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Rapid Detection of *Streptococcus mutans* in Saliva

A thesis

presented to

the faculty of the Department of Allied Health Sciences

East Tennessee State University

In partial fulfillment

of the requirements for the degree

Master of Science in Allied Health

by

Catherine E. Holtman

August 2012

Dr. Ester Verhovsek, Chair

Dr. Randy Byington

Dr. Debbie Dotson

Keywords: *Streptococcus mutans*, rapid detection, saliva, caries, specificity

ABSTRACT

Rapid Detection of *Streptococcus mutans* in Saliva

by

Catherine E. Holtman

Documentation exists that mothers can pass the cariogenic bacteria *Streptococcus mutans* to their infants. The newest technology to identify *Streptococcus mutans* is a rapid detection saliva test. Two hundred patients above the age of 18 were targeted using random selection in a Louisville, Kentucky dental office. Patients signed an informed consent form and were given a qualifying questionnaire. Patients received 2 bitewing x-rays and a charted DMFT index and were administered the saliva test. While the null hypothesis was rejected using the chi square test, the results were inconclusive due to expected values. However, other chi square results revealed that the test worked or had the potential to work. Furthermore, it was concluded that the test had high specificity. Further research is warranted; however, the saliva test in combination with the DMFT and x-rays are instrumental tools for the dental professional in educating patients and prevention.

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DEDICATION

I dedicate this thesis to my husband John Bernard Holtman. Your constant support, understanding, and love gave me so much strength to achieve my goals. I am so thankful for you. Your continuous belief in me and encouragement gives me so much strength and makes all things possible. Thank you so much for helping my dreams and goals become a reality.

ACKNOWLEDGEMENTS

Words cannot express my gratitude to the many people who have mentored, guided, and supported me during the past few years in achieving my higher education goals. I want to sincerely express my gratitude to my thesis chair Dr. Ester Verhovsek for guiding, supporting, and mentoring me through this entire research project. Thank you so much for the prompt emails, responses, and endless days that you spent helping me with this project. Your belief in me has given me so much strength to see this project to the end.

I am equally as grateful to Dr. Randy Byington, a member of my graduate committee who guided me through the statistics of this research project. I knew immediately with my first encounter in your class that you were going to challenge me and make me think outside the box. Your patience, understanding, mentoring, and help are greatly appreciated. I have learned to think from a global perspective and I appreciate that quality on a daily basis.

I sincerely express my gratitude to Dr. Debbie Dotson, a member of my graduate committee. Your encouragement early on in my higher education journey gave me the courage to start achieving my goals. In addition, your belief in me has given me such strength. Thank you so much for being such an important mentor in my life. I hope that I can be a mentor to students and encourage them as you have encouraged me.

Lastly, I would like to express my gratitude to my children Brandon and Blake Hunt. It has been such a pleasure going back to school during your college years. We have laughed, cried, and supported one another through our college experiences. Moreover, we will all graduate within a year of one another and create yet another wonderful family memory. I can't thank both of you enough for your support and for believing in your mother.

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CHAPTER 1

INTRODUCTION

Dental caries are a concern of every person regardless of race, age, gender, or socioeconomic level. The ability to determine a person's susceptibility to dental caries has become a prominent area of research to identify both the cause and the effect. The U.S. Surgeon General's Report: *Oral Health in America* (2000) stated that the *silent epidemic* of oral disease is becoming prevalent in poor children, the elderly population, and many racial and ethnic minority groups. The same report also revealed that dental caries is the single most common chronic childhood disease. It is estimated that more than 51 million school hours are lost each year to dental-related illness (United States Department of Health and Human Services, 2000). In addition, The Academy of Pediatrics (2003) reported that human dental flora is site specific, and an infant is not colonized with normal dental flora until the eruption of the primary dentition. This occurs at approximately 6 to 30 months of age. It has been well documented that mothers who have a high caries risk can pass the cariogenic bacteria *Streptococcus mutans* to their infants. This automatically predisposes the infant to a high caries risk (American Academy of Pediatrics, 2003). On the other hand, the U.S. Surgeon General's Report (2000) revealed that employed adults lose approximately 164 million hours of work each year due to dental disease. It is apparent that the ability to assess caries susceptibility would be advantageous for all populations groups.

With regard to adults, The National Institute of Dental and Craniofacial Research (2011) reported that although dental caries in both treated and untreated adults has declined since the 1970s, significant disparities are found in some population groups. In addition, the National Health and Nutrition Examination Survey from 1999-2004 stated that the prevalence of untreated

caries in permanent teeth among adults 20-64 is: 28% in the 20-34 group, 26% in the 35-49 group, and 22% in the 50-64 group (National Institute of Dental and Craniofacial Research, 2011a). It is also reported that 49.7% of the 75 years or older age group had root caries that affected at least one tooth (National Institute of Dental and Craniofacial Research, 2011b). It is important to note racial and ethnic differences as well. “Regardless of poverty level status, adult non-Hispanic Blacks and Mexican Americans have higher proportions of untreated decayed teeth than their non-Hispanic White counterparts”(National Institute of Dental and Craniofacial Research, 2011b, p.5). It is apparent that over the past 5 decades major improvements in oral health have occurred for many Americans. However, research is needed to create and identify interventions that will eliminate oral health disparities (National Institute of Dental and Craniofacial Research, 2011b).

The approach to decreasing dental decay is to assess the mother’s caries risk, educate about oral hygiene for mother and child, optimize systemic and topical fluoride use, and nutritional counseling (American Academy of Pediatrics, 2003). However, assessing the caries risk may be beneficial to future generations and allow for more effective interventions. The National Institute for Dental and Craniofacial Research (2011a) reported that 93% of females and 91% of males in the 20–64 age range have caries in their permanent teeth. Therefore, assessing the caries risk through the presence of *Streptococcus mutans* may present information to lower the transmission rates, decrease caries, and increase knowledge about caries susceptibility.

The oral cavity is home to many different types of bacteria. However, *Streptococcus mutans* is considered to be a major human cariogen (Dasanayake, Caufield, Cutter, Roseman, & Köhler, 1995, p.345). Therefore, having an increase in the number of *Streptococcus mutans* in

the oral cavity is “considered as a risk factor for the onset of caries” (Giacaman, Araneda, & Padilla, 2010, p.550). Moreover, given the uncertainty about the association between *Streptococcus mutans* and the entire caries experience in adults, Giacaman et al. (2010) decided to research if high colonies of *Streptococcus mutans* and the presence of biofilm could predict caries susceptibility in adults. Results showed that the “cariogenic potential of *Streptococcus mutans* may rely on individual pathogenic traits of the microorganism, including biofilm formation capabilities” (Giacaman et al., 2010, p.553). Indicating further studies are necessary.

Saliva has been studied for decades. However, the physiological importance of saliva has only come into focus recently. Schipper, Silletti, and Vingerhoeds (2007) stated that the “interest in saliva even more increased with the finding that saliva was filled with hundreds of components that may serve to detect systemic disease or evidence of exposure to various harmful substances, as well as provide biomarkers of health and disease status” (p.1115). In addition, “many salivary proteins offer great potential in clinical and epidemiological research, in oral as well as in general health studies” (Chiappin, Antonelli, Gatti, & De Palo, 2007, p.38).

Many methods have been developed to identify *Streptococcus mutans* and predict caries susceptibility. The earlier detection methods of *Streptococcus mutans* used several different media to grow colonies. These media were mitis-salivarius-bacitracin agar (MSB), trypticase yeast-extract-cysteine-sucrose-bacitracin agar (TYCSB), and glucose-sucrose-potassium tellurite-bacitracin (GSTB) agar (Dasanayake et al., 1995). Early research by Dasanayake et al. (1995) were comparisons of a number of different studies that involved the detection of *Streptococcus mutans* in unstimulated saliva versus plaque, in stimulated saliva versus plaque, the enumeration of *Streptococcus mutans* in stimulated versus unstimulated saliva, and the detection and enumeration of *Streptococcus mutans* in MSB versus GSTB. Dasanayake et al.

(1995) concluded that “of the two culture media we compared, MSB seems to be more sensitive because the GSTB medium failed to detect *Streptococcus mutans* in about one half of the individuals in whom mutans Streptococci were detected with MSB medium” (p.350). In addition, Dasanayake et al. (1995) stated that “quantitative assessment of *mutans Streptococci* should be based on stimulated rather than unstimulated saliva samples” (p.350). With this in mind, the newest technology suggests an immunochromatography process that uses two monoclonal antibodies for rapid detection and accurate results. This test is currently available from GC America (Saliva-Check Mutans) and will deliver results in 15 minutes (GC America, 2007).

Giacaman et al. (2010) researched 96 patients between 15 and 27 years of age to determine if biofilm (dental plaque) formation by *Streptococcus mutans* is associated with higher caries susceptibility in young adults. The descriptive data of age and gender were collected. In addition, the World Health Organization (WHO) criterion was used to collect a decayed, missing, and filled teeth (DMFT) index. Bitewing radiographs were also used to confirm interproximal caries. The patients were asked to chew on paraffin wax for 2 minutes to stimulate saliva flow. The saliva samples were collected and brought immediately to the microbiology laboratory for testing. The samples of saliva were tested using the trypticase-yeast-cysteine-sucrose-bacitracin medium (TYCSB). The samples were incubated at 37° C for 48 hours in anaerobic jars to determine the number of colony forming units per millilitre (cfu/ml) of saliva. The levels of *Streptococcus mutans* were then divided into three categories: $< 1 \times 10^5$, 1×10^5 to 1×10^6 , and $> 1 \times 10^6$ cfu/ml (Giacaman et al., 2010, p. 551).

