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Evaluation of Bacterial Enumeration of CariScreen Versus Traditional Selective Culture

by

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THESIS

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MASTER OF SCIENCE

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[redacted]

Dedications and Acknowledgements

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Special thanks to my partner in life, Elliott Smith, for his unwavering support throughout my personal and professional career.

Evaluation of Bacterial Enumeration of CariScreen Versus Traditional Selective Culture

Gina Graziani, D.D.S.

Purpose: The purpose is to evaluate whether the CariScreen meter is a reliable tool in quantifying cariogenic bacteria and a valuable addition to caries risk assessment.

Methods: Sixty children, aged 6-17 years, were recruited. Caries status was recorded utilizing ICDAS system. CariScreen scores were evaluated from two sites: the lingual surfaces of the mandibular anterior dentition per manufacturer's instruction and the mandibular buccal surface of one posterior molar. Stimulated saliva samples were collected for bacterial enumeration (Mitis Salivarus plate for total oral streptococci, Mitis Salivarus Sucrose Bacitracin plates for Mutans Streptococci (MS), Rogosa tomato juice plates for *Lactobacillus* (LB), and Blood agar plates for total viable bacteria). Statistical analysis was conducted using SPSS 22.

Results: CariScreen scores from anterior and posterior sites revealed a moderate correlation (Correlation Coefficient = 0.42). CariScreen scores for both anterior and posterior sites revealed poor correlation with logarithmic counts of Mutans Streptococci, Total oral streptococci, *Lactobacilli*, and total viable bacteria (Pearson Correlation coefficient between -0.30 to 0.03, $P>.02$ for anterior sites and between -0.10 to 0.18, $P>.18$ for posterior sites). For anterior sites, caries-free subjects had higher log CariScreen scores than caries-active subjects (mean±SE as 3.78±0.11 and 3.34±0.06, respectively, $P=.12$) while, for posterior sites, caries-free subjects' log CariScreen scores were similar to caries-active subjects (mean±SE as 2.95±0.19 and 3.06±0.08, respectively, $P=.76$). There were acceptable sensitivities (66-70%), but poor specificity (33-

37%) and high false positive rate (45-75%) in diagnosing MS, LB and MS+LB levels compared with gold standard culture methods.

Conclusion: Compared to bacterial culture, CariScreen meter showed poor prediction of cariogenic bacteria levels. Furthermore, CarieScreen scores in anterior sites reveal a trend of negative correlation to cariogenic bacteria levels and caries status.

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Introduction

Dental caries is the most common chronic disease of childhood (1). Distribution of dental caries in children is skewed (2) that 25% of children aged 5 to 17 years account for 80% of caries load in permanent teeth while 7% of children account for 80% of decay load in primary teeth (3,4). Dental decay is particularly worrisome in children as it may lead to disruption of dental development, tooth pain, malnutrition, infections, and high caries risk for permanent dentition.

Given oral health disparities in children, there has been an increased interest in the development of caries risk assessments for prevention practices. Caries risk assessment determines the probability of caries incidence in a certain period (5). The etiology of dental caries is multifactorial. Factors in the commencement of dental caries include fermentable carbohydrates, oral microflora, presence of teeth, ethnicity, socioeconomic status, salivary flow, genetic factors, health history, medications, among others (6). Caries risk assessment functions to identify risk factors and protective factors associated with dental caries. Based on the findings, specific treatment recommendations with stricter preventive and therapeutic treatments will be recommended (7).

Dental caries is an infectious disease with Mutans streptococci (MS) and *Lactobacilli* identified as two main groups of cariogenic bacteria (8). MS has proven to be a significant contributor in dental caries development (9). Reduction of the MS population on teeth is followed by a reduction in caries activity (10). Furthermore, caries free children have less than one percent of MS in their oral flora (11). Therefore, identification and quantification of MS has been considered as one of the strongest single risk factors associated when predicting caries development (12), and is utilized as an important component of caries risk assessments (13). A variety of microbiological test assays are available for measuring the amount of MS and

Lactobacilli in saliva, including bacterial culture, molecular techniques, and ATP-driven bioluminescence (14). Although these techniques may offer superior specificity and sensitivity over traditional culture techniques, they are expensive and require trained and specialized skills as well as laboratory equipment (15). Therefore, these methods are not readily available for chair-side use by dental clinicians.

Chair-side cultural tests are reported to simplify the bacterial quantification process by making it readily available to the practicing dentists. MS quantification by so-called dip slide chair-side culture tests in children showed significant correlation with MS counts on conventional selective media for MS (16). However, the chair-side cultural test requires laboratory equipment (namely incubators) and requires 72-hour incubation prior to bacterial quantification. Therefore, it does not allow dentists to provide a same-day preventive treatment plan and education to patients. Although the chair-side cultural tests have been available for decades, clinicians rarely employ them in their caries risk assessments due to these inherent limitations. There continues to be a need for a reliable, easy-to-use chair-side rapid assay method for practicing dentists to use when assessing oral bacterial counts for caries risk assessments.

The CariFree CariScreen Caries Risk Testing meter has been introduced as an alternative rapid chair-side assay for cariogenic bacterial quantification. The CariScreen utilizes ATP-driven bioluminescence technology, which uses the presence of ATP to estimate the number of viable cells. ATP is a universal constituent of all cellular organisms, occurs in proportion to cellular mass, is rapidly degraded, and can be assayed specifically with exquisite sensitivity (17). Rapid ATP-driven bioluminescence assays have proven to be accurate in the enumeration of bacteria in dental plaque (18). A recent study has revealed that the ATP-driven bioluminescence

technique *in vitro* is predictive of numbers of total bacteria and total *Streptococcus* counts, reflective of MS (19). However, there are limited published studies to support the CariScreen meter as a reliable tool for cariogenic bacterial enumeration for caries risk assessment. The lack of clinical evidence calls for the need of further research to verify the CariScreen meter as a useful and reliable tool for cariogenic bacterial quantification in caries risk assessments. A comparison of CariFree CariScreen Caries Risk Testing meter enumeration of oral, cariogenic bacteria to the gold standard, selective culture on stimulated salivary samples will provide evaluation of whether the CariScreen tool is an accurate and efficient chair-side rapid assay test in determining caries risk.

Aims and Hypotheses

The overall goals of the current study are to evaluate whether CariFree CariScreen Caries Susceptibility Testing meter can be a reliable clinical chair-side tool to enumerate oral, cariogenic bacteria levels. The specific aims of this study are to 1) study the correlation of the CariFree CariScreen Caries Susceptibility Testing Meter in oral cariogenic bacteria quantification with the gold standard, selective culture enumeration on stimulated saliva samples; 2) calculate the sensitivity, specificity, and false negative rate of the CariScreen meter scores when compared to culture enumeration, and 3) evaluate the correlation of cariogenic bacteria level measured by CariFree CariScreen Caries Susceptibility Testing Meter with a subject's caries status. We hypothesized that cariogenic bacteria levels measured by CariFree CariScreen Caries Susceptibility testing meter will strongly correlate with oral bacterial levels measured by gold standard, bacterial cultures on selective media, have good sensitivity and specificity in diagnosing cariogenic bacterial levels and be correlated well with subjects' caries status.

Materials and Methods

The research protocol was approved by the UCSF Committee on Human Research (Approval number 14-13982). Informed consent was obtained from the guardian of all study subjects. Sixty subjects were recruited from the UCSF Pediatric Dental clinic from August to December 2015. The inclusion criteria of the subjects were: 1) 6-17-year-old children who are patients at UCSF pediatric pre-doctoral and post-graduate dental clinics; 2) subjects that are able to cooperate for the study procedure; 3) have six fully erupted mandibular anterior teeth; 4) subjects reside within a 30 mile radius of the associated study clinics to increase the retention rate at follow-up appointments of future studies. The exclusion criteria were: 1) Less than 6 or greater than 17 years of age; 2) lack of erupted mandibular six anterior teeth; 3) use of antimicrobials within past three months with the exception of topical antibiotics used for caries prevention (e.g. Chlorhexidine Gluconate); 4) severe gingival bleeding; 5) special needs patients with the lack of ability to cooperate; and 6) subjects that reside outside a 30 mile radius of the associated clinics. All study appointments were scheduled at 1 hour after eating and 2 hours after the last tooth-brushing.

