ORIGINAL RESEARCH

Detection of Oral Microbial Levels with Chair Side and Laboratory Method in Children with Caries: A Randomized Double-blind Controlled Study

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Abstract

Aim: To enumerate the *Streptococcus* (*S.*) *mutans* count in children with and without early childhood caries (ECC) by three methods, i.e., laboratory *S. mutans* culture, oratest and saliva check mutans kit test, and correlate them with each other.

Materials and methods: The study was designed as a randomized, double-blind controlled trial and was carried out in children under 71 months of age. About 10 children without caries and 10 with a DEFT score of equal to or more than 5 were selected and subjected to all three tests, i.e., Global Corporation (GC) Saliva Check Mutans kit test, oratest and the laboratory culture of *S. mutans* individually.

Results: Both the groups were subjected to Chi-square test and Pearson's correlation analysis. All the tests performed showed statistically significant results ($p < 0.05$). In group I, i.e., the test group, the mean DEFT score was 7 ± 1.054. The mean *S. mutans* laboratory count was found to be 6.39×10^5 CFU. In group II, where the DEFT score was zero, the mean *S. mutans* count was 1.68×10^4 CFU. The mean time taken by Oratest to test positive was 40.50 ± 13.632 minutes in the test group, whereas in the control group, the mean oratest time taken was 279 ± 34.545 minutes. The sensitivity of GC saliva check test was 80% and the specificity was 100% with a Youden's index of 0.8.

Conclusion: All the tests correlated well with the DEFT scores of all subjects, as well as with each other. The choice of the test is dependent upon the availability, cost-effectiveness, time constraints, and the requirement of the individual patient.

Clinical significance: This study can serve as potent educational and motivational tool for patients in assessing their oral hygiene status with help of *S. mutans* count evaluation using the different tests. Hence, these *S. mutans* can be used as alarming biomarkers for prevention and treatment urgency.

Keywords: Global corporation saliva check mutans kit test, Laboratory culture test, Oratest, *Streptococcus mutans*.

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Introduction

Throughout the world, dental caries is among the most prevalent infectious diseases.[1] When one or more decayed (cavitated or non-cavitated) lesions, missing (cavitated), or filled tooth surfaces occur in any primary tooth of a child 71 months of age or younger, it is referred to as early childhood caries (ECC).[2]

Managing ECC requires developing both restorative and preventive strategies. Since caries is a multifactorial disease, predicting caries risk and the effectiveness of preventive measures depends on numerous factors, including the microbial count.

Since *Streptococcus mutans* (*S. mutans*) is the primary causative agent of dental caries, a quantitative measurement of *S. mutans* is considered a reliable indicator of actual caries activity.[3] Various techniques, both traditional and contemporary, are employed to measure *S. mutans* counts. The traditional method, considered the "gold standard," involves culturing *S. mutans* from saliva samples in a laboratory to count the colony-forming units. The preferred medium for this culture is Mitis-Salivarius-Bacitracin (MSB) agar, as established by Gold et al. in 1973.[3]

The Oratest is a microbiological assay that assesses aerobic microbe activity by measuring the rate at which methylene blue in a milk substrate changes from red to leucomethylene blue. *Aerobic* microbes use oxygen through the process of oxygen-mediated electron transfer, or aerobic dehydrogenase.

A well-equipped laboratory and at least 48 hours of incubation are needed for the culture test to identify detectable amounts of *S. mutans*. In contrast, chairside strip tests, used to measure the number of colony-forming units (CFU) of *S. mutans*, have been available in various forms for some time. A new rapid chairside method, the Saliva-Check Mutans immunoassay system, utilizes a monoclonal antibody system where detection is based on antigen-antibody reactions. This system is designed to provide highly specific and immediate results within 15 minutes, offering a quicker and more convenient approach for *S. mutans* detection that is both fast and easy to perform.[4]

The present study was designed to compare and correlate the efficacy of three salivary microbial detection tests, i.e., laboratory