In conclusion, Giacaman et al. (2010) found that “multiple variables may confound a potential use of MS counts as a risk factor for caries, such as different methods for caries

examination, dissimilar populations i.e., race, age, education, technical variations in processing the samples, lack of standardized counting methods amongst others” (p.553). In addition, Giacaman et al. (2010) concluded that they “failed to find a direct relation between MS and DMFT” (p.553). “While the relation between MS and dental caries remains debateable, the cariogenic potential of MS is indisputable” (Giacaman et al., 2010, p.550). Further research should “consider differences in the number of colonies with and without biofilm in order to determine if a quantitative relation also exists” (Giacaman et al., 2010, p.553).

Statement of the Problem

It has been well documented that mothers who have a high caries risk can pass the cariogenic bacteria *Streptococcus mutans* to their infants (American Academy of Pediatrics, 2003). This automatically predisposes the infant to a high caries risk (American Academy of Pediatrics, 2003). The approach to decreasing dental decay is to assess the mother’s caries risk, educate about oral hygiene for mother and child, optimize systemic and topical fluoride use, and provide nutritional counseling (American Academy of Pediatrics, 2003). Assessing the caries risk of males and females in the caregiver or parental age range may be beneficial to future generations. By increasing knowledge about caries susceptibility this may lower the transmission rates and, in turn, decrease dental caries for adults and children.

The purpose of this study is to determine the specificity of the chair side GC America Rapid Detection *Streptococcus mutans* saliva test in a middle-high socioeconomic single practitioner dental office in Louisville, Kentucky. An 18 year old and above population group was targeted using random selection. By determining the specificity of *Streptococcus mutans*, the researcher can provide education to patients and decrease rates for dental caries.

Research Question

The following question guides this study:

1. Does the presence of *Streptococcus mutans* relate with caries susceptibility as indicated by the GC America Rapid Detection *Streptococcus mutans* saliva test using the 500,000 cfu/ml biomarker?

Significance of the Study

With the ability to determine caries susceptibility through rapid detection, the hypothesis is that the GC America Rapid Detection *Streptococcus mutans* saliva test will be able to relate caries susceptibility with the bitewing radiographs and DMFT, therefore showing high specificity in the testing procedure. This research is additive to oral hygiene education for the patient and would begin to restore the patient to good oral health through possible adjunctive medicinal therapies to decrease the colonies of *Streptococcus mutans* present. Moreover, the benefits of determining the specificity of the rapid detection test would validate the purchase and use of the testing product. In addition, the ability to administer the rapid detection test without the need of a laboratory is advantageous for both patient and healthcare professional. According to the U.S. Surgeon General's Report (2000), "oral diagnostics, using saliva or oral tissue samples, will contribute to overall health surveillance and monitoring" (Action 3).

Using the GC America Rapid Detection *Streptococcus mutans* saliva test chair side could change the home care regimen for the patients. This chairside test could decrease the transmission of the bacteria from caregiver to infant and increase educational interventions for the healthcare professional and the patient.

Delimitation and Limitations

Several delimitations for this study were identified. The delimitations included the geographic location of the single dental practitioner located in the East End of Louisville, Kentucky. The socioeconomic level of East End residents is considered to be middle to high. In addition, no minors under the age of 21 or pregnant patients were allowed to participate in the study. Moreover, the data only contained results collected during the time frame specified for the study from May 2012 through June 2012.

The limitations for this study included patients who refused to participate in the study due to various concerns and the ability to access a larger population size comprised of various socioeconomic levels.

Assumptions

It is assumed that the data collected by the chairside GC America Rapid Detection *Streptococcus mutans* saliva test were complete and accurate for each patient.

Operational Definitions

Biofilm: “Research over the past decade has led to recognition of dental plaque as a biofilm – a highly organized accumulation of microbial communities attached to an environmental surface” (Gurenlian, 2007, p.5).

Caries (tooth decay): “A localized bacterial disease process which destroys tooth structures and produces a cavity” (Gagliardi, 2007, p.237)

Craniofacial Complex: Includes the oral, dental, and craniofacial tissues (Petersen, 2003).

DMFT: The World Health Organization (WHO) oral survey index that is based on decayed, missing, and filled teeth. This survey measures the “lifetime experience of dental caries in the permanent dentition” (Peterson, 2003, p.5).

False Negative: The results indicate that the individual does not have the disease, but the gold standard indicates that the disease is present (Pretty & Maupomé, 2004).

False Positive: The results indicate that the individual has the disease, but the gold standard indicates that the disease is not present (Pretty & Maupomé, 2004).

mitis-salivarius-bacitracin agar (MSB), trypticase yeast-extract-cysteine-sucrose-bacitracin agar (TYCSB), and glucose-sucrose-potassium tellurite-bacitracin (GSTB) agar: Different types of culture media most commonly used to test for *mutans streptococci* (Dasanayake et al., 1995).
mutans Streptococci: “Microorganisms associated with the development of caries” (Baca et al., 2008, p.751).

Specificity: The proportion of patients without caries who are correctly identified by the test (Pretty & Maupomé, 2004).

Streptococcus mutans: The most frequently isolated member of the *mutans Streptococci* group in humans (Baca et al., 2008).

Stimulated saliva: Oral saliva that is generated by gentle mastication. Examples include chewing on paraffin wax, using citric acid, and chewing on a neutral gum base to produce saliva (Dasanayake et al., 1995).

Sucrose: “A disaccharide composed of fructose and glucose” (Caufield & Griffen, 2000, p, 1009).

True Negative: The results indicate that the person does not have the disease; the results are confirmed by the gold standard (Pretty & Maupomé, 2004).

True positive: The results indicate that the individual has the disease, and this is confirmed by the gold standard (Pretty & Maupomé, 2004).

Unstimulated saliva: Oral saliva that is collected without mastication. Examples would include expectorating in a cup and passive drooling (Dasanayake et al., 1995).

CHAPTER 2

REVIEW OF LITERATURE

Dental Caries

“Dental caries is a modern, lifestyle-dependent disease of humans caused by excess consumption of fermentable carbohydrate” (Caufield & Griffen, 2000, p.1009). “Humans have always been colonized by potentially cariogenic bacteria; what changed in recent centuries, leading to disease, is the availability of vast quantities of refined sugar” (Caufield & Griffen, 2000, p.1009). Dental caries are caused by oral bacteria. It is important to note that by definition dental caries are considered an infectious disease (Caufield & Griffen, 2000). According to a 1996 bulletin from the Centers for Disease Control and Prevention, dental caries may be one of the most prevalent infectious diseases that affect humans (Caufield & Griffen, 2000). This can be attributed to the agricultural practices that have made sucrose omnipresent. Oral bacteria contain extracellular enzymes that bond with the sucrose. The bacterial pathogens produce a lactic acid from the fermentation of the carbohydrates. The lactic acid dissolves the mineral matrix of the tooth. “If mineral continues to be lost because of acid challenge, the surface is eventually broken or cavitated, and the lesion cannot be reversed” (Caufield & Griffen, 2000, p.1002).

Although tests have been developed to investigate oral bacteria, the ability to predict dental caries has been a topic of interest in oral health care for decades. Tests have been developed to investigate oral bacteria. These tests used for caries-risk predictors have been evaluated “by means of studies providing data on test sensitivity, specificity, and predictive values” (van Houte, 1993, p.87). However, many of these tests have not been deemed acceptable in the field of dentistry (van Houte, 1993).

The review of the literature consisted of information gathered from a variety of databases. These databases were accessed from East Tennessee State University's Sherrod Library and Advanced Goggle Scholar. In addition, keywords to access the research articles included childhood caries; decayed, missing, and filled teeth index (DMFT); detection; microbiology; polymerase chain reaction (PCR); population; radiographs; saliva; selective mediums; specificity of *Streptococcus mutans*; and transmission.

Children

“Caries is a cumulative disease that is not initiated until teeth erupt into the oral cavity, and it may not be obvious until sometime, perhaps years, after teeth emerge” (Caufield & Griffen, 2000, p.1004). *Streptococcus mutans* colonize the primary dentition at approximately 2 years of age. This period of time is known as the window of infectivity (Caufield & Griffen, 2000,). Generally the primary dentition is fully erupted at 2 years of age. “Among children in the United States, dental care is the largest unmet health care need, as reported in a large-scale study based on National Health Survey data” (Caufield & Griffen, 2000, p.1001). Interestingly enough, more than \$40 billion is spent each year in the United States on the treatment of dental caries (Caufield & Griffen, 2000). “Among 5-to-17 year olds, dental caries is more than 5 times as common as a reported history of asthma and 7 times as common as hay fever” (National Institute of Dental and Craniofacial Research, 2011b, p.3).

Adults

The percent of adults 17 years old who have had at least one carious lesion or filling in the coronal portion of a permanent tooth is 77.9. The percent increased to 84.7 in adults 18 or older (National Institute of Dental and Craniofacial Research, 2011b). It is also reported that 49.7% of the 75 years or older age group had root caries that affected at least one tooth (National

Institute of Dental and Craniofacial Research, 2011b). As people age, oral disease can become progressive and cumulative causing poor oral health. “Poor oral health can increase the risks to general health and, with compromised chewing and eating abilities, affects nutritional intake” (Petersen, 2003, p.17). “The craniofacial complex allows us to speak, smile, kiss, touch, smell, taste, chew, swallow, and to cry out in pain” (Petersen, 2003, p.4). Detection of caries can lead to the prevention of eating, speaking, and learning difficulties and potentially diminish self-esteem and self-confidence concerns.

