Effectiveness of an Antigen-Specific *Streptococcus mutans* Chairside Test as Compared to a Culture-Based *S. mutans* Test

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Abstract

Purpose: To compare the effectiveness of an antigen-specific *Streptococcus mutans (S. mutans)* chairside test to a culture based *S. mutans* test.

Methods: Fifty-three patients receiving dental hygiene care at the University of Missouri-Kansas City, School of Dentistry were enrolled in the study. Stimulated saliva was collected from the patients and utilized for both bacteria tests. The antigen-specific test was compared to the culture-based bacteria test and to a caries risk assessment measuring sensitivity and specificity.

Results: The majority of participants were male (53%) with high caries risk (60%). The culture based test results were primarily negative (62%); while the antigen-specific test had more positives (76%). The sensitivity and specificity comparing the antigen-specific test to the culture based test was high (88%, 95% CI = (78%, 97%) and low (25%, 95% CI = (13%, 37%), respectively. The sensitivity and specificity comparing the antigen-specific test to caries risk was high (83%, 95% CI = (72%, 93%) and low (38%, 95% CI = (24%, 51%) respectively.

Conclusions: While the sensitivity of the antigen-specific test was high for both the culture- based test and caries risk, the specificity was low for both. These results suggest that the antigen-specific test tends to give a higher proportion of false positive results.

Keywords: caries risk assessment, salivary testing, culture-based bacteria test, antigen-specific assay test

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Introduction:

Dental caries is a multifactorial and infectious disease of microbial origin impacting every specialty within dentistry.[1,2] One factor in the etiology of dental caries is *mutans streptococci* (MS).[2-4] The *mutans streptococci* include the *Streptococcus mutans* and *Streptococcus sobrinus* species of bacteria. *Mutans streptococci* colonize the host only after the first teeth erupt[5-7] and can be passed to children simply by the transference of saliva.[5, 8-10]

S. mutans species are elevated in saliva of individuals who are susceptible to dental caries.[11] Chairside tests are available to assist in determining caries risk due to the presence or absence of *S. mutans* through the use of stimulated saliva.[12, 13] Chairside tests come in two categories: culture-based tests and antigen-specific assays. The culture-based *S. mutans* tests are conducted on collected saliva and are sensitive enough to provide a degree of low, medium, or high cariogenic bacterial challenge.[12, 13] The most common culture- based medium to test for streptococci is the Mitis-Salivarius agar or Mitis Salivarious agar with bacitracin (MSB).[12] The agar is inoculated with stimulated saliva elicited by chewing on a small paraffin block to dislodge bacterial plaque while dispersing it into the saliva. Caries risk test, CRT® (Ivoclar Vivadent Inc, Amherst, NY), is a dental chairside test utilizing a blue Mitis-Salivarius agar with bacitracin to detect *mutans streptococcus* and a Rogosa agar to evaluate the presence of lactobacilli.[14] Comparisons of CRT® with standard microbial laboratory methods demonstrate similar results.[15-17] The same is true for comparison with a similar system, Dentocult® (Orion Diganostica, Espoo, Finland), which had been considered to be the standard chairside caries risk testing procedure until the late nineteen-nineties.[17]

Another type of caries risk test is based on an antigen-specific assay. These tests utilize highly specific monoclonal antibodies which provide absolute specificity for the bacteria of choice. The rapid detection *S. mutans* dental chairside test, Saliva-Check Mutans (GC America; Alsip, IL), is considered to be an antigen-specific assay. The test uses a combination of three highly specific anti-*S. mutans* monocolonal antibodies to increase binding and reduce the detection limit for 100,000 bacterial colonies per mL of saliva, the recognized level for increased caries risk.[12] Unlike culture-based tests, viable bacteria are not needed for this type of testing.[12] The purpose of this study is to compare the effectiveness of an antigen-specific *Streptococcus mutans* (*S. mutans*) chairside test to a culture-based *S. mutans* test.

Methods

This study was a cross-sectional clinical trial conducted at the University of Missouri-Kansas City, School of Dentistry, utilizing subjects who were patients of record, seeking care in the dental hygiene clinic. All patients voluntarily chose to participate in the University of Missouri-Kansas City, Institutional Review Board approved study. A consent script was read to each subject and verbal consent was obtained by the clinical research assistants. Fifty-three (n=53) subjects consented to participate in the study and the electronic patient records were utilized to gather information from the medical history and dental records for subject demographics and caries risk.

