2004

PCR Detection of Streptococcus Mutans and Streptococcus Sobrinus in Dental Plaque Samples from Low, Moderate, and High Caries Risk Children

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October 18, 2004

PCR DETECTION OF *STREPTOCOCCUS MUTANS* AND *STREPTOCOCCUS SOBRINUS* IN DENTAL PLAQUE SAMPLES FROM LOW, MODERATE, AND HIGH CARIES RISK CHILDREN

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

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Richmond, Virginia
October, 2004

Acknowledgement

The author would like to thank several people. I would like to thank my parents and my sisters for their love and support throughout my life. I would like to extend sincere acknowledgements and thanks to my Thesis Committee Members, Dr. Arthur Mourino, Dr. Tegwyn Brickhouse and Dr. Francis Macrina for their much appreciated assistance with this research project. Your guidance and mentorship has been integral in completing my research successfully. Thank you for all of your help and support. I would like to give a special thanks to Kevin Jones for help with the laboratory aspect of this project. Your hard work was much appreciated. The author would also like to thank Dr. Al Best for calculating the statistical data presented in this study. Most of all, I would like to thank my wife, Manisha Patel, who has been very patient and supportive throughout my professional development.

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Abstract

PCR DETECTION OF *STREPTOCOCCUS MUTANS* AND *STREPTOCOCCUS SOBRINUS* IN DENTAL PLAQUE SAMPLES FROM LOW, MODERATE, AND HIGH CARIES RISK CHILDREN

By Ajit A. Patel, D.D.S.

A Thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

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Major Director: Arthur P. Mourino, D.D.S., M.S.D.
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Purpose: The purpose of this study was to correlate the presence of *Streptococcus mutans* and *Streptococcus sobrinus* with the incidence of dental caries in children as detected by the polymerase chain reaction (PCR).

Methods: Subjects between the ages 2 to 16 years of age were used in this study. After the subjects received a dental examination, dental plaque samples were collected by rubbing a toothpick on the buccal surface of the most posterior tooth in each quadrant of the mouth. Children were grouped into low, moderate, and high caries risk categories by two different Caries Risk Assessment Tools (CAT 1 and CAT 2). PCR was used to

amplify the dextranase (Dex) gene of *S. mutans* and *S. sobrinus*. Dextranase is an enzyme that hydrolyses glucans in a plaque matrix and is believed to be involved in the pathogenesis of dental caries. Identification of the amplified Dex gene sequences was made by gel electrophoresis which in turn was used to determine the presence of *S. mutans* and *S. sobrinus*.

Results: *S. mutans* was detected in 57% of patients (65/115), *S. sobrinus* was detected in 10% of patients (12/115) and both organisms were present in 9% of patients (10/115). According to CAT 1, the incidence of *S. mutans* was 64.6%, 9.2%, and 26.2% in low, moderate, and high caries risk, respectively. The incidence of *S. sobrinus* was 66.6%, 16.7%, and 16.7% in low, moderate, and high caries risk, respectively.

Conclusion: Presence of *S. mutans* and/or *S. sobrinus* as evaluated in this study had no direct correlation with caries risk level in children.

Introduction

Dental caries has been a highly prevalent and costly disease in the United States and around the world.[1] Dental caries remains the single most common disease of childhood that is neither self-limiting nor amendable to short-term pharmacological management.[2] United States Surgeon General's report shows that 45% of children ages 5 to 17 have dental caries.[3]

Mutans streptococci (MS) is group of principal micro-organisms involved in the etiology of human dental caries and is divided into 5 species and 8 different serological types (a through h).[4,5] Among the mutans streptococci, *S. mutans* (serotypes c, e and f) and *S. sobrinus* (serotype d and g) are the species closely associated with human dental caries.[4,6,7]

Streptococcal species are the first to colonize the tooth surfaces. In doing so, certain species use specific receptors to attach to the salivary pellicle.[8,9] The pellicle is derived from salivary constituents which are selectively adsorbed onto the tooth surface. Components of the dental pellicle include albumin, lysozyme, amylase, immunoglobulin A, proline-rich proteins and mucins. The formation of pellicle is the first step in plaque formation.[10]

Dental plaque is a soft deposit that accumulates on the teeth. Plaque can be defined as a complex microbial community, with greater than 10^{10} bacteria per milligram. It has

been estimated that as many as 400 distinct bacterial species may be found in plaque. In addition to the bacterial cells, plaque contains a small number of epithelial cells, leukocytes, and macrophages. The cells are contained within an extracellular matrix, which is formed from bacterial products and saliva.[10]

After the initial colonization of the tooth surface, plaque increases by two distinct mechanisms: 1) the multiplication of bacteria already attached to the tooth surface, and 2) the subsequent attachment and multiplication of new bacterial species to cells of bacteria already present in the plaque mass.[10] When provided a suitable metabolic substrate, this infection produces organic acids (primarily lactic acid) that are capable of dissolving the mineral calcium from the crystalline enamel matrix.[1] Continued and repeated dissolution leads to frank cavitation and the subsequent need for repair.[11]