Ethnicity

Differences exist by race, ethnicity, and poverty level; however, “Regardless of poverty level status, adult non-Hispanic Blacks and Mexican Americans have higher proportions of untreated decayed teeth than their non-Hispanic White counterparts”(National Institute of Dental and Craniofacial Research, 2011b, p.5). It has been noted that “although there have been gains in oral health status for the population as a whole, they have not been evenly distributed across subpopulations” (National Institute of Dental and Craniofacial Research, 2011b, p.15). The poorest oral health of any racial and ethnic groups in the United States includes non-Hispanic Blacks, Hispanics, American Indians, and Alaska Natives (National Institute of Dental and Craniofacial Research, 2011b). Detection of caries can increase quality of life. “There is considerable evidence implicating *Streptococcus mutans* as a specific infectious agent in the initiation of dental caries” (Zickert, Emilson, & Krasse, 1983, p.982).

Streptococcus mutans

Streptococcus mutans (*S. mutans*) was discovered to be a significant pathogen for human caries. In 1924 Clarke isolated *S. mutans* from human carious lesions and described his discovery as the following:

S. mutans was isolated from 36 of the 50 teeth. Acid is very rapidly produced, the medium, originally pH 7, giving a reaction of pH 4.2 in about 24 hours. All the strains isolated ferment glucose, lactose, raffinose, mannite (manitol), inulin, and salicin with production of acid. There is usually neither haemolysis nor discoloration on blood-agar. The fact that the colonies of *S. mutans* adhere closely to the surface of the teeth appears to be of great importance (Hamada & Slade, 1980, p.333).

Clarke established that *S. mutans* was a significant pathogen found in human carious lesions and *S. mutans* is considered one of the major bacterium used to predict caries.

Within the last 10-15 years there has been a dedicated interest in the concept of caries predictability. It has been well established that *S. mutans* is a transferable bacteria from mother to infant and from caregiver to infant (Caufield, Cutter, & Dasanayake, 1993). However, the development of a rapid accurate test is still a work in progress. GC America developed a rapid detection *S. mutans* saliva test using monoclonal antibodies with immunoassay. This study determined the specificity of the GC America rapid detection test (GC America, 2007).

Microbiology

Hamada and Slade (1980) revealed through extensive taxonomic studies that the *S. mutans* organisms “formed a fairly homogeneous group of nonmotile, catalase-negative, gram-positive streptococci” (p.333). Moreover, *S. mutans* has been found to be a natural inhabitant of

the human mouth. Researchers determined that streptococcal strains exhibiting the following biochemical characteristics (ferment manitol and sorbitol as well as various other sugars, and synthesize adherent water-soluble glucan from sucrose) were considered to be the organism *S. mutans* (Hamada & Slade, 1980).

Species and Serological Types

As research continued it was determined that there were five subspecies and 8 different serological types of *mutans streptococci*. These species and serological types are: “*Streptococcus cricetus* (serotype a), *Streptococcus rattus* (serotype b), *Streptococcus mutans* (serotypes c, e, and f), *Streptococcus sorbrinus* (serotypes d and g), and *Streptococcus downei* (serotype h)” (Igarashi, Yamamoto, & Goto 1996, p.294). With this in mind, it has been proven that *S. sorbrinus* and *S. mutans* are the species that are related to human dental caries with *S. mutans* the species identified most frequently in human dental plaque. In addition, it has been discovered that the “colonization of *S. mutans* occurs after tooth eruption” (Loesche, 1986, p.371). Leading to the theory that the interruption of the colonization process of *S. mutans* would have “a profound effect on the incidence of dental decay in human populations” (Loesche, 1986, p.371).

Transmission

Caufield et al. (1993) studied 46 mother-child pairs to determine the initial acquisition of *S. mutans*. The mother-child pairs were studied from infant birth to 5 years of age. It was revealed in this study that 38 children with a median age of 26 months acquired *S. mutans* from their mothers. In addition, these 38 children were from a predicted high-risk caries population. Caufield et al. (1993) stated that “a major finding of this study was that the initial acquisition of MS in infants occurred during a well-delineated age range we have designated as the window of infectivity” (p.42). Moreover, Davey and Rodgers (1984) found that “the early childhood years

are a critical period for the acquisition of *S. Mutans*” (p.453). In researching intra-family transmission, Davey and Rodgers (1984) stated in the conclusions of their study that “maternal transmission has been suggested although the results were not clear-cut” (p.457).

In using a cryptic plasmid as the epidemiological marker, the families of four plasmid-positive children were studied. The study revealed in three families, that “the mothers’ *Strep mutans* strains were plasmid-negative and in the fourth both parents carried the plasmid-positive strain” (Davey & Rodgers, 1984, p.459). Furthermore, Duchin and van Houte (1978) concluded in their study that “although the formation evidence about the relative susceptibility of infants, children, and adults to infection by *S. mutans* is lacking, the evidence suggests that this organism will readily colonize newly exposed teeth of children and adults in the presence of suitable salivary levels” (p.124).

“Because mothers are the major source of cariogenic bacteria to their children and sucrose consumption modulates the expression of disease, approaches aimed at interfering or preventing mother-child transmission hold promise” (Caufield & Griffen, 2000, p.1010). It is evident that many studies have been performed over the years with similar conclusions that cariogenic bacteria transmission is a concern with dental caries.

Duration

Zickert et al. (1983) conducted a study to “examine the level and duration of *S. mutans* infection in relation to the development of caries in teenagers” (p.982). Ninety-one children ranging in age from 13-14 years old at the beginning of the study participated in the clinical trial. The control group (n=47) and the test group (n=44) completed the clinical trial. The elementary children were followed for 3 years in a negligible fluoridated community in Mölndal Sweden. Samples of stimulated saliva were taken at the beginning, middle, and end of the year. In

addition, dental caries examinations were performed after 1, 2, and 3 years. The results revealed that “the children with a mean colony count values of $> 10^6$ *S. mutans* per ml of saliva showed considerably higher caries activity than those with lower levels of *S. mutans* infection” (Zickert et al., 1983, p.982). In addition, children who sustained longer periods of high counts of *S. mutans* proved to have more destruction on their teeth than those with intermittent high counts of *S. mutans*. In conclusion, the results of this study demonstrated that the level and duration of *S. mutans* strongly correlates with the incidence of caries in the Swedish teenagers and supported the theory that *S. mutans* is a significant organism in the development of human caries. Furthermore, it was determined that 10^5 - 10^6 colony forming units per ml (cfu/ml) of saliva are considered a high level of *S. mutans*, therefore, indicating a high caries risk (Jordan, Laraway, Snirch, & Marmel, 1987).

Saliva

Saliva has been studied throughout history. Moreover in the past 50 years saliva research has become more prominent with the development of new techniques that analyzed the biochemical and physicochemical properties of saliva. Saliva is important in speech, lubrication, digestion of food, and maintaining oral and general health (Schipper et al., 2007). Saliva is produced by the contra-lateral glands (parotid, submandibular, and sublingual) as well as minor salivary glands present in the mucosa of the tongue, cheeks, lips, and palate (Schipper et al., 2007). Saliva consists of 99.5% water, 0.3% proteins, and 0.2% inorganic and trace substances (Schipper et al., 2007).

Saliva secretions ranges from 0.3 to 7 ml of saliva per minute (Schipper et al., 2007). In addition “saliva pH can range from 6.2 to 7.4, with the higher pH exhibited upon increased secretion” (Schipper et al., 2007, p.1115). Several methods exist to collect saliva; however, there

are two preferred methods. The unstimulated method requires a person to expectorate into a tube. The stimulated method requires a person to chew on paraffin wax for 5 minutes and then expectorate into a tube. In addition, saliva samples (unstimulated and stimulated) have been collected by swabbing, wooden spatula method, a tongue loop, or with a plastic strip (Dasanayake et al., 1995). It was reported that stimulated saliva samples were superior for the reason that they yielded higher levels of mutans streptococci with lower sample variance than with unstimulated saliva (Gu et al., 2002).

Detection

Giacaman et al. (2010) performed a study to determine whether biofilm formation by *S. mutans* was associated with high caries susceptibility in young adults. A cross-sectional study using 96 randomly selected patients ranging in age 15-17 years was performed. A sample of stimulated saliva was collected. In addition, patients were examined under a dental light using a mouth mirror and curved probe. The World Health Organization (WHO) criterion was followed as means of assessment. The WHO criterion records a tooth (T) or its surfaces (S) as decayed (D), missing (M), and filled (F) to obtain the decayed, missing, and filled (DMFT) index. In addition, bitewing radiographs were obtained on each patient for accurate clinical diagnosis (Giacaman et al., 2010). Wenzel (2004) stated that “in the clinical situation, caries lesions have traditionally been diagnosed by visual inspection in combination with radiography” (p.C73). Moreover, Shi, Jewett, and Hume (1998) stated that dental caries are detected “by changes in translucency, color, hardness, or x-ray density of tooth structure” (p.370).