The following information was collected by a questionnaire: age, gender, race/ethnicity, medications within past 3 months, demographic data, and the time from last known toothbrushing, together with a standard caries risk assessment form. Dental caries scores were collected using International Caries Detection and Evaluation System (ICDAS) scoring system for each child by one examiner. Bacterial enumeration by CariScreen meter was performed on anterior mandibular lingual sites per manufacturer's instruction as well as a mandibular right posterior molar buccal site. Stimulated whole saliva was collected for each child and transported on ice for microbiological assays within 24 hours. A plaque index was generated utilizing the

criteria of the Simplified Oral Hygiene Index (20). All examinations and specimen collections were completed before any dental procedure performed for each patient to eliminate potential treatment modalities, such as a dental prophylaxis, from interfering with the oral environment.

Sample Size Calculation

In the Fazilat study, a correlation coefficient of 0.471 between ATP bioluminescence readings (derived from CariScreen meter) and total MS in plaque samples (18). Therefore, at least 26 subjects will be needed at a type I error, $\alpha = 0.05$, and a power of 80%. A sample size of 60 will provide a 95% confidence interval for the correlation coefficient of 0.47 with a half width of 0.20 and will show the significance of a correlation coefficient of 0.47 at the Type I error of 0.05 and power of 0.97.

Bacterial Enumeration by CariScreen Meter

Bacterial scores by CariScreen meter were collected from two sites: 1) the lingual surfaces of six mandibular anterior teeth, per CariScreen manufacturer's instruction; 2) the buccal surface of a posterior mandibular tooth. This second site was added because the manufacturer's recommended site of the lingual surfaces of mandibular anterior teeth are associated with clinically low caries activity. For both sites, the samples were collected by carefully swabbing the mid-lingual or buccal surfaces of respective surfaces without contacting the gingival or any soft tissue with any part of the swab. The scores of CariScreen were then obtained following manufacturer's instructions for the device (21).

Saliva sample collection, storage, transportation, culture and enumeration

The stimulated saliva samples were collected by asking the participants to chew on a paraffin wax tablet until 5-mL of saliva was collected. Samples were stored on ice or at 4ºC and processed for culture within 24 hours. Saliva samples were inoculated on Mitis Salivarius

sucrose bacitracin plates for MS enumeration, Mitis Salivarius plates for total streptococci enumeration, Rogosa tomato juice plates from LB enumeration and Brain Heart Infusion blood plates for total viable bacterial enumeration as previously described (22). The plates were incubated anaerobically at 37°C for 72 hours before enumeration under a dissecting microscope (22).

Dental caries examination and enumeration

A dental examination, caries risk assessment, and plaque score were performed for all 60 subjects by one provider. The International Caries Detection and Evaluation System (ICDAS) scores were generated and recorded for all teeth present (23).

Plaque Index

A plaque index score was completed for each participant utilizing the Simplified Oral Hygiene Index (OHI-S) (21). Six tooth surfaces were examined, including the buccal surfaces of the maxillary primary second molar/permanent first molar, the lingual surfaces of the mandibular primary second molar/permanent first molar, and the labial surfaces of the upper right and lower left central incisors. The score for each surface recorded as "0" for no plaque debris; "1" for plaque covering less than one third of the tooth surface; "2" for plaque covering one third to two thirds of tooth surface; and "3" for plaque covering more than two thirds of the exposed surface. An average plaque score of all surfaces was calculated for each subject.

Data Analysis

Data analyses were performed using SPSS 22. Descriptive analyses were performed to summarize the data. Means, standard deviations (SDs), median and quartiles were calculated for continuous variables, while frequencies and percentages were calculated for categorical variables.

The logarithmic transmission of the bacteria levels by culture and CariScreen were used for all tests to achieve normal distribution of the data. Pearson correlation coefficients between CariScreen scores and bacteria levels by culture in stimulated saliva samples were computed. Based on the manufacturer's instructions, CariScreen scores were categorized as low (CariScreen score<1500) or high (CariScreen score≥1500)) for bacterial challenge per manufacturer instruction. For MS levels by selective culture, <100,000 CFU/ml were categorized as low (combined low and moderate challenge group for MS) or ≥100,000CFU/ml as high. For LB levels by culture, <1,000 CFU/ml were categorized as low (combined the low and moderate challenge group for LB) or ≥1,000CFU/ml as high (24). The sensitivity, specificity, false positive rate and false negative rate were calculated for CariScreen data using manufacture instruction of mandibular anterior teeth for MS, LB and combination of MS and LB. The bacteria levels by culture, caries scores (dmfs/DMFS), and plaque scores between subjects with high or low CariScreen scores were analyzed by Student t test or non-parametric based on the normality of the data distribution. In addition, the bacteria levels by culture and CariScreen were also compared between subjects with or without caries lesions by Student t test or non-parametric tests based on the normality of the data distribution.

Results

Subject demographics, bacterial levels and caries status

Summaries of subject demographic, bacterial levels and caries data are illustrated in Table 1. The mean age of the study population was 9.3 years old (SD: 2.7) with more females (68.3%) and diverse race/ethnicity as the populations resided in the San Francisco Bay area. The majority of the subjects presented with active decay including white spot lesions with a high mean of DS

(10.4±6.9) and dmfs/DMFS scores (21.3±15.1).

Correlation among CariScreen Scores, Bacterial Counts by Culture, caries status and plaque index

Figure 1 through 4 present the scatter plots of the bacterial levels by culture and the CariScreen scores for anterior and posterior sites. Table 2 shows the correlation coefficiencies among log CariScreen scores, log bacterial levels by culture, caries scores (ds/DS and dmfs/DMFS), and plaque scores. There was moderate significant correlation between log CariScreen scores from anterior and posterior sites (correlation coefficient=0.47, $P<0.01$). However, correlation between log CariScreen scores from either anterior and posterior sites, and levels of logLB, logTS and logTVC were poor except a negative correlation between log anterior CariScreen scores and logMS levels (Correlation coefficient=0.30, $P<0.05$).

For correlations of bacterial counts and caries status, only logMS by culture showed a moderate significant correlation with dmfs/DMFS scores (Correlation coefficient=0.28, $P<0.05$).

Bacterial levels by culture, ds/DS, dmfs/DMFS and plaques scores between subjects with high or low CariScreen scores were compared to further evaluate whether CariScreen can be used as a good measure of cariogenic bacterial and whether there is any association with caries level. No statistical significant differences were found, but there was a borderline negative trend for anterior CariScreen scores with logMS levels (Table 3, $P=0.07$).

Sensitivity and Specificity of Bacterial Challenge by Anterior CariScreen (Figures 6 through 8)

Figures 6 through 8 depict the sensitivity, specificity and false negative rate for anterior CariScreen scores compared to MS, LB, and MS+LB counts, respectively. Our data reveal there is fair sensitivity but poor specificity and high false negative rates between anterior CariScreen scores and cariogenic bacteria levels by culture.

CariScreen scores, bacterial levels, and plaque score by culture in caries-free and caries-active subjects (Table 4)

For anterior sites, caries-free subjects had higher log CariScreen scores than caries-active subjects but it is not statistically significant. For posterior sites, caries-free subjects' log CarieScreen scores were similar to caries-active subjects. LogMS and logLB were higher in caries active subjects than caries free subjects, but the difference was not significant ($P>0.05$). LogTS, logTVC and plaque scores were similar between caries free and active subjects.