Normal standard of care procedures for patients presenting to the dental hygiene clinic includes the collection of saliva and determination of caries risk by using the Caries Management by Risk Assessment (CAMBRA) criteria[3] which is entered in the electronic patient record. The determination of caries risk is as follows: Low (no disease indicators, <2 risk factors, has protective factors), Moderate (no disease indicators, > 2 risk factors, but no caries), and High (cavitated lesion(s)/disease indicators or > 3 risk factors). For the purposes of this study, caries risk was evaluated without the *S. mutans* component.

Patients enrolled in the study were instructed not to smoke, eat, drink, brush their teeth or use a mouth rinse one hour prior to their appointment. The routine saliva collection process facilitated the determination of stimulated and unstimulated pH and the buffering capacity required for the caries risk assessment. The antigen-specific assay chairside test, Saliva-Check Mutans (GC America, Inc.; Alsip, IL) and the culture-based bacteria test, CRT® (Ivoclar Vivadent, Inc.; Amherst, NY), were administered during a routine appointment in the dental hygiene clinic as part of the participant's intra-oral examination. Clinical research assistants, calibrated on the manufacturer instructions for collection by the chief examiner, collected the saliva and conducted the tests according to the manufacturers' specifications.

The clinical research assistants also collected and recorded the following information from the dental records into a password protected spreadsheet: caries risk, tobacco use status, current or recent use of antibiotic (within 2 weeks) and any use of an antimicrobial rinse. Bacteria tests conducted as part of the study were not factored into the caries risk determination, since bacteria tests can either be utilized as a baseline reference or for suspicion of high bacterial challenge.

The antigen-specific test for colony count of *mutans Streptococcus* levels was used in this study. The patient chewed on paraffin wax for one minute and then expectorated into a calibrated plastic medicine cup for a stimulated saliva sample. Stimulated saliva from the cup was poured into the saliva collection vessel provided in the antigen-specific test kit, up to the indicator line. One drop of reagent one (alkaline agent) was then added to the stimulated saliva and the container was tapped 15 times**.** Four drops of reagent two (neutralizing agent) were then added to the stimulated saliva and the container was shaken until the sample turned green, indicating that the solution had gone from alkaline to neutral pH. The manufacturer included pipette was used to draw saliva up from the container and three drops of the sample were dispensed into the window of the test device. After 15 minutes time, the test device was observed for a positive or negative test result for *S. mutans*. A positive reading (red line is shown on the test line) indicated *S. mutans* levels were > 500,000 cfu/ml and a negative reading (no line shown on the test line) indicated < 500,000 cfu/ml of *S. mutans*. An invalid test reading signified the test did not clearly indicate a positive or negative reading.

The same stimulated, unmodified saliva collected for the antigen-specific test was used for the culture-based test. During this test procedure, a sodium bicarbonate ($NaHCO_3$) tablet was placed in the manufacturer supplied test vial and carbon dioxide (CO_2) was released upon contact with moisture. The protective foil was then removed from the cultured-based test agars. Using the supplied pipette, both agars were then covered with saliva taking care not to scratch the culture media. The agar carrier was held slightly oblique to prevent the saliva from flowing off too quickly and to allow for thorough wetting of the surface. The agar carrier was immediately placed in the test vial, which was then tightly sealed according to the manufacturer's directions. The agar carriers were then labelled with date and time, and placed in an incubator (37°C /99°F) located in the oral biology lab at the institution for 48 hours. The culture-based *S. mutans* test comparison diagram was used to determine negative or positive results for *S. mutans*.

The antigen-specific test results were compared to the culture-based *S. mutans* test results and to the caries risk assessment obtained using CAMBRA criteria (excluding the *S. mutans* component) using sensitivity and specificity.

Results

Fifty-three volunteer subjects enrolled in the study. The majority were male 53% (n=53), 60% had high caries risk, 79% were non-smokers, 100% had not used any antibiotics within the last 2 weeks, and 96% had not recently used an antibacterial mouth rinse (Table I). The culture-based *S. mutans* test results were primarily negative (62%), while the antigen-specific test had more positive results (76%). The was one invalid result antigen- specific test (Table I).

Overall, the sensitivity when comparing the antigen-specific test to the culture-based *S. mutans* test was high (88%, 95% CI=(78%, 97%). Comparisons of specificity of the antigen-specific test to the culture-based test was low (25%, 95% CI= (13%, 37%). The sensitivity of the antigen-specific test compared to caries risk was high (83%, 95% CI=(72%, 93%) while the specificity for this same comparison was low (38%, 95% CI = (24%, 51%). Sensitivity and specificity comparing antigen-specific to culture-based test and to caries risk is shown in Table II.