X-rays

Caries detection is not limited to the levels of surface cavitation that can be seen upon clinical observation. Radiography (bitewing) can enhance caries detection by showing information of the clinical stages of the caries on the approximal surfaces and the advanced stages on occlusal surfaces (Wenzel, 2004). The bitewing examination should show the crowns of the teeth from the distal surface of the canine to the distal surface of the most posterior erupted molar. In an adult patient, a #2 size film should be used. Two or four bitewing radiographs may be used; however, in a recent study it was concluded that one bitewing radiograph on each side placed horizontally was sufficient for diagnosis (Wenzel, 2004). Results showed very little disease was evident in the canines and first premolars, therefore, recommending one bitewing radiograph (Wenzel, 2004). Becker, Levin, Shochat, and Einy, (2007) concluded that it is imperative to include radiographs in a dental examination for an accurate diagnosis.

DMFT

“The DMFT index was created to express the caries experience” (Becker et al., 2007, p.677). The study performed by Becker et al. (2007) compared the DMFT index as a diagnostic tool on its own and the DMFT as a diagnostic tool with radiographs. The study included 376 young male recruits of the Israeli Air Defense Artillery ranging in age from 18-20 years old. The mandatory dental screenings consisted of clinical examinations for caries and bilateral bitewing radiographs. The DMFT index was calculated with and without radiographs to compare the DMFT scores. It was concluded that “when performing dental examination without the use of oral radiographs, there is a 44 percent probability that the caries decay value will be lower than the actual one” (Becker et al., 2007, p.680). Therefore, it is important to include radiographs “when conducting an epidemiological examination in dentistry” (Becker et al., 2007, p.680).

Selective Mediums

S. mutans studies have evolved over the decades. Many different culture media have been used to examine *S. mutans* and involved the collection of dental plaque samples, saliva samples, or both (Dasanayake et al., 1995). The most commonly used culture media has been mitis-salivarius bacitracin agar (MSB), trypticase-yeast-extract-cysteine- sucrose-bacitracin agar (TYCSB), and glucose-sucrose-potassium tellurite-bacitracin agar (GSTB). Dasanayake et al. (1995) compared MSB and GSTB media to “identify a suitable method of assessment of mutans streptococci for epidemiological studies and to provide a basis for adjustment of estimates when comparing results across the studies” (p.346). “Of the two culture media we compared, MSB seems to be more sensitive because the GSTB medium failed to detect mutans streptococci in about one half of the individuals in whom *mutans streptococci* were detected with MSB medium” (Dasanayake et al., 1995, p.350). It was concluded that stimulated saliva samples were superior for the reason that they yielded higher levels of mutans streptococci with lower sample variance than with unstimulated saliva. More importantly, Gu et al. (2002) determined that “there is no selective medium that allows only one bacterial species to grow” (p.225). Therefore, indicating that *S. mutans* tests using culture media are inaccurate and may contain false-positive data. With this in mind, selective culture assays evolved. Gu et al. (2002) found that culture assays “can only detect viable, cultivable cells” (p.225). Therefore, research has advanced and the current testing methods use monoclonal antibodies rather than culture techniques.

Polymerase Chain Reaction Technique

Kindt, Goldsby, and Osborne (2007) stated that “the polymerase chain reaction (PCR) is a powerful technique for amplifying specific DNA sequences even when they are present at extremely low levels in a complex mixture” (p.559). Igarashi et al. (1996) determined that *S. mutans* produces an extracellular dextranase. This extracellular dextranase has been determined to be one of the virulent factors of *S. mutans*-induced human dental caries. Igarashi et al. (1996) cloned the dextranase gene (*dexA*), the serotype c *S. mutans* strain. Igarashi et al. (1996) performed a study that designed a pair of PCR primers specific for the *dexA* gene. The goal was to “establish a simple, rapid, and specific detection and identification method of *S. mutans* from clinical isolates and human dental plaque” (Igarashi et al., 1996, p.294). The study concluded that the PCR method proved to be highly specific for *S. mutans* however; the diagnosis using the PCR method took one day. Therefore, the PCR method proved to be more useful than the selective culture mediums used in previous studies.

With this in mind, Oho, Yamashita, Shimazaki, Kushiya, and Koga (2000) reported that dental plaque may not be the preferred collection method for epidemiological studies due to difficulty in collection. Oho et al. (2000) performed a study including 60 people who were exactly 20 years of age. Stimulated saliva was collected for the study instead of dental plaque. Using saliva samples made it impossible to differentiate between *S. mutans* and *S. sorbinus*. With this in mind, more information to differentiate between the two was necessary. To further help identify the correct bacterium, *S. mutans* “produces three types of glucosyltransferases (GTF-1, GTF-S1, and the GTF-S), and the *gtfB* gene encodes GTF-1, which primarily synthesizes water-insoluble glucan from sucrose” (Oho et al., 2000, p.261). *S. sorbinus* has four types of glucosyltransferases. The PCR primers in this study were designed to recognize the *gtfB*

gene. In addition, the caries risk of individuals was categorized into four levels from the results of counting colonies. These levels were negative, not detected; low, about 5×10^3 cfu/ml; moderate, about 5×10^4 cfu/ml; and high, over 10^5 cfu/ml (Oho et al., 2000, p.261). In conclusion, the PCR method allowed the study to identify *S. mutans* at a level of $\geq 10^4$ cfu/ml. Therefore, it was proven that the sensitivity of the PCR method could sufficiently evaluate the caries risk of an individual (Oho et al., 2000, p.261).

Monoclonal Antibodies

Kindt et al. (2007) stated that “a monoclonal antibody is derived from a single B-cell clone and has a single binding site” (p.107). Gu et al. (2002) discovered that “three highly species-specific monoclonal antibodies (MAbs) against *S. mutans* (SWLA1-3 antibodies) were recently developed” (p.225). These MAbs demonstrated high specificity and sensitivity in detecting *S. mutans* resuspended in phosphate-buffered saline (PBS) (Gu et al., 2002). In the study performed by Gu et al., they analyzed the ability of the MAbs in regards to detecting *S. mutans* in saliva. This study included 2,000 human saliva samples that were collected using both stimulated and unstimulated collection methods. The study revealed a wide variation in *S. mutans* counts within the population. The study concluded that “there is no statistically significant difference in the number of *S. mutans* in unstimulated saliva versus stimulated saliva and that there is no significant correlation between the number of *S. mutans* in unstimulated and in stimulated saliva” (Gu et al. 2002, p. 230). In addition, the study concluded that “MAb-based bacterial detection is now considered to be one of the most accurate bacterial detection methods” (Gu et al. 2002, p.231). Moreover, this method can provide rapid results and does not take days like the selective culture media methods. Gu et al. (2002) suggested that more research needed to be performed to examine whether there is a correlation between high salivary *S. mutans* counts

and high caries incidence. Additionally, Shi et al. (1998) stated that “monoclonal antibodies can be linked to colloidal gold colorimetric system on test strips” (p.370). The rapid detection simple assay of *S. mutans* indicated by a color change would be a convenient method to determine caries risks for the dental practitioner and the patient’s household. It is important to note that “the sensitivity and accuracy of the method is largely dependent on the specificity of the MAb produced” (Shi et al., 1998, p.369).

Summary

Upon reviewing the comparative literature and research studies, the researcher believes it is important to determine the specificity of the immunoassay monoclonal rapid detection *S. mutans* saliva test. It was revealed through the research that the testing methods to determine caries risk predictability have continuously been evolving. It has been proven that stimulated saliva and monoclonal assay reveal quick chair side results. However, determining the specificity of the GC America rapid detection *S. mutans* saliva test has proven its worth to the dental professional. “The accurate and objective assessment of caries risk state and /or caries activity with any of these similar technologies will permit targeted preventive and curative treatment, thereby significantly improving human dental health” (Shi et al., 1998, p.370).

CHAPTER 3

DESIGN AND METHODOLOGY

Overview

The oral cavity is home to many different types of bacteria. However, *Streptococcus mutans* is considered to be a major human cariogen (Dasanayake et al., 1995, p.345). Therefore, having an increase in the number of *Streptococcus mutans* in the oral cavity is “considered as a risk factor for the onset of caries” (Giacaman et al., 2010, p.550). Moreover, given the uncertainty about the association between *Streptococcus mutans* and the entire caries experience in adults, Giacaman et al. (2010) decided to research if high colonies of *Streptococcus mutans* and the presence of biofilm could predict caries susceptibility in adults. It was concluded that “while the relation between MS and dental caries remains debateable, the cariogenic potential of MS is indisputable” (Giacaman et al., 2010, p.550). Further research should “consider differences in the number of colonies with and without biofilm in order to determine if a quantitative relation also exists” (Giacaman et al., 2010, p.553).

Many methods have been developed to identify *Streptococcus mutans* and predict caries susceptibility. Earlier detection methods of *Streptococcus mutans* used several different media to grow colonies. These media were mitis-salivarius-bacitracin agar (MSB), trypticase yeast-extract-cysteine-sucrose-bacitracin agar (TYCSB), and glucose-sucrose-potassium tellurite-bacitracin (GSTB) agar (Dasanayake et al., 1995). With this in mind, the newest technology suggests an immunochromatography process that uses two monoclonal antibodies for rapid detection and accurate results. This test is currently available from GC America (Saliva-Check Mutans) and will deliver results in 15 minutes.

It is well noted that within the last 10-15 years there has been a dedicated interest to the concept of caries predictability. It has been well established that *Streptococcus mutans* is a transferable bacteria from mother to infant and from caregiver to infant (Caufield et al., 1993). However, the ability to develop an accurate test is still in progress. GC America has developed a rapid detection *Streptococcus mutans* saliva test utilizing monoclonal antibodies with immunoassay (GC America, 2007). This study determined the specificity of the GC America rapid detection test.