Mutans streptococci (MS) are the group of microorganisms most associated with the dental caries process.[12] MS are believed to contribute to caries because of their ability to adhere to tooth surfaces, produce copious amounts of acid, and survive and continue metabolism at low pH conditions.[7]

While many carbohydrates may be utilized by plaque bacteria to generate acids, sucrose is recognized as being particularly important in the caries process because not only can it be fermented, but it also serves as substrate for extracellular enzymes of plaque bacteria which synthesize sucrose-derived polymers. These polymers are of central importance in adhesive interactions in plaque, where they mediate attachment of bacteria to the tooth surface and to other bacteria thus stabilizing the plaque biofilm, serve as

energy stores aiding the survival of plaque bacteria and modulate the permeability of plaque and hence the level of acid at the enamel surface.[13]

Several epidemiological studies have suggested that subjects harboring both *S. mutans* and *S. sobrinus* have a significantly higher level of past caries experience than those harboring *S. mutans*[14-17] or *S. sobrinus* alone[14]. Other studies have suggested that in a population with high caries prevalence *Strep. mutans* must be the significant organism since it is mostly found in the absence of *Strep. sobrinus.*[13]

However, there have, been suggestions that *Strep. sobrinus* is more virulent than *S. mutans*.[18] de Soet *et al.*[19] observed that *S. sobrinus* strains were capable of higher acid production at low pH than *S. mutans* strains. Experimental animal studies have demonstrated that strains of *S. sobrinus* have a high cariogenic potential.[20,21] Studies also show that the prevalence of *S. sobrinus* in saliva was more closely associated with future caries activity, especially with smooth-surface caries increment, than the prevalence of *S. mutans* and that the subjects in the *S. sobrinus* group had both higher caries and higher decayed teeth and surface increments than the *S. mutans* group.[17]

Prevalence of *S. sobrinus* and *S. mutans* in the human oral cavity has been reported by epidemiological studies in which the isolation frequency of *S. mutans* from dental plaque is much higher than that of *S. sobrinus*.[22-24] Studies from around the world have shown that >98% of adults carry *Strep. mutans*, while different studies have found *Strep. sobrinus* in 7-35% of individuals.[12] It should be noted that the primary differentiation of these cariogenic species is usually based on the colonial morphology on mitis-salivarius (MS) or MS-bacitracin (MSB) agar.[24-27] However, this procedure is

sometimes inaccurate, and also time-consuming and laborious.[28] It has been reported that MSB inhibits the growth of *S. sobrinus* to a greater extent than *S. mutans.*[25,29] Therefore, it is conceivable that the actual incidence of *S. sobrinus* may be higher than currently reported.[28] The application of the polymerase chain reaction (PCR) method to human dental plaque showed that the prevalence of *S. sobrinus* (83%) in oral cavities was higher than currently reported (0-50%).[28] It is speculated that the difference depends on the detection method used: the detection method with PCR was performed directly on dental plaque without the culture step and biochemical tests.[28]

The results from previous studies have shown PCR oligonucleotide primers (SD1 and SD2) specific to dextranase gene of *S. mutans*[30] and pair of PCR primers (SOF14 and SOR1623) specific to dextranase gene of *S. sobrinus*[28]. Dextranase is an enzyme that hydrolyses glucans in a plaque matrix and is believed to be involved in the pathogenesis of dental caries.[31,32] Dextranase (Dex) is able to break down glucans to isomaltosaccharides 3-4 glucose units long by cleaving α(1-6)-linkages within the dextran chain.[33,34] This activity may modify glucans by altering the ratio of α(1-6)- to α(1-3)-linked chains, hence influencing solubility and adhesive properties. Dex activity therefore, influences sucrose-dependent adherence by reducing the number of α(1-6)-linkages in the glucan.[13]

Apart from its effect on the adhesiveness of glucans, the dextranase of *Strep. mutans* breaks down glucans to isomaltosaccharides which may then be transported into the cell via the multiple sugar metabolism (msm) operon,[35,36] where they are further degraded to glucose by a dextran glucosidase (DexB)[31].

The PCR was able to amplify the DNA fragment from the *S. mutans* sample containing as few as 12 colony-forming units (cfu) prepared by the lysis method[30] and was able to detect as few as 9 cfu of *S. sobrinus*[28]. These results indicate that the primer pairs of *S. sobrinus* and *S. mutans* amplified the species-specific amplicons with different lengths, indicating that the present PCR method is useful for detection and identification of the human cariogenic species, *S. sobrinus* and *S. mutans*.[28]

Besides the precision of identifying the bacterial species present, there are a few other things that need to be considered to obtain an accurate caries risk status. Present literature has used the following factors to assess caries risk.

1). Number of teeth present (including their sealant status).