Table I. Test results and covariates of interest by caries risk assessment.

	Caries Risk			
	Low (N = 8) N (%)	**Moderate (N = 10) N (%)**	**High (N = 35) N (%)**	**Total (N = 53)**
Culture Based *S. mutans* Results				
Negative	7 (87.5%)	7 (70.0%)	19 (54.3%)	33 (62.3%)
Positive	1 (12.5%)	3 (30.0%)	16 (45.7%)	20 (37.7%)
Antigen-Specific Results				
Negative	3 (37.5%)	3 (30.0%)	6 (17.1%)	12 (22.6%)
Positive	5 (62.5%)	7 (70.0%)	28 (80.0%)	40 (75.5%)
Test Invalid	0 (0%)	0 (0%)	1 (2.9%)	1 (1.9%)
Gender				
Female	5 (62.5%)	6 (60.0%)	14 (40.0%)	25 (47.2%)
Male	3 (37.5%)	4 (40.0%)	21 (60.0%)	28 (52.8%)
Smoker				
No	8 (100%)	9 (90.0%)	25 (71.4%)	42 (79.2%)
Yes	0 (0%)	1 (10.0%)	10 (28.6%)	11 (20.8%)
Any Antibiotic Use				
No	8 (100%)	10 (100%)	33 (94.3%)	51 (96.2%)
Yes	0 (0%)	0 (0%)	2 (5.7%)	2 (3.8%)
Any Antibiotic Use within 2 Weeks				
No	8 (100%)	10 (100%)	35 (100%)	53 (100%)
Yes	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Any Recent Use of Antibacterial Mouth Rinse				
No	8 (100%)	10 (100%)	33 (94.3%)	51 (96.2%)
Yes	0 (0%)	0 (0%)	2 (5.7%)	2 (3.8%)

Discussion

S. mutans is a major contributor to the development of dental caries.[13,18-20] Chairside testing for *S. mutans* is one component of the caries risk assessment tool utilized by dental professionals to determine patients' caries risk level. The caries management by risk assessment tool (CAMBRA) assists clinicians in managing caries through preventive counseling or clinical interventions.[3] Patients who have one or more disease indicators (cavities present, interproximal enamel lesions on radiographs, white spot lesions on smooth surfaces, and restorations placed within the last 3 years) fall into the high-risk category for caries.[3] Bacterial testing is recommended for these patients to determine their colonization levels of specific bacteria. Patients recruited for this study had not been screened for disease indicators which would have recommended the use of a bacterial test to determine their caries risk levels. The culture-based test had not been factored into the determination of the patients' caries risk, which might have changed the caries risk reflected in their initial assessment. For the purposes of this study, caries risk was utilized to compare the antigen-specific *S. mutans* chairside test to the culture-based test. Therefore, the bacteria test results were not utilized to determine caries risk in order to protect from unnecessary influence in the outcomes of the study.

Contraindications for the culture-based test indicate that patients who had recently received antibiotics would need to wait for at least two weeks before completing the test and patients who had used an

Table II. Sensitivity and specificity comparing antigen-specific to culture-based test and to caries risk (low vs. moderate or high)[1]

Culture Based *S. Mutans* Test						
	Negative (N = 33) N (%)	**Positive (N = 16) N (%)**	**Sensitivity***	**95% CI**	**Specificity***	**95% CI**
Antigen-Specific Test			87.50%	(78.14%, 96.86%)	25.00%	(12.75%, 37.25%)
Negative	8 (24.2%)	2 (12.5%)				
Positive	24 (72.7%)	14 (87.5%)				
Test Invalid	1 (3.0%)	0 (0%)				
Caries Risk						
	Low (N = 8) N (%)	**Moderate/ High (N = 41) N (%)**	**Sensitivity***	**95% CI**	**Specificity***	**95% CI**
Antigen-Specific Test			82.5%	(71.75%, 93.25%)	37.5%	(23.8%, 51.2%)
Negative	3 (37.5%)	7 (17.1%)				
Positive	5 (62.5%)	33 (80.5%)				
Test Invalid	0 (0%)	1| (2.4%)				

[1] Excluding patients with any antibiotic or any recent antibacterial mouth rinse use (n = 49)

*Note: Sensitivity and Specificity calculations do not count the test invalid categories

antibacterial mouth rinse would need to wait at least twelve hours before culture-based testing could be performed. Antibiotic and antibacterial use can alter the effectiveness of the culture-based testing due to the associated reduction in the number of bacterial colonies. The study data indicated that 2% (n=2) of the patients had a history of antibiotic use, but none within 2 weeks. The data also indicated 2% (n=2) had recent used an antibacterial mouth rinse. Therefore, the analysis excluded these four subjects from the sensitivity and specificity calculations.