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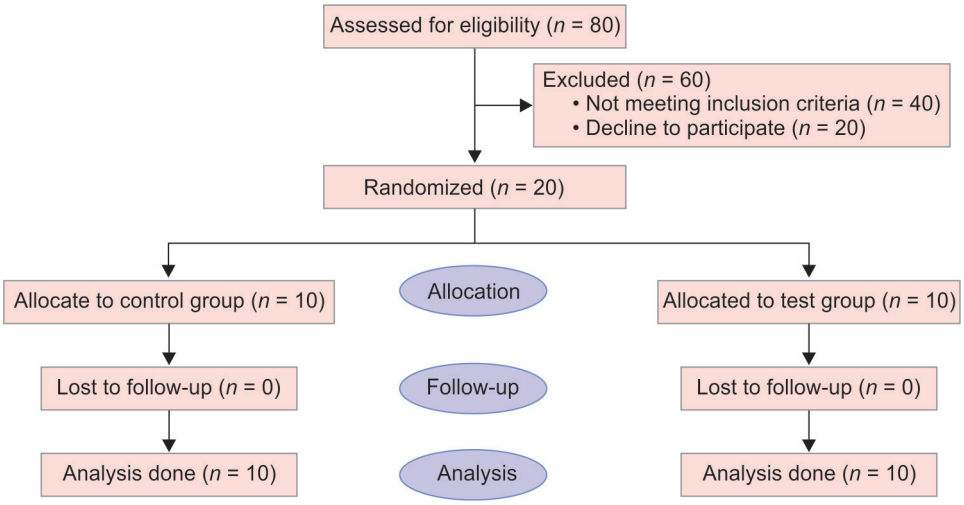

Fig. 1: Consort flowchart of the study

S. mutans culture test, Oratest and Global Corporation (GC) Saliva Check Mutans kit test, in children with and without ECC. The goal of the research is to assess the efficacy of chair side methods as an effective motivational and educational tool for patients. The aim of the study is to find the correlation of the tests for *S. mutans* with the caries status of the children. The objectives are (a) To enumerate the *S. mutans* count in children with and without ECC by laboratory *S. mutans* culture and the GC Saliva Check Mutans kit test, (b) to detect the level of aerobic microbial activity in the saliva through Oratest in the same children.

Materials and Methods

Ethical clearance for the study was granted by the institute, and all the Institutional Ethical Committee's and the Central Ethics Committee on Human Research's (India) ethical criteria were followed.[5] The study was conducted in the city of Modinagar, for a period of 1 month. The study was designed as a randomized double-blind controlled trial (Fig. 1) and was conducted on children under 71 months of age who visited the Out Patient Department (OPD) of the Department of Pediatric and Preventive Dentistry at the Institute of Dental Studies and Technologies, Modinagar. All children with caries were examined by one single examiner using visual and tactile methods. Intra-oral examination was done using a mouth mirror, and an explorer. DEFT scores were recorded as per WHO criteria. Gingival index was noted for each patient. The children with a maximum age of 71 months with no caries and equal to more than 5 carious lesions who were able to spit saliva in the vials were selected. To include the children in the study it was required that they should not have a medical history or a history of long-term drug use, have a gingival index score of zero, and show no signs of abscess, draining sinus, or cellulitis in relation to any tooth.

Exclusion Criteria

- Gingival index score exceeding zero
- Presence of an abscess, draining sinus, or cellulitis related to any tooth
- Use of antibiotics within the last month
- Children with a medical history or ongoing use of medications

A total of 10 children having DEFT score of equal to or more than 5 (test group) group I and 10 children without caries (control group) group II were selected on the basis of the inclusion and exclusion criteria, so that a total of 20 children were participating in the study.

Written consent was obtained for the child's involvement in the study, and the parents were informed about the child's condition as well as the rationale behind the tests and the process involved.

All the children in both the groups were subjected to the three tests individually.