Research Design

A cross sectional quantitative research design was used in this study to determine the specificity of the GC America Rapid Detection Saliva *Streptococcus mutans* test. Giacaman et al. (2010) performed a cross-sectional study using 96 randomly selected patients ranging in age 15-17 years to determine whether biofilm formation by *S. mutans* was associated with high caries susceptibility. “Patients were randomly chosen for the study amongst those in treatment by the students” (Giacaman et al., 2010 p.551). Informed consent was requested and age and gender were collected. Patients were examined clinically. Bitewing radiographs and a DMFT were used to supplement the clinical examination. Stimulated saliva was collected using paraffin wax and seeded on TYSCB agar plates to culture MS counts. The collection of data allowed for anonymity and confidentiality. “Anonymity exists when there is no link between personal information and the research participant” (Cottrell & McKenzie, 2011, p.111). “Confidentiality exists when there is a link between personal information and the research participant’s identity but that information is protected from others” (Cottrell & McKenzie, 2011, p.111). Therefore, the research design of this study allowed for confidentiality. Confidentiality was achieved by assigning each participant a confidential number and storing all collected data on a password

protected laptop. Age and gender were collected from the patient's dental chart and the patient was assigned a confidential number. All data were stored on a password protected laptop that remained in the researcher's possession at all times. Using these techniques protects the privacy of the research participants (Cottrell & McKenzie, 2011). Demographic data was collected including age and gender to determine if differences among various population demographics exist. Other data collected included, two horizontal bitewing radiographs, a DMFT index, and the administration of the GC America Rapid Detection *Streptococcus mutans* Saliva test. The researcher was trained to administer the saliva test by a representative from GC America. The dentist used the DMFT index to chart the existing condition of the oral cavity of the patient. A Hu-Freidy 22 explorer was used to diagnose cavitation in the enamel. The radiographs were used to determine carious lesions that were evident in the enamel, dentin, and cementum. The results were recorded on a standard clinical dental charting form. All x-rays, charts and test results were only identified by the confidential patient number. The master list was kept in the researcher's possession on a password protected laptop. Prior approval from East Tennessee State University's (ETSU) Institutional Review Board (IRB) was obtained and data collection followed [Appendix A]. The ETSU IRB approval number is 0312.18s.

Population

The population for this study included patients in an east end Jefferson County, Louisville, Kentucky single practitioner dental office. The practitioner signed a consent form allowing the study to take place in his office using his patient population [Appendix B].

The practice currently has approximately 3,000 active patients and has been in existence for 35 years. During a single month, approximately 288 patients receive dental hygiene treatment and 125 patients receive restorative treatment. The demographics of the practice population

include patients ranging from pediatric to geriatric ages. The general dental practice concentrates on comprehensive dental care including both preventive and restorative procedures. A random sampling of patients above the age of 18 was used in this study.

Informed Consent Consideration

Each participant was presented with a letter detailing the purpose of the study, associated risks, and rewards upon arrival for their appointment [Appendix C]. Patients were escorted to a consultation room and allowed to review the information. Patients remained in the consultation room and notified the researcher after a decision had been made. In the event a patient could not read, someone read the information out loud to the patient. Patients were given an opportunity to ask questions and the researcher answered all questions prior to receiving final approval to participate in the study.

Data Collection Procedure

Patients in this single practitioner dental office are on a 1, 3, 4, and 6 month recall system to receive dental hygiene treatment from two hygienists on staff depending upon their needs. The patients' recall appointments are scheduled each time the patient receives dental hygiene treatment. Patients were selected by alternating every other appointment between the two hygienists. Two hundred patients were randomly selected from the dental hygiene schedule and were above the age of 18. The patients were randomly selected from recall appointments and new patients entering the practice. Minors under 18 and pregnant women were excluded from the study and not considered in the random sample. To reduce researcher bias, data were collected from the patient's record concerning age and gender. Each participant was presented with a letter detailing the purpose of the study, associated risks, and rewards. Patients were escorted to a private consultation room and given information to review the letter. The patients were allowed

the opportunity to ask questions about the study. After patients agreed to participate in the study, each patient was assigned a number for data collection to ensure confidentiality.

The population was identified and confidential descriptive data were collected using a confidential assigned number. A confidential questionnaire was given to all participants prior to test administration to ensure that GC America Rapid Detection *Streptococcus mutans* Saliva test could be administered. The questions ensured that the test results would not be jeopardized by eating, drinking, smoking, or brushing prior to the test [Appendix D].

The GC America Rapid Detection *Streptococcus mutans* Saliva test contains 10 kits. The kits contain one strep mutans test device, one piece of paraffin wax, one pipette, one mixing container, one 2 ml bottle of reagent #1, and one 4 ml bottle of reagent #2. Reagent bottle #1 contains distilled water, sodium hydroxide, and sodium azide. Reagent bottle #2 contains distilled water, organic acid, poloxyethylene octylphenyl ether, sodium azide, and bromothymol blue. The reagents are designed to remove impediments in the saliva to obtain accurate results. Saliva has a high viscosity and the *Streptococcus mutans* bacteria are covered by glucans. The glucans can inhibit the reaction to the antibody (GC America, 2007).

The *S. mutans* testing device contains a colloidal gold- labeled anti-*Streptococcus mutans* monoclonal antibody. The particles attach to the surface of the *Streptococcus mutans* bacteria. This causes a reaction with another anti-*Streptococcus mutans* antibody to form a red line in the window of the testing device. The colloidal gold- labeled anti-*Streptococcus mutans* monoclonal antibody that did not react with the *Streptococcus mutans* bacteria reacts with the anti-mouse immunoglobulin in the window to form a control red line (GC America, 2007).

Upon acceptance into the study, the patient chewed on the paraffin wax for 1 minute to stimulate the secretion of saliva. “Quantitative assessment of *mutan Streptococci* should be based on stimulated rather than unstimulated saliva samples” (Dasanayake et al., 1995, p.350). After 1 minute the patient expectorated the piece of paraffin wax into a tissue. The patient then expectorated stimulated saliva into the mixing container. The level of saliva reached the line indicated on the mixing container. Holding reagent bottle #1 vertically, one drop was added into the mixing container. The container was tapped with a finger 15 times over a 10-second period to ensure proper mixing of the reagent. Holding reagent bottle #2 vertically, four drops were applied to the saliva and shaken for several seconds to ensure mixing. The saliva sample demonstrated a light green color. Using the pipette, a saliva sample was drawn to the line indicated on the pipette. The saliva sample was then dispensed into the sample window at the end of the testing device. The testing device was allowed to sit in a secure location in the dental hygiene operatory at room temperature for 15 minutes. A thick red line appeared in the control window to indicate that the testing device was working accurately. In the event the test was positive, a thin red line was evident in the test window. In the event the test was negative, no line would appear. A positive reaction to the test indicated a high *Streptococcus mutans* count of ($>5 \times 10^5$ cfu/ml saliva).

Research Question

1. Does the presence of *Streptococcus mutans* relate to caries susceptibility as indicated by the GC America Rapid Detection *Streptococcus mutans* saliva test using the 500,000 cfu/ml biomarker?

Data Analysis Procedure

Specificity is an operating characteristics that indicates the accuracy of a diagnostic procedure. In other words it is the ability to correctly identify individuals without the disease process in question. Looking at the specificity of the *Streptococcus mutans* test, we recognize that “specificity of a diagnostic procedure is the percentage of disease-free individuals who are diagnosed correctly” (Pretty & Maupomé, 2004, p.254). Pretty and Maupomé (2004) stated that “a typical diagnostic situation allows for two outcomes: either the person has or does not have the disease” (p.253). However, when the test is compared to a gold standard there are four outcomes. The gold standard with this study is two horizontal bitewing radiographs and a DMFT index. There are four possible outcomes:

1. True positive (TP), the results indicate that the individual has the disease, and this is confirmed by the gold standard.
2. False positive (FP), the results indicate that the individual has the disease, but the gold standard indicates that the disease is not present.
3. False negative (FN), the results indicate that the person does not have the disease, but the gold standard indicates that the disease is present.
4. True negative (TN), the results indicate that the individual does not have the disease; the results are confirmed by the gold standard (Pretty & Maupomé, 2004).

Pretty and Maupomé (2004) stated that “reliability is equivalent to repeatability or reproducibility” (p.251). In other words, “a reliable procedure is one that is consistent, stable and dependable” (Pretty & Maupomé, 2004, p.252). Moreover, reliability is associated with the precision of the procedure. Inter-rater reliability was used in this study. Inter-rater reliability is the measure that tells how much the two raters agree on their judgment of the outcome (Salkind,

2011). Reliability can range from 0 to +1. It cannot be negative. The two raters for this study were the researcher and the dentist in the Louisville, Kentucky dental practice.

Concerning validity, Pretty and Maupomé (2004) stated that “the validity of a diagnostic procedure is the extent to which it measures what it claims to measure” (p.253). Moreover, validity is associated with accuracy. However, Pretty and Maupomé (2004) remind us that “ideally a diagnostic procedure should be both accurate and valid” (p.253). It is important to note that “a procedure can be accurate without being valid, but it cannot be valid if it is inaccurate” (Pretty & Maupomé, 2004, p.253). Concurrent criterion validity was used in this study, indicating that it is dealing with the data present at this period of time.