Precautions for the antigen-specific test indicate that patients are to be instructed not to smoke, consume food or drink, nor brush their teeth one hour prior to their appointment. Contraindications for both the culture-based test and the antigen specific-test may have altered the results. All subjects were instructed by the clinician to adhere to the same precautions as indicated for the routinely conducted salivary testing which mirrored the precautions for the antigen-specific test. However, the researchers needed to rely on the subject's word regarding adherence to the precautions. Future studies should require that the subject report to the clinic setting one hour prior to the salivary testing to ensure compliance to the required precautions.

Caries risk is correlated to the levels of MS on the teeth.[18] The MS level detection limit is 100,000 /mL of colony forming units (cfu), the recognized level for increased caries risk.[17,21] The antigen-specific chairside test indicated a positive result with bacteria counts at 500,000 cfu/ml while the culture-based test detected MS 100,000 cfu/mL or ≥ 10^5 cfu.[12,17] At these levels, for the antigen-specific test to result in a positive indication for high levels of *S. mutans,* a level of 500,000 cfu would be necessary, whereas the culture-based test recognizes the *S. mutans* risk at 100,000 cfu. These limits are noteworthy since MS

level detection is recognized to increase at 100,000 cfu/mL or ≥ 10^5 cfu.

When comparing the antigen-specific test values with the culture-based *S. mutans* test and the MS threshold levels of detection, the following scenarios must be considered:

- If the antigen-specific test results in a positive (high risk) and the culture-based *S. mutans* test read positive (high risk), it indicates that the MS concentration has reached 500,000 cfu.
- If the antigen-specific test results in a negative (low risk) and culture-based *S. mutans* test reads positive (high risk), it indicates that the MS concentration has reached 100,000 cfu.
- If the antigen-specific test results in a negative (low risk) and culture-based *S. mutans* test reads negative (low risk), it indicates that the MS concentration was less than 100,000 cfu.
- If the antigen specific test does not read either negative (low risk) or positive (high risk) and the culture based *S. mutans* test is positive (high risk) or negative (low risk), it indicates that the antigen-specific test is not reliable for reading (missing data point).
- If the antigen-specific test reads positive (high risk) and culture-based *S. mutans* test reads negative (low risk), a concern is raised considering whether the culture-based *S. mutans* test is the more sensitive test. Further testing ensuring that the contraindications for the culture-based *S. mutans* test were not the factor would be needed, as those can confound the results of the test.

The culture-based *S. mutans* test was utilized in this study due to its proven reliability.[16,17,22] When comparing the two tests, one must take into consideration that the antigen-specific test is a newer product on the market, utilizing highly specific anti-*S. mutans* monoclonal antibodies designed to increase binding and reduce the detection limit.[12] The saliva sample collected for the antigen-specific test reacts with buffers to establish a constant pH and detergents for proper dispersal of the sample. The saliva sample is placed on a nitrocellulose strip with impregnated monoclonal antibodies which trap the S. *mutans* bacteria, triggering a detection reaction. Control reactants are utilized to ensure proper functioning of the detection chemistry.[12]

Dental offices should consider factors such as cost, time, reliability, and effectiveness when considering a chairside test for MS bacteria counts. The culture-based *S. mutans* test utilizes viable bacteria requiring incubation for 48 hours at 37°C; counter top incubators are available for dental practices to purchase. The antigen-specific test contains monoclonal antibodies to detect select *S. mutans* species and can be completed chairside in five minutes, enabling the clinician to share the test results with the patient before the end of the appointment. The instant results of the antigen-specific assay provide clinicians with a rapid, valid test for the quantification of *mutans streptococci*. However, data from this study reveal that the antigen-specific test tends to yield a higher proportion of false positive results.

Conclusion

While the sensitivity of the antigen-specific test was high for both the culture-based test and caries risk, the specificity was low for both types of tests. These results suggest that the antigen specific test tends to yield a higher proportion of false positive results.

Disclosures

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