2). Previous caries experience: Studies show that "past caries experience is the most significant predictor"[37-42], but young children may have no cavitations simply due to the fact that the disease has not had time to express itself.[12]

3). Smooth surface vs. Pit and fissure caries: Patients that exhibit only pit and fissure caries and not smooth surface caries have less risk of future caries than those who have extension of the disease onto smooth surface.[1]

4). Presence of white spot lesions or enamel defect: White spot lesions are the precursors to cavitated lesions. In young children, where the disease has not had the time to express itself, white spot lesions may be the only sign of caries experience. Presence of developmental structural defects in enamel may increase the caries risk in children by increasing plaque retention and acid production, thus increasing the susceptibility to tooth demineralization.[12]

5). Presence of visible plaque: Several studies have shown that there is a correlation between visible plaque on primary teeth and caries risk.[43-45] One study found that 91% of the children are correctly classified at to caries risk solely based on the presence or absence of visible plaque.[44]

6). Family history: Children who have parents/siblings with high level of caries are likely to have higher chances of getting cavities.

7). Sugar consumption: Frequency of carbohydrate consumption and sugar intake through fruit juices and flavored drinks has been implicated to cause caries. Models developed for young children who had little caries history include bacterial levels and sugar use as the strongest predictors.[46-48]

8). Socioeconomic level of family: National data shows that caries is highly prevalent in poor and near poor US preschool children.[12]

9). Fluoride exposure: If the fluoride content of drinking water is low and systemic fluoride supplementation is given, data from over 20 clinical trials show a caries reduction of 30%-80% in primary teeth from fluoride supplements, provided they are started close to birth and continued for 5 or more years.[49-53]

Recently, tools have been developed by National Institute of Health (NIH) and American Academy of Pediatric Dentistry (AAPD) to assess caries risk in children. The tool developed by NIH (CAT 1)[8] classified children's caries risk as follows: Children with decayed (D/d) or filled (F/f) surfaces less than half their age were classified as low risk. The letters D and F are used to designate decayed and filled permanent teeth. The letters d and f are used to designate decayed and filled primary teeth. Children with DFs more than

half their age were classified as moderate risk group. Finally, children with DFs more than their own age were considered as a high risk group.

The caries risk assessment tool suggested by AAPD (CAT 2)[54] classifies children as follows: Children with no carious teeth in the past 24 months were considered low risk. Children with carious teeth in the past 24 months were considered moderate risk. Lastly, children with carious teeth in the past 12 months or radiographic enamel caries were considered high risk.

In this study, plaque samples were collected from children ages 2 to 16 years of age. Children were grouped into low, moderate, and high caries risk categories by two different Caries Risk Assessment Tools. PCR primer pairs were used to amplify the Dex gene for detection and identification of *S. mutans* and *S. sobrinus*. The purpose of this study was to correlate the presence of *Streptococcus mutans* and *Streptococcus sobrinus* with the incidence of dental caries in children.

Methods

One hundred fifteen children, ages 2-16 years old, who were patients of Virginia Commonwealth University, Department of Pediatric Dentistry, were included in the study. Consent for participation was obtained from at least one parent/guardian before the study. The consent form was approved on 2/9/04 and protocol number 03440 was assigned by VCU IRB (see Appendix A). If the child was between the ages seven and sixteen, an assent was also obtained from the child. The enrolled subjects received a dental examination with appropriate radiographs by a resident or a faculty member of the department. The caries risk level in patients was determined by two different caries risk assessment tools CAT 1 and CAT 2.

CAT 1 (Table 1) classified children with decayed (D/d) or filled (F/f) surfaces less than half their age, no new lesions in 1 year, no white spot lesions, no enamel defects, low titers of mutans streptococci, and high socioeconomic status as low risk group. Children with Dfs more than half their age, one or more lesions in 1 year, infrequent white spot lesions, enamel defects, moderate titers of mutans streptococci, middle socioeconomic status, and visible plaque were classified as moderate risk group. The criteria for high risk group included Dfs more than their own age, two or more lesions in 1 year, numerous white spot lesions, enamel defects, high titer of mutans streptococci, low socioeconomic setting, visible plaque, appliances in mouth and frequent sugar consumption.

Only the age component from CAT 1 is used in this study to determine the caries risk status. Patients with decayed (D/d) or filled (F/f) surfaces less than half their age were classified as low risk. Patients with DFs more than half their age were classified as moderate risk group. Finally, patients with DFs more than their own age were considered as a high risk group.

The revised Caries-risk assessment tool (CAT 2) suggested by AAPD (Table 2) includes three different criteria: clinical conditions, environmental characteristics, and general health conditions, to assess caries risk. Children with no carious teeth in the past 24 months, no enamel demineralization ("white spot lesions"), no visible plaque, no gingivitis, optimal systemic and topical fluoride exposure, consumption of simple sugars primarily at mealtimes, high caregiver socioeconomic status, and regular use of dental services are considered to be at low risk. Children with carious teeth in the past 24 months, one area of enamel demineralization, presence of gingivitis, suboptimal systemic fluoride exposure with optimal topical exposure, 1-2 between meal exposures to simple sugars, midlevel caregiver socioeconomic status, and irregular use of dental services are considered to be at moderate risk. Children with carious teeth in the past 12 months, more than one area of enamel demineralization, visible plaque on anterior teeth, radiographic enamel caries, high titers of mutans streptococci, wearing dental or orthodontic appliances, enamel hypoplasia, suboptimal topical fluoride exposure, 3 or more between meal exposures to simple sugars, low-level caregiver socioeconomic status, no usual source of dental care, and active caries present in mother are considered to be at high caries risk.