Discussion

Our results revealed poor correlations of CariScreen scores, anterior or posterior, to cariogenic and total bacteria selective culture counts in our mostly high risk subjects. This is in contrast to Fazilat 2010 who found fairly good correlation between ATP bioluminescence technology and bacteria levels. However, there are several differences from this study to Fazilat 2010. First, Fazilat 2010 results were produced using real laboratory techniques, namely the luciferase-based assay system, in addition to the CariScreen technology. Results produced from CariScreen in their study produced lower correlations to bacteria levels when compared to the luciferase-based assay, albeit better correlations than the present study. Additionally, their method of sample collection differs from the present study and CariScreen manufacturer's instructions. In the Fazilat 2010 study, plaque samples were collected using a toothpick swept across one entire surface of one tooth surface in four different areas of the mouth and subsequently dispersed into solution. The small, compact and firm tip of a toothpick allows for collection of plaque from interproximal and gingival sites where cariogenic bacteria tend to be found, without contacting the gingiva. This is in contrast to the swab utilized in the CariScreen

system, as supplied by the manufacturer. The tip of the swab is blunt and rounded, preventing the user from collecting plaque from interproximal sites. Also, the user is unable to precisely swab the gingival portion of the tooth, where most plaque accumulates, due to the blunt end of the swab and fear of contacting the gingival soft tissue, which would inherently skew the results by including human cells. Fazilat utilized two different samples in the CariScreen: a dispersed plaque solution sample and a composite of plaque and saliva sample. Neither utilized the CariScreen swab nor was the sample collected per CariScreen manufacturer's instructions, as used in the present study. However, our study proposal represented a true clinical usage of the CariScreen meter in dental practice per manufacturer's instruction. Our study results do not support the use of the CariScreen technology as a reliable tool for cariogenic bacterial quantification compared to the culture methods.

Although a majority of the data produced lacked significance, several surprising trends were noted. Especially, a significant negative correlation between log anterior CariScreen scores and log levels of MS was observed. This trend is consistent with the finding that subjects with high CariScreen score had lower logMS counts than those with low CariScreen scores. Additionally, caries-active subjects had lower log Cariscreen scores than Caries-free subjects ($P=0.12$). These poor and negative correlation coefficients of the anterior site lingual surfaces of mandibular anterior teeth, as recommended by manufacturer, may result from the least-caries-prone nature of this site in oral cavity. This region is in close proximity to the opening of the sublingual and submandibular salivary gland ducts. These teeth are constantly being rinsed and bathed in saliva with buffering and cleaning capacity. Cariogenic bacteria are classically acidogenic and acidophilic, and would not compete well in such environments with other commensal bacteria. We, therefore, selected the more cariogenic, mandibular posterior tooth for

the 2nd site for CariScreen bacteria enumeration. Although this site did not show reverse correlation with bacterial levels by traditional culture, it produced poor correlation overall as well.

Further, as a diagnostic tool for cariogenic bacterial level measurement, the current CariScreen meter for clinical use yields fair sensitivity but poor specificity and more concerning high false negative rates, that can lead to incorrect categorization of patients into low-risk group. Therefore, it can not be used as an effective tool in caries risk assessment to guide effective prevention plans.

The CariScreen meter has had a positive impact in dental practice by introducing the concept of including microbiological risk factors into caries risk assessment and by providing a valuable educational tool for dentists during consultation and the development of prevention plans with patients as a instant chair-side measurement. It is disappointing that we did not find evidence to show it as a reliable tool for accurate cariogenic bacterial quantification and is also concerning especially in the false negative readings found in the present high-risk children population. Based on our and Fazilat 2010 study results, it is possible that, with modification of sampling technique and revisiting on the ATP-driven bioluminescence technology, the CariScreen meter might be used as a reliable chair-side instant tool for cariogenic bacterial measurement.

There are a few limitations in our study. We have a small sample-size for caries-free individuals. We used a convenience sample from the UCSF Pediatric Dentistry clinic, where a majority of patients are at high caries risk. Therefore, only three caries-free subjects were included in the study. Even with three caries free subjects, the CariScreen posterior score was lower than the caries active subjects. Currently, more caries-free subjects are being recruited

into the ongoing study to verify correlations between CariScreen data with caries status and bacteria levels. Another limitation is that the present study is cross-sectional. This is only the first phase of the study; research subjects will be followed up at one year to study the longitudinal prediction value of bacterial testing of CariScreen.

Conclusion

1) The concept of CariScreen is attractive; its handheld, easy to use and produces instant results. Our data reveal there is fair sensitivity, but poor correlation, poor specificity and a high false negative rate between CariScreen numbers and cariogenic bacteria levels although there was some promising association between the dichotomized CariScreen posterior score and caries status. Further modifications in sampling technique and ATP bioluminescence technology may be needed before the CariScreen meter can be used as a reliable and effective tool for cariogenic bacterial measurement in the clinic.
2) There continues to be a need for a reliable, easy-to-use, chair-side, rapid assay method for practicing dentists to use when assessing oral bacterial counts for caries risk assessments.

Table 1 Subject Demographics, bacterial levels, caries status, and plaque scores

Characteristic	Baseline Sample (N=60)[1]
Baseline age, mean (SD)	9.3 (2.7)
Sex, n (%)	
Female	41 (68.3%)
Male	19 (31.7%)
Race/Ethnicity, n (%)	
Asian	13 (21.7%)
African American	8 (13.3%)
Latino/Hispanic	8 (13.3%)
Caucasion	9 (15.0%)
Other/No Answer	22 (36.7%)
CariScreen Score, median (quartile)	
Anterior	2733 (1072-6040)
Posterior	1151 (582-3537)
Bacteria counts (log), mean (SD)	
Mutans Streptococci	4.9 (1.7)
Lactobacillus	2.7 (2.1)
Total Streptococci	7.5 (0.3)
Total Viable Bacteria	8.3 (0.4)
Caries, mean (SD)	
Decayed surfaces	10.4 (6.9)
dmfs/DMFS	21.3 (15.1)
% of subjects with active decay	95%
Plaque Score, mean (SD)	1.82 (0.5)

Table 2. Correlation between log bacteria levels, log CariScreen scores, caries scores, and plaque scores

		logMdPost	LogMS	LogTS	LogLB	LogTVC	ds/DS	Dmfs/DMFS	Plaque Score
logMdAnt	Pearson Correlation	.472**	-.303*	-.115	.010	.027	-.164	-.188	.024
	Sig. (2-tailed)	.000	.019	.380	.939	.840	.212	.150	.857
logMdPost	Pearson Correlation		-.101	.034	.045	.175	.114	.041	-.097
	Sig. (2-tailed)		.443	.796	.733	.181	.385	.757	.459
LogMS	Pearson Correlation			.262*	.385**	.297*	.155	.284*	.091
	Sig. (2-tailed)			.043	.002	.021	.236	.028	.487
LogTS	Pearson Correlation				.169	.612**	.035	.186	.052
	Sig. (2-tailed)				.198	.000	.790	.154	.693
LogLB	Pearson Correlation					.264*	.352**	.305*	-.040
	Sig. (2-tailed)					.041	.006	.018	.763
LogTVC	Pearson Correlation						.110	.159	.049
	Sig. (2-tailed)						.404	.224	.708
ds/DS	Pearson Correlation							.518**	.113
	Sig. (2-tailed)							.000	.388
Dmfs/DMFS	Pearson Correlation								-.047
	Sig. (2-tailed)								.723

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table 3. Bacterial levels by culture, caries levels, and plaque scores in subjects with high or low CariScreen scores

	Anterior CariScreen			Posterior CariScreen		
	<1500 (low) (n=40) mean(SD)	≥1500 (high) (n=20) mean (SD)	P value	<1500 (low) (n=34) mean(SD)	≥1500 (high) (n=26) mean(SD)	P value
logMS	5.4 (0.8)	4.7(2.0)	0.07	5.1 (1.3)	4.6 (2.2)	0.32
logLB	2.3 (2.1)	2.9 (2.1)	0.25	2.7 (1.9)	2.7 (2.4)	0.95
logTS	7.6 (0.3)	7.5 (0.3)	0.39	7.5 (0.3)	7.5 (0.3)	0.78
logTVC	8.3 (0.4)	8.3 (0.4)	0.48	8.3 (0.4)	8.4 (0.4)	0.16
DS	10.7 (5.7)	10.2 (7.5)	0.78	9.5 (6.9)	11.5 (6.9)	0.26
dmfs/DMFS	20.1 (14.5)	21.8 (15.5)	0.68	22.4 (16.4)	19.8 (13.4)	0.51
Plaque Score	1.8 (0.5)	1.8 (0.5)	0.80	1.8 (0.6)	1.8 (0.5)	0.76

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table 4. CariScreen scores, bacterial levels, and plaque score by culture in caries-free and caries-active subjects

	Caries Free N=3 Mean (SD)	Caries Active N=57 Mean (SD)	P Value
Log Anterior CariScreen	3.78 (0.11)	3.34 (0.06)	0.12
Log Posterior CariScreen	2.95 (0.19)	3.06 (0.08)	0.76
logMS	4.3 (2.6)	4.9 (1.7)	0.53
logLB	0.8 (1.3)	2.8 (2.1)	0.10
logTS	7.6 (0.2)	7.5 (0.3)	0.57
logTVC	8.4 (0.4)	8.3 (0.4)	0.64
Plaque Score	1.8 (0.6)	1.8 (0.5)	0.96

* Correlation is significant at the 0.05 level (2-tailed).

