends on the specific clinical questions, for example, quantifying the correlation between the level of *S. mutans* colonisation and caries outcome, as demonstrated in this study. The results suggest that using the lowest and highest scores is sufficient for caries risk assessment; the information can be useful for the future development of a molecular-based diagnostic test (point-of-care or "lab-on-a-chip" technologies for *S. mutans* assessment). These new technologies will facilitate chairside testing and provide simple, inexpensive, and accurate measurements directly from saliva for caries risk early prediction, the evaluation of caries treatment and intervention, and patient oral hygiene education and consultation. Future research is needed to determine

Table 4 Comparison of the chairside Dentocult SM Strip test and the qPCR method with regard to *S. mutans* assessment.

Factors \ Tests	Dentocult SM Strip mutans test	Real-time qPCR
Method	Culture-dependent method based on selective media with bacitracin added	Culture-independent method based on DNA amplification with specific primers and real-time signal detection using florescent dye
Special equipment and supplies required	Sample collection and process: 4°C refrigerator Portable incubator at 37°C Data analysis: Computer Statistical programme	Molecular lab basic equipment: Incubator Micro-centrifuge -20°C freezer qPCR machine and software programme Data analysis: Computer Statistical programme
Time consumption	Total time required: 48 h or 2 working days: Preparation, 15 min Sample collection, 15 min Incubation period, 47 h Results evaluation and analysis, 30 min	Total time required: 15 h, or 2 working days: Preparation, 15 min Sample collection, 15 min DNA extraction, 8 h qPCR preparation and process, 6 h Results evaluation and analysis, 30 min
Material cost	$55 for 10 tests (Orion Diagnostica)	$220 per 20 samples, $11 per test
Personnel skill required	Clinically trained and standardized research assistant	Molecular biology trained research assistant or technician
Personnel cost	~$360 (2 working days x $180)	~$600 (2 working days x $300)
Sensitivity	10 colony forming units per millilitre (ml) of saliva	0.01 nanogram of bacterial DNA per micro-litre (µl) of saliva
Specificity	Cannot differentiate *S. mutans* from *S. sobrinus*	Can be specific for *S. mutans* or *S. sobrinus*
Outcome analysis	Visualised check based on colony forming units and compared with a standard chart	Software programme based on targeted DNA concentration compared with an internal DNA standard
Other advantages	Non-invasive sample collection procedure *S. mutans* estimation of whole saliva samples Able to test many samples simultaneously Less complicated testing procedures Suitable for field study, dental clinic or personal use Affordable cost	Non-invasive sample collection procedure Clinical samples can be transported at room temperature Bacterial DNA can be obtained and saved for other tests Able to test many samples simultaneously High sensitivity, specificity, accuracy, efficiency, and reproducibility Can study not only whole saliva or pooled plaque samples but also site-specific samples Quality of data is enhanced
Other disadvantages	Depends on living cells and their metabolism; therefore, can affect the accuracy and consistency of the count for the microbial load Less reliable Special care is needed for the test media and bacitracin Requires a nonstop 2-day process between sample collection and results evaluation at any study site Not suitable for *S. mutans* site-specific sampling or study	Requires special equipment Requires specially trained technicians PCR is an error-prone technique Relatively expensive

the most suitable devices and tools that can be applied across different clinical settings.

The major advantages and disadvantages are summarised in Table 4, considering such factors as sensitivity, specificity, cost per test, time and effort, special equipment and supplies needed, and personnel skill required. If the SM Strip test is selected, clinicians and researchers should consider using a simplified scoring system as described in this study. Although a chairside qPCR test for *S. mutans* assessment is currently not commercially available, the technology has revolutionised clinical microbiology practice. To date, qPCR assays have become a common platform in clinical microbiology laboratories for the diagnosis of bacterial diseases[34]. It is our hope that simplified clinical sample handling procedures will be developed and that a low-cost qPCR test for *S. mutans* that is especially suitable for use in clinical settings or field epidemiology studies will be commercially available in the future.

In conclusion, this study not only shows that *S. mutans* is a critical biomarker for caries risk assessment, especially in young children, but also provides new evidence that both the chairside SM Strip test and the molecular-based qPCR assay are reliable methods for the detection and quantification of *S. mutans* colonisation in the oral cavity. Thus, future planning for caries risk assessment, caries treatment evaluation, oral hygiene education and patient consultation should include one of these tests as the analytical approach of choice in clinical and epidemiological studies.

Conflicts of interest

The authors reported no conflicts of interest related to this study.

Author contribution

Drs Yihong Li, Prakaimuk Saraithong, and Komkham Pattanaporn contributed to the conception and design of the study and to data acquisition; Drs Prakaimuk Saraithong and Komkham Pattanaporn contributed to the chairside SM Strip test analysis; Drs Prakaimuk Saraithong and Zhou Chen contributed to the qPCR experiments. Drs Yihong Li, Prakaimuk Saraithong, Zhou Chen, and Erica Leung contributed to data analysis and interpretation and drafted the manuscript; Dr Ananda Dasanayake helped critically revise the manuscript.

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