The variables included inferential data of age and gender. The independent variable was *Streptococcus mutans* and the dependent variables were DMFT index and two horizontal bitewing radiographs. Looking at the results of the test, a chi square test was performed to determine each of the outcome variables. The level of significance represented was $p < .05$. Specificity was determined using the test results.

Data collected were analyzed using the statistical package for the social sciences (SPSS) version 18.

Hypothesis

The hypothesis is that a relationship will exist between the two variables.

Null Hypothesis

The null hypothesis is that no relationship exists between the two variables.

Background of the Researcher

The researcher holds a Bachelor of Science degree in Dental Hygiene from East Tennessee State University (ETSU) in Johnson City, Tennessee. She has worked in the field of dental hygiene for 20 years. After 17 years working in dental hygiene, she returned to ETSU and is currently pursuing a Master of Science degree in Allied Health with concentrations in education and administration. During her career she worked in direct patient care, education in the classroom, and as a sales and marketing representative and volunteered with many missions concerning direct patient care and education. She also owns her own dental consulting company.

The researcher believes that education is the key to increasing quality of life and decreasing oral health disparities. She also believes that the findings in this study can contribute and encourage dental health care professionals to improve the quality of life for many people and improve the knowledge of caries susceptibility through education and other adjunctive therapies.

CHAPTER 4

DATA ANALYSIS

The purpose of this study was to determine the specificity of the chair side GC America Rapid Detection *Streptococcus mutans* saliva test in a middle-high socioeconomic single practitioner dental office in Louisville, Kentucky. An adult (18 year old and above) population group was targeted using random selection. By determining the specificity of *Streptococcus mutans* test, the results could determine if the test is useful as a chair side tool. The results would be advantageous to the dental community concerning patient education and decreasing dental caries.

Depending upon their needs, patients in this single practitioner dental office were on a 1, 3, 4, and 6 month recall system to receive dental hygiene treatment from two hygienists on staff. The patients' recall appointments were scheduled each time the patient received dental hygiene treatment. Patients were selected by alternating every other appointment between the two hygienists. This sampling technique yielded 200 adult patients randomly selected from the dental hygiene schedule. The patients were randomly selected from recall appointments and new patients entering the practice. Minors under 18 and pregnant women were excluded from the study.

Participants

The demographic variables were age and gender, and study's dependent variable was the test result of the GC America *Streptococcus mutans* saliva test. Of the 200 participants in the study, women comprised the majority of the participants with 58% participation.

When examining the age of the participants, the ages were slightly skewed to the left with 54.91 the mean, 57.00 the median, and 63 the mode. This indicated that the participants in this

study was comprised more of patients in the caregiver age than actual patients of childbearing age. There was 100% acceptance in the study. (No patients declined to participate or failed the questionnaire allowing them to participate in the study). The data indicated that 70% of the patients were diagnosed as true negative, 26.5% were false negative, 3% were true positive, and .5% were false positive. In looking at the positive and negative results of the test only, it was revealed that 96.5% were negative for *Streptococcus mutans* and 3.5% were positive for *Streptococcus mutans*.

Results

The research question stated: Does the presence of *Streptococcus mutans* relate to caries susceptibility as indicated by the GC America Rapid Detection *Streptococcus mutans* saliva test using 500,000 cfu/ml biomarker? In addition, the research hypothesis stated that there is a relationship between the variables. The null hypothesis stated that there is no relationship between the variables.

Chi Square

Two hundred patients were randomly sampled and evaluated as to whether the category of the result (true negative, false negative, true positive, and false positive) ($f = 200$) was an indicator of the DMFT ($f = 200$) and x-rays ($f = 200$). Data were analyzed using the Pearson chi square tests. While the null hypothesis would be rejected using this statistical test, the number of cells with values less than 5 (4 of 8 or 50%) renders the test inconclusive.

Tables 1-4 demonstrate the results for the Pearson chi square tests.

Table 1 *Category of Result DMFT Result*

Crosstab

			DMFT Result		
			Positive	Negative	Total
Category of Result	True Negative	Count	0	140	140
		% of Total	.0%	70.0%	70.0%
	False Negative	Count	53	0	53
		% of Total	26.5%	.0%	26.5%
	True Positive	Count	6	0	6
		% of Total	3.0%	.0%	3.0%
	False Positive	Count	0	1	1
		% of Total	.0%	.5%	.5%
Total		Count	59	141	200
		% of Total	29.5%	70.5%	100.0%

Table 2

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	200.000 ^a	3	.000
Likelihood Ratio	242.627	3	.000
Linear-by-Linear Association	153.662	1	.000
N of Valid Cases	200		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is .30.

Table 3 *Category of Result X-Ray Results*

Crosstab

			X-ray Results		
			Positive	Negative	Total
Category of Result	True Negative	Count	0	140	140
		% of Total	.0%	70.0%	70.0%
	False Negative	Count	53	0	53
		% of Total	26.5%	.0%	26.5%
	True Positive	Count	6	0	6
		% of Total	3.0%	.0%	3.0%
	False Positive	Count	0	1	1
		% of Total	.0%	.5%	.5%
Total		Count	59	141	200
		% of Total	29.5%	70.5%	100.0%

Table 4

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	200.000 ^a	3	.000
Likelihood Ratio	242.627	3	.000
Linear-by-Linear Association	153.662	1	.000
N of Valid Cases	200		

a. 4 cells (50.0%) have expected count less than 5. The minimum expected count is .30.

Specificity

Concerning the category results for the GC America Rapid Detection *Streptococcus Mutans* saliva test, it was revealed that the test has high specificity. The formula for specificity is

$Sp = TN / (TN + FP)$. In other words, specificity is equal to the number of true negatives divided by the sum of true negatives plus false positives. The test result of 1.00 demonstrated that the test did identify the proportion of patients without caries who were correctly identified by the test.

$$1.00 = 140 / (140 + 1)$$

Discussion

The *Streptococcus mutans* study was conducted from April 2012 to May 2012. The dental hygiene patients in a middle-high socioeconomic single practitioner dental office in Louisville, Kentucky were the target population. Results of the GC America Rapid Detection *Streptococcus mutans* saliva test was used as well as results from radiographs and the DMFT. Age and gender were also collected as descriptive data for the population group.

The random sampling consisted of 116 female patients and 84 male patients respectively. There was 100% acceptance into the study. The median age of the patients was 57 indicating that patients were more in the caregiving roles of children rather than the childbearing years. However, regardless of age, the GC America Rapid Detection *Streptococcus mutans* test can be a useful tool in detecting caries susceptibility, increasing education, and decreasing dental caries.

The chi square tests were inconclusive, but the results of the cross tabulation indicated that 26.5% of the time there was a false positive result (i.e. the *Streptococcus mutans* test was positive and the DMFT index or radiographs were negative).

Examining specificity revealed that high specificity existed with this chair side test. Therefore, it was determined that the portion of patients without caries was correctly identified. In summary, the test would be a useful tool to the dental professional and the patient.

CHAPTER 5

CONCLUSIONS, DISCUSSION, AND RECOMMENDATIONS

Dental caries is a concern of every person regardless of race, age, gender, or socioeconomic level. The ability to determine a person's susceptibility to dental caries is a prominent area of research to identify both cause and effect. It is estimated that more than 51 million school hours are lost each year to dental-related illness (United States Department of Health and Human Services, 2000). In addition, The Academy of Pediatrics (2003) reported that human dental flora is site specific, and an infant is not colonized with normal dental flora until the eruption of the primary dentition. It has been well documented that mothers who have a high caries risk can pass the cariogenic bacteria *Streptococcus mutans* to their infants (American Academy of Pediatrics, 2003). In other words, this automatically predisposes the infant to a high caries risk (American Academy of Pediatrics, 2003). On the other hand, the U.S. Surgeon General's Report (2000) revealed that employed adults lose approximately 164 million hours of work each year due to dental disease. It is apparent that the ability to assess caries susceptibility is advantageous for all populations and groups.

“The oral cavity is home to many different types of bacteria. However, *Streptococcus mutans* is considered to be a major human cariogen” (Dasanayake et al., 1995, p.345). Therefore, having an increase in the number of *Streptococcus mutans* in the oral cavity is “considered as a risk factor for the onset of caries” (Giacaman et al., 2010, p.550). It has been well established that *S. mutans* is a transferable bacteria from mother to infant and from caregiver to infant (Caufield et al., 1993).

Many methods have been developed to identify *Streptococcus mutans* and predict caries susceptibility. Earlier detection methods of *Streptococcus mutans* used several different media to grow colonies. With this in mind, the newest technology is an immunochromatography process

that uses two monoclonal antibodies for rapid detection and accurate results. This test is currently available from GC America (Saliva-Check Mutans) and will deliver results in 15 minutes.

Saliva has been studied for decades. However, the physiological importance of saliva has only come into focus recently. Schipper et al., (2007) stated that the “interest in saliva even more increased with the finding that saliva was filled with hundreds of components that may serve to detect systemic disease or evidence of exposure to various harmful substances, as well as provide biomarkers of health and disease status” (p.1115). In addition, “many salivary proteins offer great potential in clinical and epidemiological research, in oral as well as in general health studies” (Chiappin et al., 2007, p.38).

“The DMFT index was created to express the caries experience” (Becker et al., 2007, p.677). The study performed by Becker et al. (2007) compared the DMFT index as a diagnostic tool on its own and the DMFT as a diagnostic tool with radiographs. It is evident that radiography (bitewing) can enhance caries detection by showing information of the clinical stages of the caries on the approximal surfaces and the advanced stages on occlusal surfaces (Wenzel, 2004).