Children with special health care needs and children suffering from conditions impairing saliva composition/flow are also considered to be in high caries risk group.

CAT 2 component used in this study is as follows: Patients with no carious teeth in the past 24 months were considered low risk. Patients with carious teeth in the past 24 months were considered moderate risk. Lastly, patients with carious teeth in the past 12 months or radiographic enamel caries were considered high risk.

The letters D and F are used to designate decayed and filled permanent teeth, respectively. The letters d and f are used to designate decayed and filled primary teeth, respectively. For the purposes of this study, no distinction was made between primary and permanent teeth when recording the decayed and filled teeth.

Patient Examination

Multiple people (faulty and residents) were used to perform the examination. The data was then collected from patient charting. Examiner reliability was not done due to high number of faculty and residents.

Plaque sampling

Dental plaque samples were collected by rubbing a sterile toothpick on the buccal surface of the most posterior tooth in each quadrant of the mouth. The plaque sample was then placed into the GeneMate PCR strip-tubes with caps (0.2 ml size, cat.# T-3014-1) made by ISC-Bioexpress, Kaysville, UT. The PCR tubes contained 25 µl of TB buffer. The samples were transported and stored at -4˚C.

Extraction of Genomic DNA

Frozen plaque samples were thawed by using a water bath at 55˚C. 10 µl of each sample was transported on to FTA® (Flinders Technology Associates, Clifton, New Jersey) Cards by Whatman® and labeled appropriately. The cards were allowed to dry completely. Once dried, a 2.0mm disc was removed from each sample card, which contained a dried plaque sample, and placed into a separate microcentrifuge tubes (PCR tubes). The punch was washed for 5 min. by adding 200 µl of FTA® Purification Reagent to the tube and gently pipetting the reagent up and down twice. The reagent was discarded completely and the process was repeated two more times. The punch was washed by adding 200 µl of sterile TE^{-1} buffer (10 mM Tris, 0.1 mM EDTA, pH 8.0) to the tube and gently pipetting the buffer up and down twice. The buffer was discarded completely and the process was repeated one more time. The discs were allowed to dry completely in the tubes.

Polymerase Chain Reaction (PCR)

S. mutans (ATCC 25175) and *S. sobrinus* (ATCC 33478) were used as positive controls. The strains were obtained from ATCC (American Type Culture Collection), Manassas, VA. PCR detection of these species was performed using oligonucleotide primers designed to amplify a 1272-bp and 1610-bp fragment on the dextranase genes of *S. mutans* and *S. sobrinus*, respectively. SD1 and SD2 primers were used to specifically amplify a 1272-bp fragment in *S. mutans*. The sequence of SD1 and SD2 were 5'-TAT GCT GCT ATT GGA GGT TC-3' (positions 973 to 992) and 5'-AAG GTT GAG CAA

TTG AAT CG-3' (positions 2225 to 2244), respectively.[10] SOF14 and SOR 1623 primers were used to specifically amplify a 1610-bp fragment in *S. sobrinus*. The nucleotide sequence of SOF14 and SOR1623 were 5'-TGC TAT CTT TCC CTA GCA TG-3' (position 134-153) and 5'-GGT ATT CGG TTT GAC TGC-3' (positions 1743-1726), respectively.[12]

PCR reaction mixture (50 µl) was added into the tubes before starting the PCR. PCR reaction mixture was comprised of 1 µl of each of the two primers and 2 µl of Magnesium Chloride added to Invitrogen® PCR Supermix.

PCR was performed starting with the denaturation of the reaction mixture at 95°C for 3 min followed by a series of amplification: denaturation at 94°C for 30 sec, annealing at 52°C for 12 sec and extension at 72°C for 45 sec. The series was repeated for 28 cycles. The final cycle comprised of 94°C for 30 sec, 52°C for 12 sec, and 72°C for 7 min. The PCR products were then stored at 4°C for later use.

The PCR products were subjected to electrophoresis on a 0.8% agarose gel and stained with ethidium bromide.

Statistical methods

Groups were compared using a chi-square test of independence. All tests were performed at alpha = .05.

Results

A total of n = 115 subjects received a dental examination and forty-seven percent of these subjects were male (54/115). Table 3 shows a description of patients. Their average age was 7.3 years and they ranged from 2 years 1 month to 15 years 7 months. The number of decayed teeth on a patient ranged from 0 to 16, with the average of 3.19 decayed teeth per person. Each child had anywhere from 13 to 28 teeth with the average of 21.97 teeth per person. (Table 3) The subjects were measured for the presence of *S. mutans* and *S. sobrinus*. Caries risk was classified using only the dental caries indicators of CAT 1 and CAT 2. The number decayed (D, d) and filled (F, f) teeth were categorized low, medium, or high.

CAT 1: Children with DFs less than half their age were classified as low risk. Children with DFs more than their age were classified as high risk. The remaining children were classified as medium risk.

CAT 2: Children with no carious teeth in the past 24 months were considered low risk. Children with carious teeth in the past 24 months were considered moderate risk. Lastly, children with carious teeth or radiographic enamel caries in the past 12 months were considered high risk.