** Correlation is significant at the 0.01 level (2-tailed).

Table 5. Sensitivity, Specificity and False Negative Rate of Bacterial Challenge by Anterior CariScreen Scores Compared to Cariogenic Culture Enumeration

	MS	**LB**	**MS+LB**
Sensitivity	66%	70%	67%
Specificity	33%	37%	33%
False Negative Rate	70%	45%	75%

Figure 1. Scatter plot of log mandibular anterior CariScreen scores against cariogenic bacteria culture level

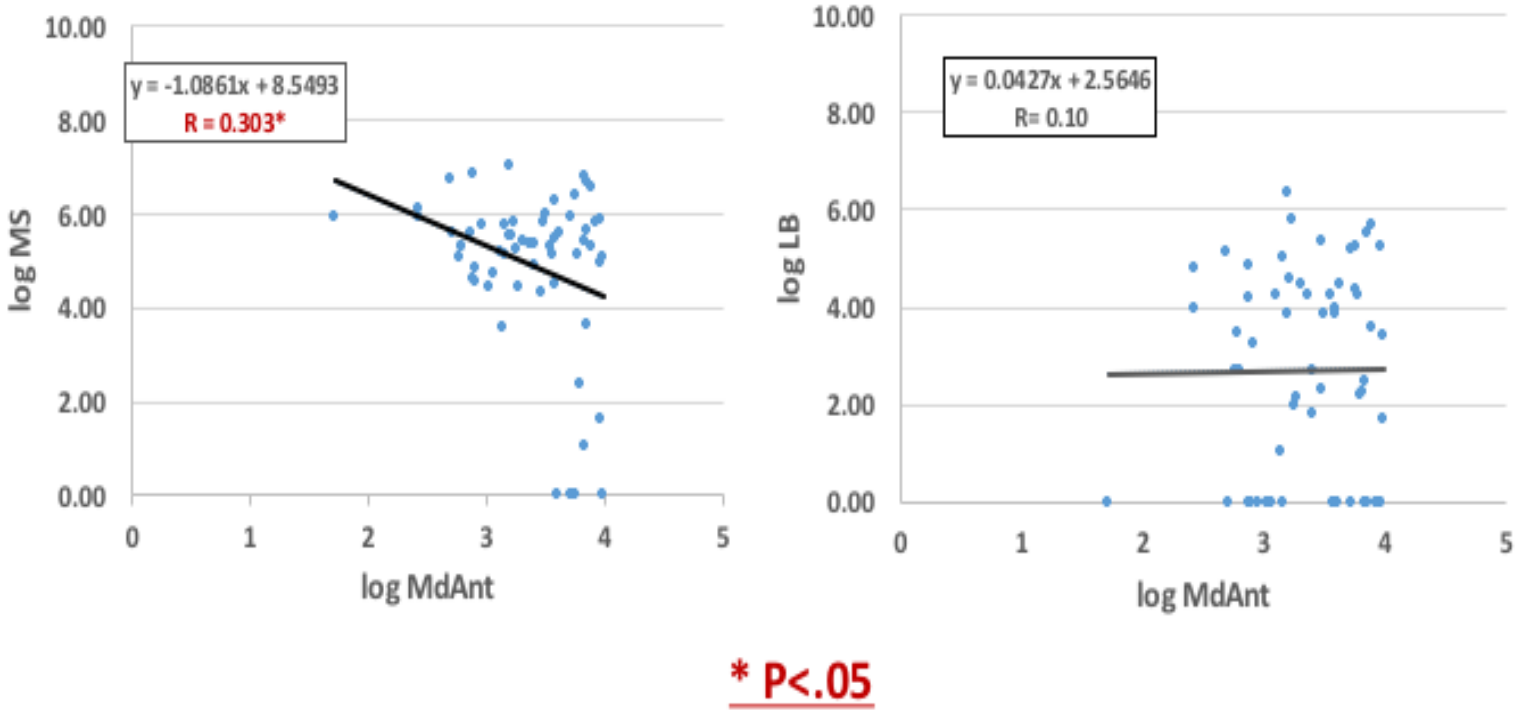


Figure 2. Scatter plot of log mandibular anterior CariScreen scores against oral bacteria culture levels

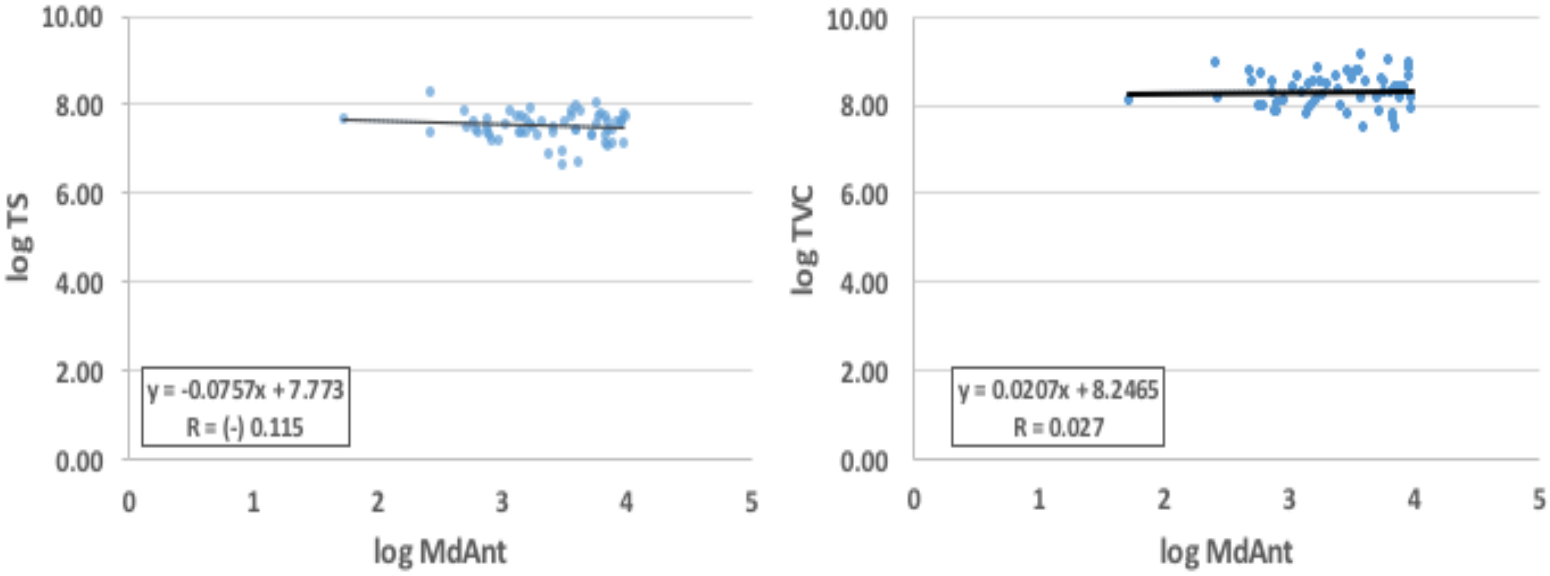


Figure 3. Scatter plot of log mandibular posterior CariScreen scores against cariogenic bacteria bacteria levels