Sub-group A: Laboratory culture for *S. mutans*
Sub-group B: Oratest
Sub-group C: GC Saliva Check Mutans kit test

Parents were advised to feed their child an early breakfast the following day in order to ensure that there was a minimum of ninety minutes between the child's last meal and the collection of ten milliliters of saliva. This is because it has been shown that the correlations are affected if the child consumes food or beverages before the test. The gap was maintained to ensure that the saliva was collected at a resting pH.[6]

Procedure for Laboratory *S. mutans* Culture Test

Method of Saliva Collection

A standardized protocol was implemented for saliva collection across all subjects. The child was seated upright in the dental chair and provided with a paraffin block to chew on. After 2 minutes of chewing, the stimulated saliva was collected in sterile containers positioned close to the mouth. This process was consistently applied to all patients. The collected samples were stored at 4°C and promptly transferred to the laboratory for further analysis.

Salivary Sampling for Estimating *S. mutans*

Using Mitis-Salivarius-Bacitracin agar, *S. mutans* was cultured (Gold et al.). The plates were prepared by placing saliva samples into wells and letting them sit there for 4 hours. The plates were incubated in an atmosphere with 5–10% CO2 for 48 hours at 37° C.[3] The colony-forming units (CFU) of *S. mutans* per milliliter of saliva were determined using the micropipette method (Westergren and Krasse). *S. mutans* colonies were identified by using a digital colony counter. These colonies were described as being elevated, round or spherical, strongly convex, dark blue in color, ranging in size from

Table 1: Mean DEFT, Laboratory count of *S. mutans* and oratest time of *S. mutans* of group I and II

	DEFT		*Laboratory count (CFU)*		*Oratest (Minutes)*	
Group	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Group I	7.00	1.054	6.39×10^5	129138.169	40.50	13.632
Group II	0.00	0.000	1.68×10^4	5788.878	279.00	34.545
Independent *t*-test	21.000		15.221		–20.309	
p-value	<0.001		<0.001		34.545	

pinpoint to pinhead, and rough-textured. Colony-forming units was used to record the results. The same researcher processed, looked over, and used a light microscope to confirm that *S. mutans* was present on all the plates.

Procedure for Oratest

Method of Sample Collection and Data Recording

The individuals were given 10 mL of ultra-high-temperature sterilized cow's milk, which they were to vigorously rinsed for 30 seconds. Next, to expectorate was gathered into a beaker. Using a disposable syringe, 3 mL of this was transferred to a test tube with a screw top. This was combined with 0.12 mL of 0.1% methylene blue, well mixed, and set aside at room temperature on a stand in front of a mirror in a well-lit environment. Every ten minutes, test tubes were checked for color changes at the bottom, which were clearly visible on the test tube stand using a mirror. The amount of time needed for the test tube's bottom to change color within a circle with a diameter of 6 mm was noted. This process was the same as described by Tal H and Rosenberg M.

S. Mutans Detection by Chairside GC Saliva Check Mutans Kit

Method of Sample Collection

For this test, the subjects were asked to chew on paraffin blocks for 3 minutes. Stimulated saliva was collected in a mixing container. About 250 microliters of saliva were measured using a pipette, and after 30 seconds of dynamic mixing with 50 microliters of Tris-NaOH (Reagent 1), 300 microliters of this reagent-mixed saliva were added to the test device vial to neutralize the pH. If no line was visible after 15 minutes, then the *S. mutans* count was considered low.[4]

Method of Statistical Analysis

This study has employed the subsequent statistical analysis techniques. A computer was used to obtain the data from a pre-coded survey, Performa. The software programs Excel and SPSS (SPSS Inc., Chicago, Version 21.0) were used for data entry and analysis. Descriptive statistics, such as percentages and numbers for discrete or categorical data and averages (mean + standard deviation) for continuous data, are shown for each parameter using tables and graphs. Every statistical test has specified significance at a probability value of 0.05 or less.

Results

Both the groups were subjected to Chi-square test and Pearson's correlation analysis.

Laboratory Culture and DEFT

In group I, i.e., the test group, the mean DEFT score was 7 ± 1.054. The mean *S. mutans* laboratory count was found to be 6.39×10^5 CFU. In group II, where the DEFT score was zero, the mean *S. mutans* count was 1.68×10^4 CFU as shown in Table 1.