The CAT 1 method was used in Figure 1. The high risk children are above the upper line and the low risk children are below the lower line. The figure also shows that

there were no high risk caries children found above 11 years of age. There was only one child categorized as moderate caries risk above the age of 12 years. However, there was no statistical correlation with age.

S. mutans was detected in 57% of patients (65/115), *S. sobrinus* was detected in 10% of patients (12/115) and both organisms were present in 9% of patients (10/115, Table 4). The mean number of decayed teeth found in patients positive for both, *S. mutans* and *S. sobrinus*, was 2.8 teeth per patient. The highest number of decayed teeth (3.9) was found in patients negative for both *S. mutans* and *S. sobrinus* (Table 4).

The relationship between the CAT 1 and CAT 2 caries risk score is shown in Table 5. There were 40 patients "low" on both risk scores, 7 patients who were "low" caries risk according to CAT 1 and "moderate" caries risk according to CAT 2. Table 5 also shows the total number to low caries risk patients according to CAT 1 (n=67) and CAT 2 (n=41). There is a notable difference in the caries risk categorization between CAT 1 and CAT 2 as shown in Table 5. The majority of patients classified by CAT 1 are low caries risk patients (n=67), while the majority of patients classified by CAT 2 are high caries risk patients (n=63).

The relationship between *S. mutans* prevalence and caries risk (CAT 1) is shown in the contingency Table 6. This table shows that from the low risk group, 62.7% of children were positive for *S. mutans* and 37.3% were negative for *S. mutans*. The table also shows that from the children who were positive for *S. mutans*, 64.6% = low risk, 9.2% = moderate risk and 26.2% = high risk group. Pearson chi-square indicated no significant relationship (chi-square = 3.04, p-value = 0.2188).

The relationship between *S. sobrinus* prevalence and caries risk (CAT 1) is shown in the contingency Table 7. Pearson chi-square indicated no significant any relationship (chi-square = 0.97, p-value = 0.6148).

The relationship between S. mutans prevalence and the Caries Risk Assessment tool (CAT 2) is shown in the contingency Table 8. There was a trend towards a relationship (chi-square = 5.29, p-value = 0.0710).

The relationship between S. sobrinus prevalence and the CAT 2 is shown in the contingency Table 9. There was no significant relationship (chi-square = 1.4, p-value = 0.4847).

Discussion

This study did not find a correlation between the presence of *S. mutans* and/or *S. sobrinus* in dental plaque in either CAT 1 or CAT 2 methods of risk assessment. Identifying "risk" is more difficult in chronic diseases that are caused by multiple factors and events over a longer period of time in individuals. In the case of dental caries, the profession knows that bacteria capable of producing acids as a metabolic by product are necessary to produce the clinical manifestations of the disease. However, they are not in and of themselves "sufficient" to produce a cavitated tooth. Also needed are a susceptible host and a metabolic substrate on which these bacteria can act over time.[1]

This study was able to incorporate the new CAT 1 and CAT 2 methods that have been reported in the literature. Due to the recent creation of CAT 1 and CAT 2, there are no published studies making comparisons or examine the validity and reproducibility of these CAT methods. Due to time and limitations of funding, this study was not able to incorporate all of the criteria used in CAT 1 and CAT 2. The study was also based on a disproportionate number of young children. The population seen in the clinic is comprised of high number of young children. This study serves as a first step in the examination of caries risk assessment tools and the relationship to plaque by genomic plaque sampling methods.

Due to the nature of the environment, multiple people (faculty and residents) were used to perform the examination. The data on decayed and filled teeth was collected from the patient's charts. Examiner reliability was not assessed due to the number of faculty and residents. Inter-examiner reliability is a limitation of this study that could result in the misclassification of subjects according to CAT 1 and CAT 2.

According to CAT 1, the incidence of *S. mutans* was 64.6%, 9.2%, and 26.2% in low, moderate, and high caries risk, respectively. The incidence of *S. sobrinus* was 66.6%, 16.7%, and 16.7% in low, moderate, and high caries risk, respectively. CAT 2 also showed high number of *S. mutans* and *S. sobrinus* in low caries risk population. These results indicate a possible flaw in plaque sampling technique and/or misclassification of risk category according to caries risk assessment tools. Due to limited time and funding, all of the components of CAT 1 and CAT 2 were not used to determine the caries risk status. The results may have been different if all or more components of CAT 1 and CAT 2 were included in the study.

Future study should also include the use of real-time PCR in order to determine the amount of *S. mutans* and *S. sobrinus* in a given plaque sample and it's relation to caries risk. Conventional PCR method used in this study revealed the presence or absence of the target template. Real-time PCR monitors the fluorescence emitted during the reaction as an indicator of amplicon production during each PCR cycle. This signal increases in direct proportion to the amount of PCR product in a reaction. By recording the amount of fluorescence emission at each cycle, it is possible to monitor the PCR reaction during exponential phase where the first significant increase in the amount of PCR product

correlates to the initial amount of target template. The higher the starting copy number of the nucleic acid target, the sooner a significant increase in fluorescence is observed. A significant increase in fluorescence above the baseline value measured during the 3-15 cycles indicates the detection of accumulated PCR product.[55]

This study was able to show a quick, accurate, and inexpensive diagnostic method of detecting the presence of *S. mutans* and *S. sobrinus*. With this new tool (PCR using FTA® cards), it is possible to diagnose within hours with a very small number of bacteria. This tool will aid in the identification of one of the variables used to determine caries risk and allow early preventive interventions. It will eliminate any inconsistency encountered by identifying the bacteria according to their colony morphology.