Specificity is an operating characteristic that indicates the accuracy of a diagnostic procedure. For example, the ability to correctly identify individuals with and individuals without the disease process in question. In addition, the “specificity of a diagnostic procedure is the percentage of disease-free individuals who are diagnosed correctly” (Pretty & Maupomé, 2004, p.254). Moreover Pretty and Maupomé (2004) wrote, that “a typical diagnostic situation allows for two outcomes: either the person has or does not have the disease” (p.253). However, when

the test is compared to a gold standard there are four outcomes. The gold standard with this study is two horizontal bitewing radiographs and a DMFT index. There were four possible outcomes:

1. True positive (TP), the results indicate that the individual has the disease, and this is confirmed by the gold standard.
2. False positive (FP), the results indicate that the individual has the disease, but the gold standard indicates that the disease is not present.
3. False negative (FN), the results indicate that the person does not have the disease, but the gold standard indicates that the disease is present.
4. True negative (TN), the results indicate that the individual does not have the disease; the results are confirmed by the gold standard (Pretty & Maupomé, 2004).

It has been well documented that mothers who have a high caries risk can pass the cariogenic bacteria *Streptococcus mutans* to their infants (American Academy of Pediatrics, 2003). Assessing the caries risk of males and females in the caregiver or parental age range can be beneficial to future generations. By increasing knowledge about caries susceptibility this could lower the transmission rates and, in turn, decrease dental caries for adults and children.

The purpose of this study was to determine the specificity of the chair side GC America Rapid Detection *Streptococcus mutans* saliva test in a middle-high socioeconomic single practitioner dental office in Louisville, Kentucky. Two hundred patients in an 18 year old and above population group was targeted using random selection. By determining the specificity of *Streptococcus mutans*, the researcher could provide education to patients and decrease rates for dental caries.

Conclusion

The GC America Rapid Detection *Streptococcus mutans* test was administered to 200 patients in a single practitioner Louisville, Kentucky dental practice. Patients signed an informed consent. The patients were given a qualifying questionnaire and upon acceptance into the study had two bitewing x-rays and a DMFT index charted. Data were collected and stored in a password protected laptop. All information gathered (age, gender, and *Streptococcus mutans* test results) were analyzed using the statistical package for the social sciences (SPSS) version 18.

The demographic data were collected and the demographic variables included age and gender. Of the 200 participants in the study, women comprised the majority of the participants with 58% participation. Because we know that maternal transmission is possible, 58% was a good representation of women for this study. However when examining the age of the participants, it was revealed that the mean was 54.91. This is more representative of the caregiver age group rather than the childbearing age group. Duchin and van Houte (1978) concluded in their study that “although the formation evidence about the relative susceptibility of infants, children, and adults to infection by *S. mutans* is lacking, the evidence suggests that this organism will readily colonize newly exposed teeth of children and adults in the presence of suitable salivary levels” (p.124). Therefore, regardless of age, the GC America Rapid Detection *Streptococcus mutans* test can be a useful tool in detecting caries susceptibility.

The study’s dependent variable was the test results of the GC America Rapid Detection *Streptococcus mutans* test. These results were identified as true positive, false positive, false negative, and true negative. The data indicated that 70% of the patients were diagnosed as true negative, 26.5% were false negative, 3% were true positive, and .5% was false positive. In other words, seventy percent of the patient’s results indicated that they did not have the disease and

this was confirmed by the gold standard (DMFT and x-rays), 26.5% of the patient's results indicated that they did not have the disease, but the gold standard indicated that that the disease was present. In addition, the data revealed that 3% of the patient's results indicated that they had the disease and this was confirmed by the gold standard, and .5% of the patient's results indicated that they had the disease and the gold standard indicated that the disease was not present.

A chi square test was used to evaluate whether the category of the result (true negative, false negative, true positive, and false positive) was an indicator for the DMFT and the x-rays. While the null hypothesis was rejected using the chi square, the number of cells with values less than 5 (4 of 8 or 50%) rendered the results inconclusive. However, the frequencies and the percentage in each cell rendered extremely important information. It was concluded that the number of positive *Streptococcus mutans* tests without a corresponding DMFT or x-ray indicated that the test was working or had the potential to work exactly as the test was designed. It is important to note, the DMFT and the x-rays are retrospective indicators of dental caries and the *Streptococcus mutans* (in combination with specificity) is the prospective indicator. Therefore, without intervention the patient will eventually have a positive result with the DMFT and x-rays.

Discussion

Looking at the specificity of the *Streptococcus mutans* test, we recognize that “specificity of a diagnostic procedure is the percentage of disease-free individuals who are diagnosed correctly” (Pretty & Maupomé, 2004, p.254). The data collected concerning specificity showed that the test did identify the proportion of patients who were correctly identified by the test. The specificity test result was 1.00. This gives the test a high specificity. By this result alone, we have concluded that the test is an accurate test to use as a chair side tool. In addition, we have

concluded that the DMFT and x-rays are important tools as well in the diagnosis of dental caries. It is important to recognize that a false positive test result could eventually result with a positive DMFT and x-rays. With this in mind, the dental professional could educate the patient and start preventive measures to decrease the microbial load in the oral cavity. Although this test was performed on an 18 and above population, it can be a useful tool on patients of all ages in the quest to detect caries susceptibility.

Recommendations for Future Research

It was concluded through this study that a relationship existed between the GC America Rapid Detection *Streptococcus mutans* test, the DMFT, and the x-rays. In addition, the specificity concluded that the test is a useful tool in the dental profession. However, this study used patients in a middle-high socioeconomic bracket. Although we concluded that the test was a valuable tool, recommendations would include using this study to further the research on caries susceptibility.

Further research could answer the following questions:

1. How would this study's results compare with a study conducted on a lower socioeconomic level? (public health facility, free clinic)
2. How would this study's results compare with a study using patients within childbearing ages only?
3. Are the results of this test specific to the patient population that visits their dentists regularly?
4. What role does education and prevention play in the results of this test?
5. Does the dental professional see this test as an important chair side tool with detecting caries susceptibility?

By determining the high specificity of the GC America rapid detection *Streptococcus mutans* saliva test, the importance has been proven to the dental professional. “The accurate and objective assessment of caries risk state and /or caries activity with any of these similar technologies will permit targeted preventive and curative treatment, thereby significantly improving human dental health” (Shi et al., 1998, p.370). In conclusion more research could be warranted; however, the GC America Rapid Detection *Streptococcus mutans* saliva test along with the DMFT and x-rays are invaluable tools to the dental healthcare professional.

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APPENDICES

Appendix A

Approval Letter

IRB APPROVAL – Initial Expedited Review

April 24, 2012

Ms. Catherine Holtman

Re: Rapid Detection of Streptococcus mutans in Saliva

IRB#: 0312.18s

ORSPA #:

The following items were reviewed and approved by an expedited process:

- xform new protocol submission, CV, questionnaire for qualification, site permission letter, brochure for saliva check mutans, ICD (version 3/1/2012)

On April 23, 2012, a final approval was granted for a period not to exceed 12 months and will expire on April 22, 2013. The expedited approval of the study will be reported to the convened board on the next agenda.

The following **enclosed stamped, approved Informed Consent Documents** have been stamped with the approval and expiration date and these documents must be copied and provided to each participant prior to participant enrollment:

- Informed Consent Document ()

Federal regulations require that a copy is given to the subject at the time of consent.

Unanticipated Problems Involving Risks to Subjects or Others must be reported to the IRB (and VA R&D if applicable) within 10 working days.

Proposed changes in approved research cannot be initiated without IRB review and approval. The only exception to this rule is that a change can be made prior to IRB approval when necessary to eliminate apparent immediate hazards to the research subjects [21 CFR 56.108 (a)(4)]. In such a case, the IRB must be promptly informed of the change following its implementation (within 10 working days) on Form 109 (www.etsu.edu/irb). The IRB will review the change to determine that it is consistent with ensuring the subject's continued welfare.

Sincerely,
George Youngberg, M.D., Chair
ETSU/VA Medical IRB

cc: Ester Verhovsek

Appendix B
Facility Consent

John B. Holtman, D.M.D.

COMPREHENSIVE GENERAL DENTISTRY

3932 Dutchman's Lane Louisville, Ky 40207
(502) 895-0707 Fax (502) 895-1225

3/16/12

I HEREBY GIVE PERMISSION FOR CATHERINE E. HOLTMAN
TO CONDUCT HER GRADUATE RESEARCH STUDY TITLED "RAPID DETECTION
OF STREPTOCOCCUS MUTANS IN SALIVA" IN MY DENTAL OFFICE.

John B. Holtman DMD

Appendix C

Informed Consent for Participant

Principal Investigator: Catherine E. Holtman RDH, BS

Title of Project: Rapid Detection of *Streptococcus mutans* in Saliva

Informed Consent for Participant

Introduction: Dear Potential Volunteer: You are invited to participate in a research study determining the accuracy of the GC America rapid detection *Streptococcus mutans* saliva test. As proven by previous studies, *Streptococcus mutans* is the main bacterium that contributes to tooth decay. With the advancement of dental technology, the GC America rapid detection *Streptococcus mutans* saliva test may be helpful chair side determining the possibility of developing tooth decay. This study has been approved by the Institutional Review Board at East Tennessee State University in Johnson City, Tennessee. It is important that you read this material carefully and then decide if you wish to volunteer.