A positive linear correlation was seen between caries rate and *S. mutans* numbers when subjected to Pearson correlation analysis as shown in Table 2 and Fig. 2.

Table 2: Correlation of DEFT, Oratest, *S. mutans* and GC saliva

	DEFT	*Oratest*	*S. mutans lab test*	*GC saliva*
DEFT				
Pearson correlation		–0.972	0.982	0.886
p-value		<0.001	<0.001	<0.001
Oratest				
Pearson correlation	–0.972		–0.960	–0.840
p-value	<0.001		<0.001	<0.001
S. mutans test				
Pearson correlation	0.982	–0.960		0.896
p-value	<0.001	<0.001		<0.001
GC saliva				
Pearson correlation	0.886	–0.840	0.896	
p-value	<0.001	<0.001	<0.001	

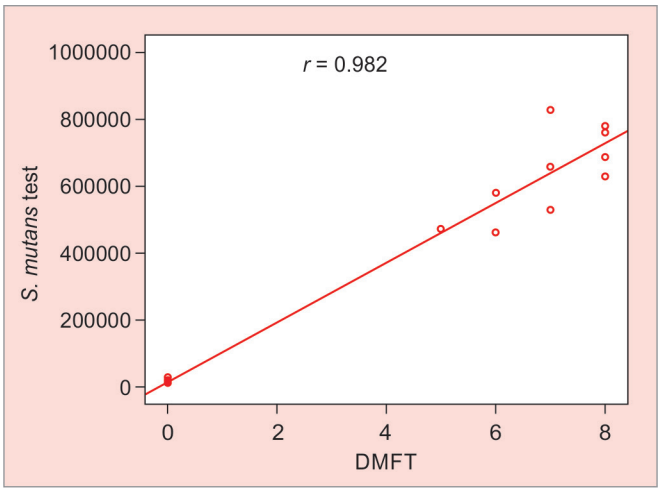

Fig. 2: Graph showing positive linear correlation between caries rate and *S. mutans*

Oratest and DEFT

The mean time taken by Oratest to test positive was 40.50 ± 13.632 minutes in the test group, whereas in the control group, the mean Oratest time taken was 279 ± 34.545 minutes. The difference in

the values between the study group and control group for both the tests was statistically significant, with a *p*-value less than 0.001 as shown in Table 1. There was a negative linear relation between Oratest and caries rate, as shown in Table 2 and Fig. 3. The lower the DEFT, the greater the time taken by the Oratest to test positive.

GC Saliva Check Mutans Kit Test with DEFT

As this test depends on the monoclonal antibodies against *S. mutans* it is a highly sensitive test for the detection of *S. mutans*. It detects *S. mutans* only when they are more than 5×10^5 of saliva. The same could be appreciated in our result. In the test group, out of 10 subjects, 8 tested positive and 2 tested negative, as shown in Table 3. In the control group, all the participants were negative. The two children who showed negative results in group 1 had a DEFT score of 5 and 6 individually and a mean DEFT of 5.50. Their *S. mutans* count was 4.7×10^5 and 4.6×10^5, respectively, with a mean of 4.65×10^5. However, the subjects who tested positive had a mean DEFT score of 7.38 and a mean *S. mutans* count of 6.82×10^5 as shown in Table 3. The sensitivity of the GC Saliva Check test was 80%, and the specificity was 100% with a Youden's index of 0.8. This test is dependable and helpful for assessing and measuring the *S. mutans* count, as evidenced by its 100% positive predictive value and 83.3% negative predictive value.

A positive linear correlation was seen between caries rate and *S. mutans* when estimated through a kit test and subjected to Pearson correlation analysis, as shown in Table 2 and Fig. 4.

All, the tests correlated well with each other and with the caries status of the child. All three tests are potent tools for *S. mutans* detection.