Table 1. Caries-risk Assessment Tool 1 (CAT 1)

Table 1. Caries-risk Assessment Tool 1 (CAT 1)		
Caries-risk indicators		
Low risk	**Moderate risk**	**High risk**
Dfs<half the childs age	Dfs>half the child's age	Dfs>child's age
No new lesions in 1 year	1 or more lesions in 1 year	2 or more lesions in 1 year
No white spot lesions	Infrequent white spot lesions	Numerous white spot lesions
No enamel defects	Enamel defects	Enamel defects
Low titers of mutans streptococci	Moderate titers of mutans streptococci	High titers of mutans streptococci
High socioeconomic setting	Middle socioeconomic setting	Low socioeconomic setting
	Visible plaque	Visible plaque
		Appliances in mouth
		Frequent sugar consumption

Adapted from NIH Consensus Development Conference on the Diagnosis and Management of Dental Caries. *J Dent Ed*. 2001;65:1133-1142.

Table 2. Caries-risk Assessment Tool 2 (CAT 2)

Table 2. AAPD Caries-risk Assessment Tool (CAT 2)		
Caries-risk indicator		
Low risk	**Moderate risk**	**High risk**
Clinical conditions		
No carious teeth in past 24 months	Carious teeth in the past 24 months	Carious teeth in the past 12 months
No enamel demineralization (enamel caries "white-spot lesions")	1 area of enamel demineralization (enamel caries "white-spot lesions")	More than 1 area of enamel demineralization (enamel caries "white-spot lesions")
No visible plaque; no gingivitis	Gingivitis	Visible plaque on anterior (front) teeth
		Radiographic enamel caries
		High titers of mutans streptococci
		Wearing dental or orthodontic appliances
		Enamel hypoplasia
Environmental characteristics		
Optimal systemic and topical fluoride exposure	Suboptimal systemic fluoride exposure with optimal topical exposure	Suboptimal topical fluoride exposure
Consumption of simple sugars or foods strongly associated with caries initiation- primarily at mealtimes	Occasional (ie, 1-2) between-meal exposures to simple sugars or foods strongly associated with caries	Frequent (ie, 3 or more) between-meal exposures to simple sugars or foods strongly associated with caries
High caregiver socioeconomic status	Midlevel caregiver socioeconomic status (ie, eligible for school lunch program or SCHIP)	Low-level caregiver socioeconomic status (ie, eligible for Medicaid)
Regular use of dental care in an established dental home	Irregular use of dental services	No usual source of dental care
		Active caries present in the mother
General health conditions		
		Children with special health care needs
		Conditions impairing saliva composition/flow

Table 3 Description of Patients

Table 3: Description of Patients				
Characteristic	Mean	Std. Dev.	Range	
Age (yrs)	7.33	3.44	2y 1mo	15y 7mo
Number of teeth:				
Decayed	3.19	4.28	0	16
Filled	0.81	2.14	0	10
Missing	0.15	0.68	0	4
Total teeth:	21.97	3.02	13	28

Table 4. Bacterial Prevalence

Table 4. Bacterial Prevalence					
			Mean		
Mutans	Sobrinus	Children (n)	Decayed	Missing	Filled
+	+	8.70%(10)	2.8	0.0	0.1
+	-	47.83%(55)	2.6	0.3	1.2
-	+	1.74%(2)	2.5	0.0	0.0
-	-	41.74%(48)	3.9	0.0	0.6

Table 5. Relationship between CAT 1 and CAT 2 (frequency count)

Table 5. Relationship between CAT 1 and CAT 2 (Frequency Count)				
	CAT 2 %(n)			
CAT 1 %(n)	Low	Moderate	High	Total
Low	34.78(40)	6.09(7)	17.39(20)	58.26(67)
Moderate	0(0)	0.87(1)	12.17(14)	13.04(15)
High	0.87(1)	2.61(3)	25.22(29)	28.7(33)
Total	35.65(41)	9.57(11)	54.78(63)	100(115)

Table 6. Contingency Table of S Mutans By CAT 1

Table 6. Contingency Table of *S. mutans* By CAT 1					
		Low	Moderate	High	Total
+	Count	42	6	17	65
	Column %	62.7	40.0	51.5	
	Row %	64.6	9.2	26.2	
-	Count	25	9	16	50
	Column %	37.3	60.0	48.5	
	Row %	50.0	18.0	32.0	
	Total	67	15	33	115

Table 7. Contingency Table of S Sobrinus By CAT 1

Table 7. Contingency Table of *S. sobrinus* By CAT 1					
		Low	Moderate	High	Total
+	Count	8	2	2	12
	Column %	11.9	13.3	6.1	
	Row %	66.6	16.7	16.7	
-	Count	59	13	31	103
	Column %	88.1	86.7	93.9	
	Row %	57.3	12.6	30.1	
	Total	67	15	33	115