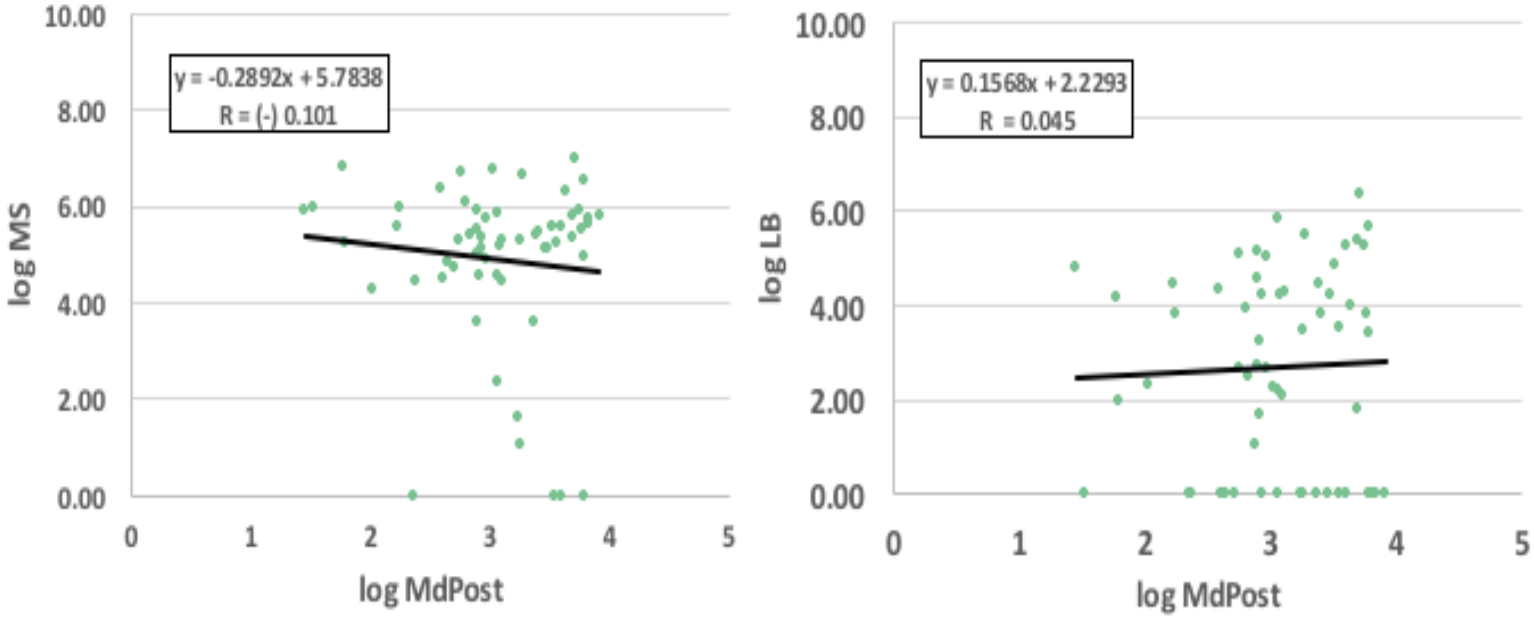


Figure 4. Scatter plot of log mandibular posterior CariScreen scores against oral bacteria culture levels