Discussion

The evaluation of all risk variables enables a more precise prediction of the development of caries, which makes caries risk assessment crucial. It aids in estimating the chance of caries occurrence within a specific time frame and in comprehending the likelihood that the lesion would alter in size or activity.[7]

Many caries prediction models often include the salivary levels of *S. mutans*.[8] Klock and Krasse originally reported on the quantitative study of *S. mutans* in saliva, and they discovered a direct correlation between the microorganisms' concentration in saliva and the presence of bacterial plaque on tooth surfaces. For dental plaque to be representative of the entire mouth, it must be gathered from numerous teeth. Saliva is therefore a better diagnostic tool for measuring the *S. mutans* count because it is easier to sample.[9] Additionally, it is simple to gather and includes systemically and locally generated markers of oral illness.[10]

In the present study, stimulated saliva was used to determine the *S. mutans* count, as stimulation of saliva results in a flushing effect and the clearance of oral debris and noxious agents.[11]

A high caries rate has been associated with an *S. mutans* count of 5×10^5 by Leal SC and Mickenautsch, who have summed up this

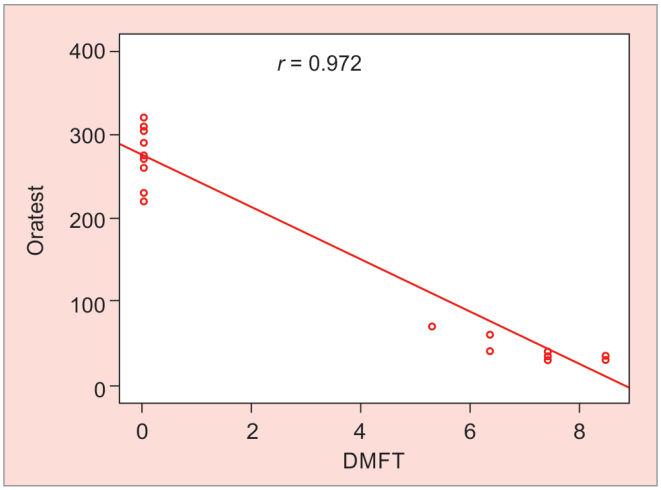

Fig. 3: Graph showing negative linear relation between oratest and caries rate

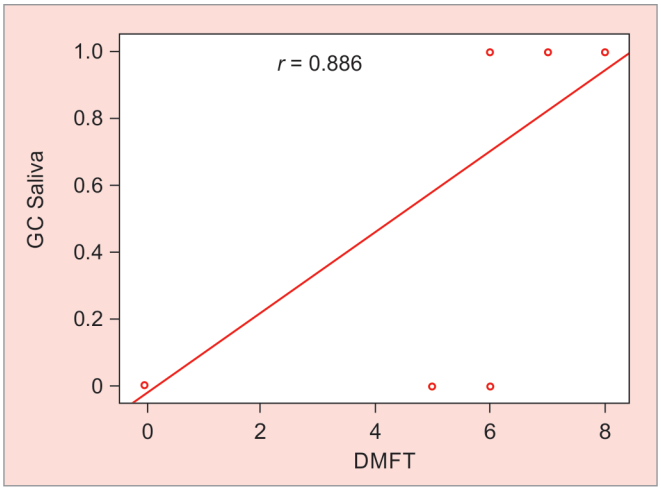

Fig. 4: Graph showing positive linear correlation between caries rate and *S. mutans*

Table 3: Correlation of GC Saliva kit with laboratory test and oratest

	DEFT		*S. mutans lab culture test (CFU)*		*Oratest (minutes)*	
GC Saliva	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*
Group I						
Negative (02)	5.50	0.707	4.65×10^5	7071.068	65	7.071
Positive (08)	7.38	0.744	6.82×10^5	103060.315	34.38	4.173
Independent *t*-test	–3.207		–2.853		8.357	
p-value	0.012		0.021		<0.001	
Group II						
Negative (10)	0.00	0.00	16800.00	5788.878	279	34.545

conclusion of high levels of *S. mutans* after reviewing and collecting the available data.[12] In the present study, a mean *S. mutans* count was seen to be 6.39×10^5 CFU with a mean DEFT score of 7.