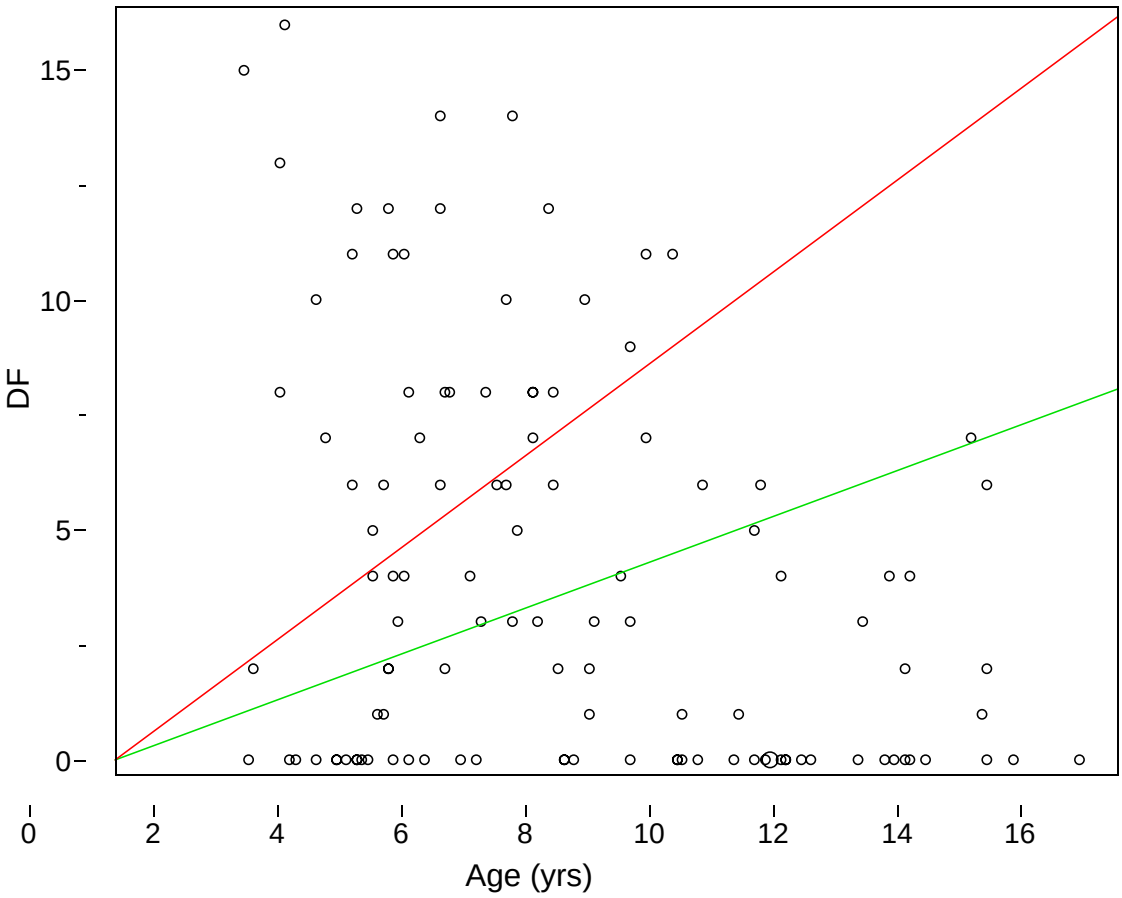
Table 8. Contingency Table of S Mutans By CAT 2

Table 8. Contingency Table of S. mutans By CAT 2					
		Low	Moderate	High	Total
+	Count	29	5	31	65
	Column %	70.7	45.5	49.2	
	Row %	44.6	7.7	47.7	
-	Count	12	6	32	50
	Column %	29.3	54.6	50.8	
	Row %	24.0	12.0	64.0	
	Total	41	11	63	115

Table 9. Contingency Table of S Mutans By CAT 2

Table 9. Contingency Table of *S. sobrinus* By CAT 2					
		Low	Moderate	High	Total
+	Count	5	0	7	12
	Column %	12.2	0.0	11.1	
	Row %	41.7	0.0	58.3	
-	Count	36	11	56	103
	Column %	87.8	100.0	88.9	
	Row %	34.9	10.7	54.4	
	Total	41	11	63	115

Figure 1. The Relationship between the number Decayed or Filled (DdFf) and Age according to CAT 1.



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APPENDIX A

RESEARCH SUBJECT INFORMATION AND CONSENT FORM
Bacteriological Samples

Title of Research: Detection of *Streptococcus mutans* and *Streptococcus sobrinus* in dental plaque samples from low, moderate, and high caries risk children.

Investigator:
Arthur P. Mourino, D.D.S.
Virginia Commonwealth University
School of Dentistry
521 N. 11th Street
Richmond, VA 23298
(804) 828-1790 (daytime)

Subject #_____ **Name**_____

This consent form may contain words that you do not understand. Please ask the study doctor or the study staff to explain any words or information that you do not clearly understand. You may take home an unsigned copy of this consent form to think about or discuss with family or friends before making your decision.