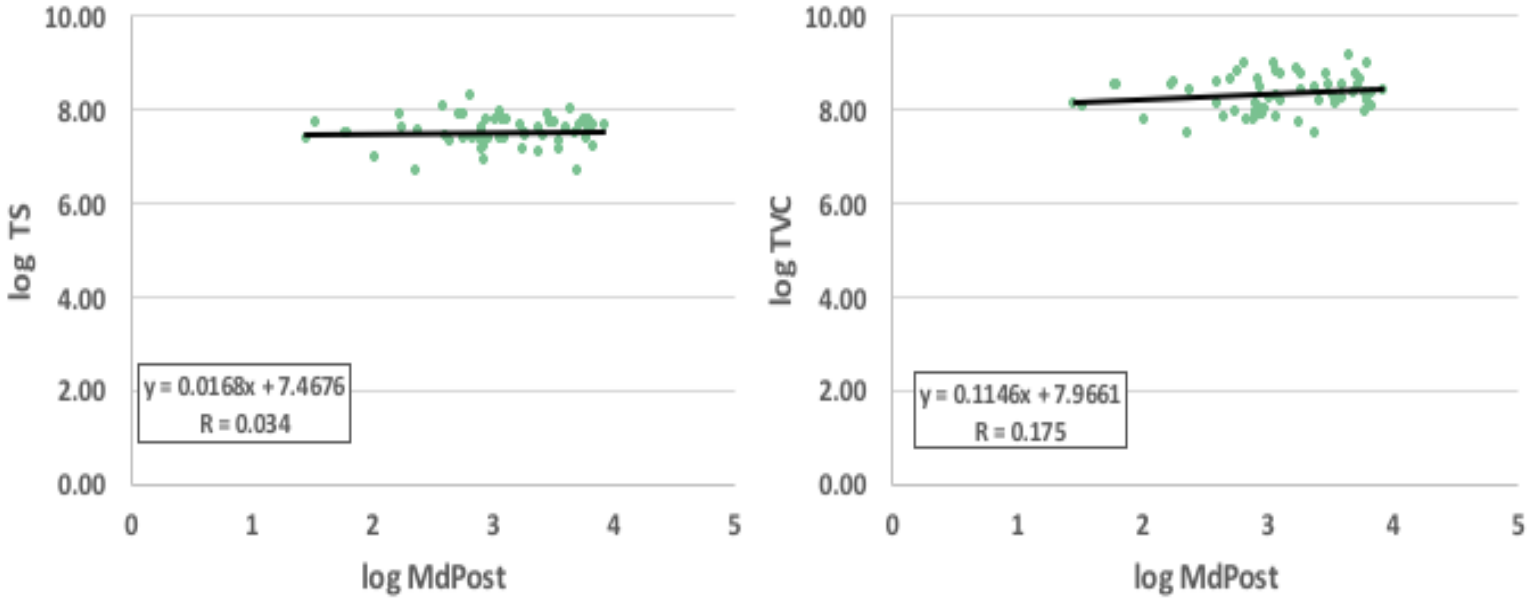


Figure 5. Correlation of mandibular anterior and posterior CariScreen Scores

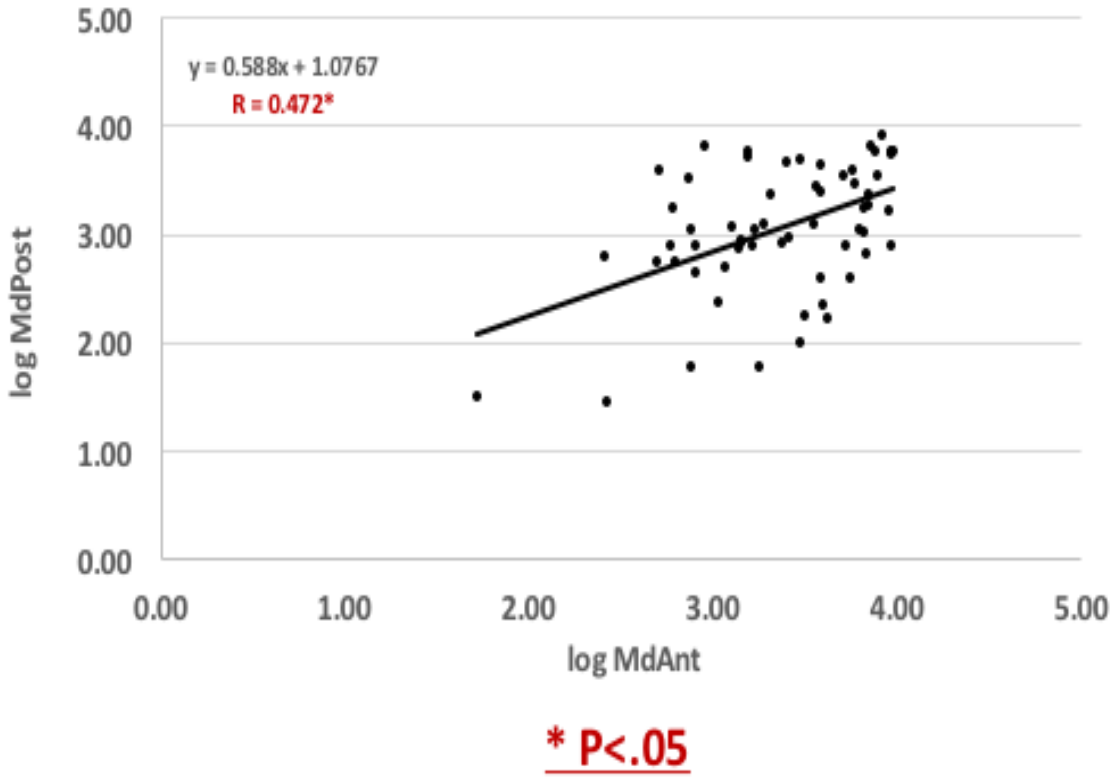


Figure 6. Sensitivity and Specificity of Bacterial Challenge by Anterior CariScreen Scores compared to MS culture enumeration

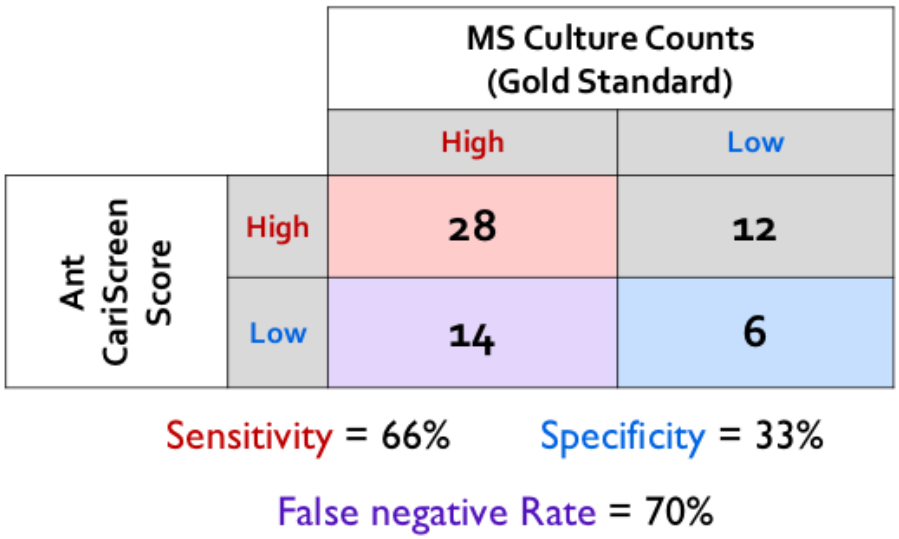


Figure 7. Sensitivity and Specificity of Bacterial Challenge by Anterior CariScreen Scores compared to LB culture enumeration

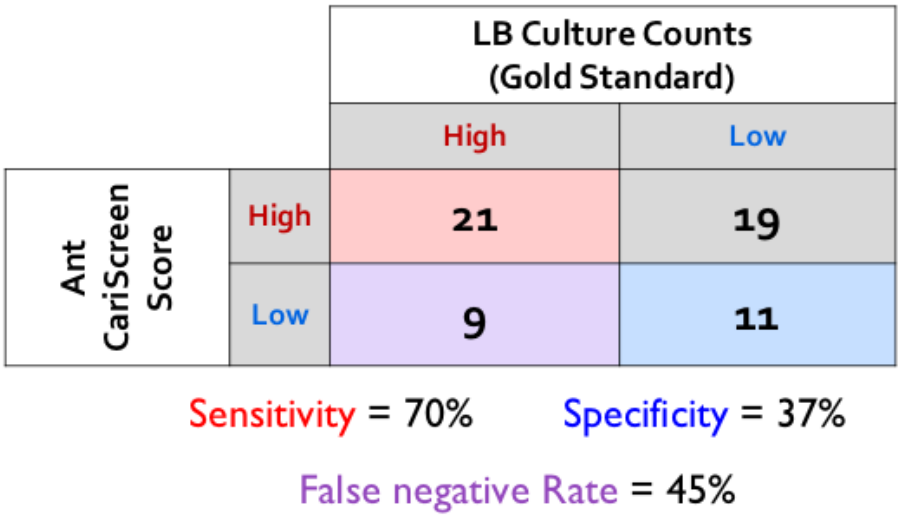
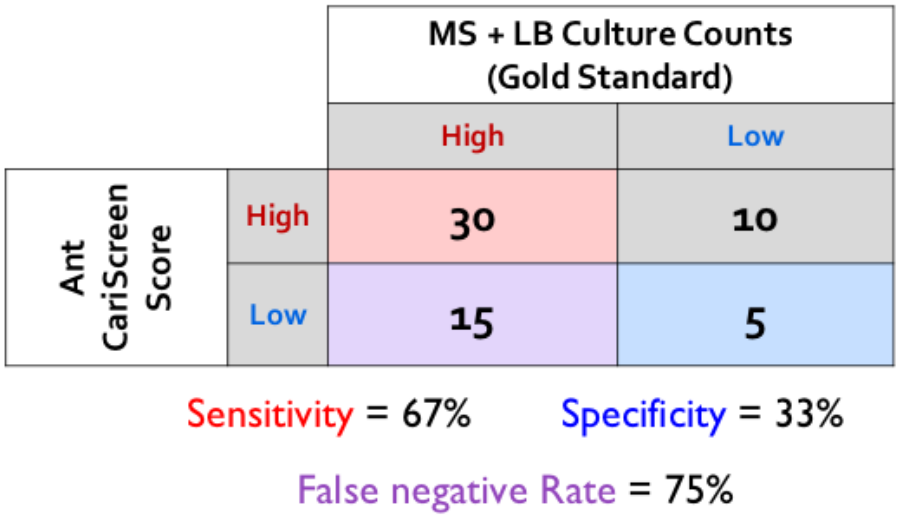


Figure 8. Sensitivity and Specificity of Bacterial Challenge by Anterior CariScreen Scores compared to MS and LB culture enumeration



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Appendices

Appendix A – CHR Approval

UCSF
University of California
San Francisco

Human Research Protection Program
Committee on Human Research

Notification of Expedited Review Approval

Principal Investigator
Dr. Ling Zhan PhD, PhD

Co-Principal Investigator

Type of Submission: Initial Review Submission Packet
Study Title: Comparison of CariScreen evaluation in determining caries risk in dental patients to bacterial plating.

IRB #: 14-13982
Reference #: 134715

Committee of Record: Laurel Heights Panel

Study Risk Assignment: Minimal

Approval Date: 08/10/2015 **Expiration Date:** 08/09/2018

Regulatory Determinations Pertaining to this Approval:

This research satisfies the following condition(s) for the involvement of children:
45 CFR 46.404, 21 CFR 50.51: Research not involving greater than minimal risk.

Parental Permission and Assent:
The permission of one parent or guardian is sufficient.

The assent of the children will be obtained.

Individual Research HIPAA Authorization is required of all subjects. Use the Permission to Use Personal Health Information for Research form.

A waiver of HIPAA Authorization and consent is acceptable for the recruitment procedures to identify potential subjects. The recruitment procedures involve routine review of medical or other records, do not adversely affect the rights and welfare of the individuals, and pose minimal risk to their privacy, based on, at least, the presence of the following elements: (1) an adequate plan to protect the identifiers from improper use and disclosure; (2) an adequate plan to destroy the identifiers at the earliest opportunity consistent with conduct of the research, or a health or research justification for retaining the identifiers was provided or such retention is otherwise required by law; (3) adequate written assurances that the requested information will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study, or for other research for which the use or disclosure of the requested information would be permitted by the Privacy Rule; (4) the research could not practicably be conducted without the waiver; and (5) the study recruitment could not practicably be conducted without access to and use of the requested information. Study participants will sign a consent form prior to participation in the study.