The purpose of this study is to determine if the GC America rapid detection *Streptococcus mutans* saliva test will provide convincing results to help in predicting tooth decay in the dental office.

Duration of this study will be during your scheduled dental appointment.

Research tests or procedures for this study: If you consent to participate in this research study, the results from your routine bitewing radiographs and your dental charting examination will be collected. In addition you will be asked to submit a saliva sample by chewing on a piece of paraffin wax for one minute and spitting a saliva sample in a container. This will be performed during your dental appointment and will not require you to return for further data collection. There will be no extra expense to you as a participant. There will be no restrictions on your normal activity.

Alternate Procedures/Treatments available to you if you elect to not participate in this study will be to have a regular cleaning, radiographs, and dental charting examination during your dental appointment. No risks are identified with this alternate treatment.

Risks and discomforts to you if you take part in this study: Your participation in this study will put you at no greater risks than the regular dental appointment you would encounter as a dental patient. However, unforeseeable risks are possible. The GC America Rapid Detection *Streptococcus mutans* testing device is not packaged in a child resistant container and should be kept out of the reach of children.

Principal Investigator: Catherine E. Holtman RDH, BS

Title of Project: Rapid Detection of *Streptococcus mutans* in Saliva

The benefits to you of taking part in this study: There are no direct benefits to you from participating in this study. The results of this study may give us knowledge to help predict tooth decay risks chair side in the dental office. In addition, it could give us knowledge to become more personalized with our homecare instructions for each patient.

Compensation for Medical Treatment: East Tennessee State University (ETSU) will pay the cost of emergency first aid for any injury that may happen as a result of your being in this study. ETSU makes no commitment to pay for any other medical treatment. Claims against ETSU or any of its agents or employees may be submitted to the Tennessee Claims Commission. These claims will be settled to the extent allowable as provided under TC Section 9-8-307. For more information about claims call the Chairman of the Intuitional Review Board at ETSU at 423-439-6055.

If taking part in this research injures you, you will be given emergency treatment. You may or may not be responsible to pay for this emergency treatment. There is no promise to pay for such emergency treatment by anyone. In the end, the decision as to who shall pay for your emergency treatment will depend on facts, the reason for the injury and state law.

Financial Costs to you as a participant in this research study are:

1. Regular expense for dental cleaning
2. Regular expense for clinical exam by the dentist
3. Regular expense for radiographs

Specific things you should understand about confidentiality: All information gathered for this research study, including the results of the GC America rapid detection *Streptococcus mutans* saliva test, will be kept confidential with regard to individual identity, and only group data will be reported. A random identification number will be assigned to you at the beginning of the study and only that number will be used on data collection forms. All data will be kept in a secure location and destroyed upon completion of the study. If you have any questions or concerns regarding your rights as a research participant, the following person may be contacted: Dr. Verhovsek, Research Advisory Committee Chair, East Tennessee State University, Johnson City, Tennessee, 37643, (423)547-0235.

Participation in this research experiment is voluntary. You may refuse to participate. You can quit at any time. If you quit or refuse to participate, the benefits or treatments to which you are otherwise entitled will not be affected. You may quit by calling Catherine Holtman, whose phone number is 502-523-0998.

Principal Investigator: Catherine E. Holtman RDH, BS

Title of Project: Rapid Detection of *Streptococcus mutans* in Saliva

Contact for Questions: If you have any questions, problems or research-related medical problems at any time, you may call Catherine Holtman at 502-523-0998, or Dr. Ester Verhovsek at 423-547-0235. You may call the Chairman of the Institutional Review Board at 423-439-6024 for any questions you may have about your rights as a research subject. If you have any questions or concerns about the research and want to talk to someone independent of the research team or you can't reach the study staff, you may call the IRB coordinator at 423-439-6055 or 423-439-6002.

Specific things you should understand about confidentiality: Every attempt will be made to see that your study results are kept confidential. A copy of the records from this study will be stored on a password protected laptop in the researcher's possession for 5 years after the end of this research. The results of this study may be published and/or presented at meetings without naming you as a subject. Although your rights and privacy will be maintained, the Secretary of the Department of Health and Human Services, the ETSU/VA IRB, and personnel particular to this research have access to the study records. Your dental records will be kept confidential according to legal requirements. They will not be revealed unless required by law, or as noted above.

I authorize the use of my bodily fluids, substance or tissues for research purposes.

The privacy law, Health Insurance Portability & Accountability Act (HIPPA), protects my individually identifiable health information (protected health information). The privacy law requires me to sign an authorization in order for researchers to be able to use or disclose my protected health information for research purposes in the study entitled Rapid Detection of *Streptococcus mutans* in saliva.

I authorize Catherine E. Holtman, RDH, BS and her research staff to use and disclose my protected health information for the purposes described below. I also permit my doctors and other health care providers to disclose my protected health information for the purposes described below.

My protected health information that may be used and disclosed includes:

1. Age
2. Gender
3. Decayed, Missing, and Filled Teeth (clinical examination results)
4. Radiograph examination results
5. Results from the GC America Rapid Detection *Streptococcus mutans* saliva test.

Principal Investigator: Catherine E. Holtman RDH, BS

Title of Project: Rapid Detection of *Streptococcus mutans* in Saliva

The investigator Catherine E. Holtman RDH, BS may use and share my health information with:

1. The East Tennessee State University Human Research Protections Program (HRPP) Institutional Review Board Administration when the researcher or the research site is undergoing Quality Improvement Program (QIP) auditing.
2. The James H. Quillen Veterans Affairs Medical Center Office of Research & Development when the researcher or the research site is undergoing Quality Improvement Program (QIP) auditing.
3. Government representatives, when required by law
4. Hospital (VAMC or Mountain States Health Alliance) representatives
5. Dupont Dental Office and GC America

Once my health information has been disclosed to anyone outside the study, the information may no longer be protected under this authorization. Catherine E. Holman RDH, BS and Dupont Dental Office agree to protect my health information by using and disclosing it only as permitted by me in this authorization and as directed by state and federal law.

I do not have to sign this authorization. If I decide not to sign the authorization:

1. It will not affect my treatment, payment or enrollment in any health plans or affect my eligibility for benefits.
2. I cannot be allowed to participate in this research study

After signing the authorization, I can change my mind and:

1. Not let the researcher disclose or use my protected health information (revoke the authorization)
2. If I revoke the authorization, I will send a written letter to Catherine E. Holtman RDH, BS, 3932 Dutchman's Lane, Louisville, Kentucky 40207 to inform her of my decision.

Principal Investigator: Catherine E. Holtman RDH, BS

Title of Project: Rapid Detection of *Streptococcus mutans* in Saliva

3. If I revoke my authorization, researchers may only use and disclose the protected health information **already collected** for this research study.
4. If I revoke this authorization my protected health information may still be used and disclosed should I have an adverse event (a bad effect, or experience something unanticipated).
5. If I change my mind and withdraw the authorization, I may not be allowed to continue to participate in the study.
6. It has been explained to me that I will not be allowed to review the information collected for the research until after the study is completed. When the study is over, I will have the right to access the information again.

This authorization does not have an expiration date.

If I have not already received a copy of the privacy notice, I may request one by contacting the Privacy Officer. If I have any questions or concerns about my privacy rights, I should contact the East Tennessee State University, James H. Quillen College of Medicine Privacy Officer, Paula Wright, at 423-433-6074.

I am the subject or am authorized to act on behalf of the subject. I have read this information, and I will receive a copy of this form after it is signed.

By signing below, you confirm that you have read or had this document read to you. You will be given a signed copy of this informed consent document. You have been given the chance to ask questions and to discuss your participation with the investigator. You freely and voluntarily choose to be in this research project.

In addition, by signing below, you are authorizing the use and disclosure of your protected health information for research purposes as described above.

Principal Investigator: Catherine E. Holtman RDH, BS

Title of Project: Rapid Detection of *Streptococcus mutans* in Saliva

_____ Date _____
Participant's Printed Name

_____ Date _____
Participant's Signature

_____ Date _____
Signature of Investigator

_____ Date _____
Signature of Witness

(ETSU, 2011)

Appendix D

Questionnaire for Qualification

Random Patient Number _____

Prior to this diagnostic test, it is important that you have not smoked, eaten, or had any form of liquid. Please verify the following information.

1. Have you smoked within one hour of this appointment Yes No

2. Have you eaten within one hour of this appointment Yes No

3. Have you consumed any liquid within one hour of this appointment Yes No

4. Did you brush your teeth within the last hour Yes No

5. Did you use mouthwash within one hour of this appointment Yes No

_____ Date _____
Signature of the Researcher

VITA

CATHERINE E. HOLTMAN

Personal Data:

Date of Birth: March 4, 1964

Place of Birth: Richmond, Virginia

Marital Status: Married

Education:

Public Schools, Summerville, South Carolina

Associate of Applied Science, East Tennessee State University,
Johnson City, Tennessee 1991

Bachelor of Science in Dental Hygiene, East Tennessee State
University, Johnson City, Tennessee 2010

Master of Science in Allied Health, East Tennessee State
University, Johnson City, Tennessee 2012

Professional Experience:

Hygienist 1991-2012

Teacher, East Tennessee State University; Johnson City,
Tennessee, 1996

Sales Representative, Professional Dental Technologies,
Batesville, Arkansas, 1996 – 2001

CEO, Dental Systems Consulting Group, Louisville,
Kentucky, 2001 – present

Honors and Awards:

Dental Hygiene Student Merit Award for Outstanding
Achievement in Community Health Dentistry