This consent form is for use in a research study that involves subjects who do not have the legal capacity to consent for their participation. Accordingly, when the subject cannot legally consent to participate, the pronouns "you" and "your" should be read as referring to the subject rather than the legally authorized representative who is signing the form to give consent for the subject.

Introduction

We are trying to determine the presence of two specific types of bacteria associated with dental caries (cavities). In order to do so, we are studying persons with dental cavities. The study is directed by Dr. Arthur P. Mourino, Professor Emeritus, Department of Pediatric Dentistry in the School of Dentistry.

Procedures

Dental caries (cavities) is caused by the bacteria (dental plaque) that grow on the surface of teeth. Dental plaque is composed almost completely of bacteria and their by-products. We wish to retrieve samples of your plaque in order to determine the presence of two different types of bacteria associated with cavities. Dental plaque will be collected by rubbing a toothpick along the outer surface of the very last tooth on each quadrant of the mouth. This will cause the plaque to cling to the toothpick. The bacteria from the plaque sample will be grown and examined in a laboratory.

Risks, Inconveniences, and Discomforts

There is minimal discomfort associated with this procedure. It is similar to that experienced with normal tooth brushing or getting your teeth cleaned at the dental office. There are no additional risks associated with the procedure.

Benefits

The tests are experimental (investigational) and will not be of immediate benefit to you. However, they may help us find out more about bacteria in dental plaque that cause dental cavities.

Payment for Participation

There will not be any compensation for your time and participation in our study.

Alternative Treatments

This is not a treatment study. You do not have to participate.

Research-Related Injury

Virginia Commonwealth University and the VCU Health System (formerly known as Medical College of Virginia Hospitals) have no plan for providing long-term care or compensation in the event that you suffer injury as a result of your participation in this research study.

If you are injured as a result of your participation in this study, contact your study doctor immediately. Your study doctor will arrange for short-term emergency care or referral if it is necessary.

Fees for such treatment may be billed to you or to appropriate third party insurance. Your health insurance company may or may not pay for treatment of injuries as a result of your participation in this study.

Confidentiality of Records

Confidentiality of personal information about you – including your medical records and personal research data gathered in connection with this study – will be maintained in a manner consistent with federal and state laws and regulations.

Information obtained for this study will solely be used for this study. Medical records which identify you and the consent form signed by you, may be inspected and/or copied by:

- Department of Health and Human Services (DHHS) agencies;
- Virginia Commonwealth University (VCU)

Because of the need to release information to these parties, absolute confidentiality cannot be guaranteed. Results of this study may be published, but your identity will not be revealed. Once the samples are 'coded', the documents which can trace the sample to the subject will be destroyed.

Voluntary Participation/Withdrawal

Participation in this study is voluntary. You are free to withdraw at any time. If you decide to withdraw from this study, you should contact Dr. Mourino. A decision not to participate, or to withdraw, will in no way affect or jeopardize the quality of care you receive now or in the future at this institution. Dr. Mourino or other investigators involve in this study may also withdraw you without your consent for medical, dental, or administrative reasons. Any significant new findings which develop during the course of the research study which in the opinion of the study doctor may affect your willingness to continue to participate will be provided to you as soon as possible.

Questions

If you have any questions concerning your participation in this study, contact:

Dr. Mourino or Dr. Patel at (804) 828-1790 or (804) 828-9095.

For information about research and subjects' rights, you may contact:

Office of Research Subjects Protection
Virginia Commonwealth University
1101 E. Marshall St., Room 1-023
P.O. Box 980568
Richmond, VA 23298
Telephone: 804-828-0868

Do not sign this consent form unless you have had a chance to ask questions and have received satisfactory answers to all of your questions.

Consent

_____ _____
Date Subject's Name

Signing this paper means that you have read this or had it read to you and that you want to be in the study. If you don't want to be in the study, don't sign the paper. Remember, being in the study is up to you, and no one will be mad if you don't sign this paper or even if you change you mind later.

If you want to be in our study, sign your name here:

Name:_____ Date:_____
Subject's Signature (if greater than 7 and less than 18 years of age)

I have been provided with an opportunity to read this consent form carefully. All my questions about this study and my participation in it have been answered to my satisfaction.

By signing this consent form, I have not waived any of the legal rights or benefits, to which I or my child otherwise would be entitled. My signature indicates that I freely give consent to my child's voluntary participation in this research study.

_____	_____
Date	Legally Authorized Representative's Signature
_____	_____
Date	Witness' Signature
_____	_____
Date	Signature of person Conducting Informed Consent Discussion
_____	_____
Date	Investigator's Signature (if different from above)

APPENDIX B

VITA

Ajit A. Patel was born on July 22, 1973 in Bombay, India and is an American citizen. He received his Bachelor of Science from University of Pittsburgh in December 1995, and his Doctor of Dental Surgery from Virginia Commonwealth University in May 2002. He is currently a postgraduate resident in the Pediatric Dentistry Program at Medical College of Virginia, Virginia Commonwealth University and upon graduation will practice in Ashburn, Virginia.