Appendix B – Informed Consent

IRB NUMBER: 14-13982
IRB APPROVAL DATE: 08/10/2015
IRB EXPIRATION DATE: 08/09/2018

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO
CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Study Title: Validity of CariScreen Caries Testing Meter in cariogenic bacteria quantification and caries risk assessment in children

This is a medical research study. The researchers, Dr. John Featherstone, Ph.D., MS, Dr. Ling Zhan, Ph.D,. D.D.S., Dr. Gina Graziani, D.D.S., Dr. Nancy Le, D.D.S. and their colleagues, from the UCSF Department of Pediatric Dentistry will explain this research to you and your child.

Medical research studies include only people who choose to take part. Take your time to make your decision about having your child participate. You may discuss your decision with your family and friends and with your health care team. If you have any questions, you may ask your study doctor.

Your child is being asked to take part in this study because your child is at risk for dental caries.

Why is this study being done?

The purpose of this study is to validate whether the use of the CariFree CariScreen Caries Testing Meter, a meter to detect the amount of cavity-causing bacteria in your child's mouth, can predict future risk of having cavities in your child.

- The study is funded by department funds from Dr. Featherstone and Dr. Zhan for the materials, consumables and reimbursement for subjects to participate the study. The investigators are not paid for the study.
- Investigators have no financial or proprietary interests and have no Conflicts of Interest related to the study.

How many people will take part in this study?

About 100 people will be asked to donate specimens for this research.

How long will my child take part in this study?

There is a one year time commitment to this study. There will be two visits required, an initial visit and then a visit one year later (9-15 months.)

What will happen if my child takes part in this research study?

If you choose for your child to take part, then your child will have the following tests and procedures completed.

- **Demographic Questionnaire**
- **Dental Exam** – A dental exam will be performed to identify cavities.

- **Caries Risk Assessment** – We will assess the likelihood of your child getting future cavities.
- **Simplified Oral Hygiene Index** – We will evaluate how much plaque is on your child's teeth.
- **Saliva Sample** – Your child will give us a saliva sample by chewing on a piece of wax and expectorating into a small tube.
- **CariScreen plaque sample** – A plaque sample will be taken from your child's teeth using a cotton swab.

When your child is finished with your dental exam and providing samples, you and your child will dismissed. There will be a follow-up visit one year (9-15 months) from the initial visit, where we will perform the same exams and tests.

- **Study location:** All study procedures will be done at UCSF Pediatric Dentistry clinics or Tenderloin Community Dental Clinic, depending on where your child is currently a patient of record.

Study Chart

There will be two visits your child will be required to attend in this study. There will be an initial visit and then a follow-up visit one year later.

Cycle 1 – Initial Visit

Visit #1	Procedures
	• *Questionnaire and form completion* • *Informed Consent* • *Dental exam* • *Caries Risk Assessment* • *Saliva Sample* • *Plaque Swab Sample*

Cycle 2 – Follow up visit (one year later)

Visit #2	Procedures
	• *Dental exam* • *Caries Risk Assessment* • *Saliva Sample* • *Plaque Swab Sample*

Can my child stop being in the study?

Yes. Your child can decide to stop at any time. Tell the study doctor if your child is thinking about stopping or decide to stop. He or she will tell you how to stop your child's participation safely.

The study doctor may stop your child from taking part in this study at any time if he/she believes it is in your child's best interest, if your child does not follow the study rules, or if the study is stopped.

What side effects or risks can your child expect from being in the study?

The risk involved in the study is similar to the risk presented during routine dental exams. There is a possibility of risk of loss of confidentiality of your child's personal information. However, we are implementing every precaution to maintain your child's privacy and confidentiality, as well as not collecting unnecessary, sensitive information, such as social security numbers.

Are there benefits to taking part in the study?

There will be no direct benefit to your child from participating in this study. However, we hope that this study will help dentists learn more about the bacteria that cause cavities, and it is hoped that this information will help in the prevention of other children getting cavities in the future

What other choices does your child have if he/she does not take part in this study?

Your child's other choices may include:

- Declining to participate in the study.

Please talk to your child's doctor about your child's choices before deciding if your child will take part in this study.

How will information about my child be kept confidential?

Participation in research involves some loss of privacy. We will do our best to make sure that information about your child is kept confidential, but we cannot guarantee total privacy. Some information from your child's medical/dental records will be collected and used for this study. If your child does not have a UCSF medical record, one will be created for him/her. Your signed consent form for your child and some of his/her research tests will be added to his/her UCSF medical record. Therefore, people involved with your child's future care and insurance may become aware of your child's participation and of any information added to your child's medical record as a result of your child's participation. Study tests that are performed by research labs, and information gathered directly from your child by the researchers will be part of your child's research records but will not be added to your child's medical record. Your child's personal information may be given out if required by law. If information from this study is published or

presented at scientific meetings, your child's name and other personal information will not be used.

Organizations that may look at and/or copy your child's medical records for research, quality assurance, and data analysis include:

- The University of California

What are the costs of taking part in this study?

Your child will not be charged for any of the study activities.

Will my child be paid for taking part in this study?

In return for your child's time, effort and travel expenses, you child will be paid a gift of $40 for taking part in this study. The gift will be delivered in two separate amounts, $10 at the initial visit and $30 at the final visit.

What happens if my child is injured because he/she took part in this study?

It is important that your child tell your study doctor, Dr. Ling Zhan, Ph.D., D.D.S., Dr. Gina Graziani, D.D.S., Dr. Nancy Le, D.D.S, if you feel that your child has been injured because of taking part in this study. You or your child can tell the doctor in person or call him/her at (415) 476-3276.

> **Treatment and Compensation for Injury:** If your child is injured as a result of being in this study, the University of California will provide necessary medical treatment. The costs of the treatment may be billed to you or your insurer just like any other medical costs, or covered by the University of California, depending on a number of factors. The University and the study sponsor do not normally provide any other form of compensation for injury. For further information about this, you may call the office of the Committee on Human Research at 415- 476-1814.

What are my child's rights if he/she takes part in this study?

Taking part in this study is your and your child's choice. You and your child may choose either to take part or not to take part in the study. If you and your child decide to take part in this study, your child may leave the study at any time. No matter what decision you and your child make, there will be no penalty to your child and your child will not lose any of his/her regular benefits. Leaving the study will not affect your child's medical or dental care. Your child can still get his/her medical and dental care from our institution.

We will tell you and your child about new information or changes in the study that may affect your child's health or your willingness to continue in the study.

In the case of injury resulting from this study, your child does not lose any of his/her legal rights to seek payment by signing this form.

Who can answer my questions about the study?

You can talk to your study doctor about any questions, concerns, or complaints you have about this study. Contact your study doctor(s) Dr. Ling Zhan, Ph.D., D.D.S., Dr. Gina Graziani, D.D.S., Dr. Nancy Le, D.D.S, at (415) 476-3276.

If you or your child wish to ask questions about the study or your child's rights as a research participant to someone other than the researchers or if you wish to voice any problems or concerns you may have about the study, please call the Office of the Committee on Human Research at 415-476-1814.

CONSENT

You have been given copies of this consent form and the Experimental Subject's Bill of Rights to keep.

You will be asked to sign a separate form authorizing access, use, creation, or disclosure of health information about you.

PARTICIPATION IN RESEARCH IS VOLUNTARY. You have the right to decline to participate or to withdraw at any point in this study without penalty or loss of benefits to which you are otherwise entitled

If you wish for your child to participate in this study, you should sign below.

_____ _____

Date Participant's Signature for Consent

_____ _____

Date Person Obtaining Consent

The person being considered for this study is unable to consent for himself/herself because he/she is a minor. By signing below, you are giving your permission for your child to be included in this study.