Multiple research have evaluated the predictive usefulness of salivary levels in *S. mutans*, with inconsistent findings. There is a noteworthy correlation between salivary levels of *mutans streptococci* and the eventual onset of caries, as reported by some researchers; however, other investigations did not find this correlation.[13] These detected differences can be attributed to the different methods of saliva collection, caries status and methods employed in estimating the *S. mutans* count.

When salivary *S. mutans* were cultured with MSB agar, it was found that with an increase in the caries rate, the levels of *S. mutans* also increased.

A positive outcome of the test is present only when the *S. mutans* count is above 5×10^5 or it can be stated that only when the DMFT/DEFT score is quite high, GC test will be positive. In the present study, 8 out of 10 children in the study group showed positive results. The culture test of the two participants who tested negative in the study group showed an *S. mutans* count of 4.7×10^5 and 4.6×10^5 CFU. Thus, the present result reveals the highly sensitive and specific nature of this test in identifying the exact number of *S. mutans*. This kit uses a highly specific immunochromatography technique to identify *S. mutans* in saliva. Salivary *S. mutans* reacts with the anti-*S. mutans* monoclonal antibody tagged with colloidal gold on the test equipment. As a result, *S. mutans*' surface is coated in gold colloidal particles. This creates the red line on the test window when it combines with another *S. mutans* antibody.

A total of 190 children ages from 3 to 4 were subjected to a chairside test comparison by Gao XL et al. They discovered that the GC Saliva Check Mutans, with a sensitivity of 97.6% and specificity of 90.6%, was more useful and reliable.[4] Twetman L and Twetman S examined the efficacy of Dentocult SM and GC Saliva Check Mutans in 89 persons aged 23–72 years, and discovered that both were efficient for chairside detection of saliva check mutans, with a sensitivity of 80% and a specificity of 88%.[14]

Oratest was initially created by Tal H and Rosenberg M as a, chairside, non-invasive technique for determining the oral cavity's bacterial population.[15]

According to an Oratest conducted by Bhasin S et al., the control group took 148 ± 38 minutes, while the mean duration for color change was 36 ± 8 minutes for 24 children with a DMFT of 6–11.[6] According to Saxena S et al., color change for 50 children with a DMFT of ≥1 took 55.6 ± 2.31 minutes, but the comparison group, with a DMFT of 0.16, took 278.5 ± 1.50 minutes.[16]

In a study with 512 children, Sunadaram M et al. found that the time it took for an Oratest to test positive was 53.6 ± 6.8 and 271.4 ± 14.4 minutes in the test group (DMFT of 6–10) and the control group (DMFT of 0), respectively.[3] Chandak S et al. found that the time it took for a color change was 76 ± 8 min in the test group with a DMFT of 6–10 and 258 ± 28 min in the control group.[17] In the current investigation, a comparable time span was noted, with the test group lasting 40.50 ± 13.63 minutes and the control group lasting 279 ± 3.45 minutes.

The Oratest was found to be an efficient and cost-effective chairside tool for caries detection by Kunte SS et al. and Sundaram M et al. after they correlated it with the *S. mutans* laboratory culture test.

As the rate of caries increases, the absolute value of *S. mutans* also increases, which is evident by both, the laboratory culture as well as Saliva Check Mutans kit test. The increased bacterial count leads to lowering of the time taken by Oratest to test positive.

This study helps to identify and assess various chairside methods for detecting *S. mutans* count that can be meaningful educational and evaluation tools for high caries risk children. This can further be used as non-invasive methods for caries prevention by early detection and preventive program implementations. The Limitations of the study were (a) Sample size was less, (b) The GC Saliva Check Mutans kit is an expensive kit hence it limits its use for routine cases. This study requires a larger sample size in future to ascertain its use an educational and motivational tool for patients.

Conclusion

Within the limits of the study, it can be stated that all the tests correlated well with the DEFT scores of all subjects as well as with each other. The choice of the test is dependent upon its availability, cost-effectiveness, time constraints, and the requirements of the individual patient. The new GC Saliva Check Mutans kit is costly but is a wonderful tool for large scale *S. mutans* count evaluation as the time required and chances of error are very low as compared to the conventional laboratory method.

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