_____ _____

Date Parent or Legal Guardian

Appendix C – Screening Form

CariScreen Data Form

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO SCHOOL OF DENTISTRY
CariScreen Study – Screening Form

PROCEDURES			
Subject Qualification			
1. Child is between 6 and 18 years of age?		**Yes**	No
2. Child has six fully erupted mandibular anterior teeth?		**Yes**	No
3. Use of systemic antibiotics within the past three months (with exception of topical use of chlorhexidine)?		Yes	**No**
4. Will the Child be able to adequately able to cooperate for the sample collection?		**Yes**	No
5. Does the child have severe gingival bleeding?		Yes	**No**
6. Will stay in the Bay Area for another 1 year?		**Yes**	No
Patient qualified for the study		**Yes**	No

Appendix D – Contact Information and Ethnicity Form

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO SCHOOL OF DENTISTRY
CAMBRA Children Study **Subject's Contact Information and Ethinicity**
Subject initials:_____. Study ID:_____.

Please fill in the following information (It will only be used for contact in this study):

Child's Name: _____
First Last Middle Initial

Child's Birth date: _____/_____/_____.
MM DD YYYY

Child's gender: Male_____ Female_____

Parent/guardian's Name: _____
First Last

Contact phone number: _____(day)

_____(night)

Contact Address:

_____
Number Street Apt#

_____
City State Zip Code

Information about you child's ethnicity:

Is your ethnic background Hispanic, Latino or other Spanish descent?

☐ No
☐ Yes

☐ Central American ☐ Puerto Rican

☐ Cuban ☐ South American

☐ Mexican ☐ Other Hispanic _____

Please select your racial background (you may select more than one):

☐ African-American / Black / Haitian

☐ American Indian / Native American / Alaskan Native

☐ Asian

☐ Bangladeshi ☐ Korean

☐ Burmese/Myanmarese ☐ Laotian

☐ Chinese ☐ Malaysian

☐ Filipino ☐ Pakistani

☐ Indian ☐ Thai

☐ Indonesian ☐ Vietnamese

☐ Japanese ☐ Other Asian _____

☐ Caucasian / White / Middle Eastern

☐ Native Hawaiian / Pacific Islander

☐ Fijian ☐ Samoan

☐ Guamanian ☐ Tongan

☐ Hawaiian ☐ Other Pacific Islander _____

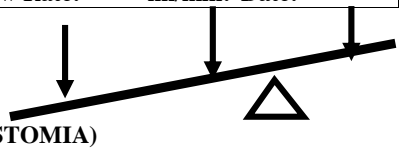
☐ Other _____

☐ Do not wish to respond

Appendix E – Caries Risk Assessment Form

Caries Risk Assessment Form for Ages 6 Years Through Adult
Study ID:
Assessment Date:

Disease Indicators (Any one YES signifies likely "High Risk" and to do a bacteria test)**	**YES = CIRCLE**	**YES = CIRCLE**	**YES= CIRCLE**
Cavities/radiograph to dentin	**YES**		
Approximal enamel lesions (E1, E2) (by radiograph)	**YES**		
White spots on smooth surfaces (Eo)	**YES**		
Restorations last 3 years	**YES**		
Risk Factors (Biological predisposing factors)		**YES**	
MS and LB both medium or high **(by culture**)**		**YES**	
Visible heavy plaque on teeth		**YES**	
Frequent snack (> 3x daily between meals)		**YES**	
Deep pits and fissures		**YES**	
Recreational drug use		**YES**	
Inadequate saliva flow by observation or measurement (**If measured note the flow rate below)		**YES**	
Saliva reducing factors (medications/radiation/systemic)		**YES**	
Exposed roots		**YES**	
Orthodontic appliances		**YES**	
Protective Factors			
Lives/work/school fluoridated community			**YES**
Fluoride toothpaste at least once daily			**YES**
Fluoride toothpaste at least 2x daily			**YES**
Fluoride mouthrinse (0.05% NaF) daily			**YES**
5000 ppm F fluoride toothpaste daily			**YES**
Fluoride varnish in last 6 months			**YES**
Office F topical in last 6 months			**YES**
Chlorhexidine prescribed/used one week each of last 6 months			**YES**
Xylitol gum/lozenges 4x daily last 6 months			**YES**
Calcium and phosphate paste during last 6 months			**YES**
Adequate saliva flow (> 1 ml/min stimulated)			**YES**
****Bacteria/Saliva Test Results: MS: LB: Flow Rate: ml/min. Date:**			



VISUALIZE CARIES BALANCE
(Use circled indicators/factors above)
(EXTREME RISK = HIGH RISK + SEVERE XEROSTOMIA)
CARIES RISK ASSESSMENT (CIRCLE): EXTREME HIGH MODERATE LOW
Doctor signature/#: Date:

1

Appendix F – Dental Exam and Plaque Score Form

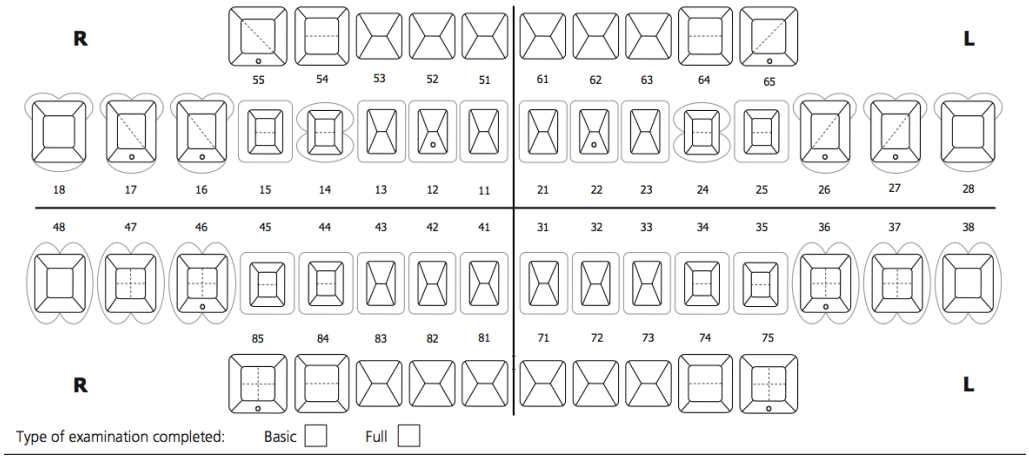
Dental Exam Form

Record ID #:

Male / Female

Examination Date Day ☐☐ Month ☐☐ Year ☐☐☐☐

Plaque Score: *A-Buccal:* *E/8-Facial:* *J-Buccal:*



Type of examination completed: Basic ☐ Full ☐

Plaque Score: *T-Lingual:* *O/24-Facial:* *K-Lingual:*

Notes:

Revised 03/26/2015. Adapted from http://www.sdcep.org.uk/index.aspx?o=3079

Appendix G – Checklist

CariScreen Data Form

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO SCHOOL OF DENTISTRY
CariScreen Study
Subject's initials: Study ID: _____.

PROCEDURES

BASELINE Exam, Sample and Data Acquisition			
Date of Visit	___/___/_____.		
Consent form signed by the parent/guardian?		Yes	No
Questionnaire completed?		Yes	No
ICDAS exam completed?		Yes	No
Plaque score completed?		Yes	No
CariScreen swab sample completed?		Yes	No
Saliva sample completed?		Yes	No
Monetary incentive distributed?		Yes	No
Last known toothbrushing?			
Scheduled date for the next allotment (include week day also): ___/___/			
Investigator's Signature:			

1 year Exam, Sample and Data Acquisition		
Date of visit	___/___/_____.	
ICDAS exam completed?	Yes	No
Plaque score completed?	Yes	No
CariScreen swab sample obtained?	Yes	No
Saliva sample obtained?	Yes	No
Monetary incentive distributed	Yes	No
Last known tooth brushing		
Investigator's Signature:		

Publishing Agreement

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Author Signature

6/8/2016

Date