

THE EFFECT OF THE ISOLITE™ SUCTION ON AEROSOLS AND SPLATTER DURING  
ULTRASONIC SCALING

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## ABSTRACT

Jessica Laurin Holloman: The Effect of the Isolite™ Suction on Aerosols and Splatter During Ultrasonic Scaling  
(Under the direction of Sally Mauriello)

This study compared the Isolite™ and saliva ejector on aerosol reduction during ultrasonic scaling. Fifty participants were randomly assigned to control (n=25, saliva ejector) or test groups (n=25, Isolite™). Plaque extent scores were recorded and aerosols were collected both during (timed period) and post (35 minute period) ultrasonic scaling in buffer in Petri dishes placed six inches from the participant's mouth. Participants were surveyed regarding device acceptance. The during and post suspensions were plated to blood agar plates and recoverable colonies (CFU) were counted following anaerobic incubation. Significant contamination occurred during ultrasonic scaling in both device groups, as indicated by high CFU and the identification of strict oral anaerobes on all plates. A Student t-test revealed that the test device did not reduce aerosol contamination compared to the control device ( $p=0.25$ ). Additional caution should be exercised with these devices to reduce the risk of exposure to potential pathogens.

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## **ABBREVIATIONS**

ADA	American Dental Association
CDC	Centers for Disease Control
CFU	Colony Forming Units
CRI	Cotton Roll Isolation
DPBS	Dulbecco's Phosphate Buffered Saline
DUWL	Dental Unit Waterline
HIPPA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HVE	High-Volume Evacuator
IRB	Institutional Review Board
µm	micrometer
OSHA	Occupational Safety and Health Administration
SARS	Severe Acute Respiratory Syndrome
TB	Tuberculosis

## **CHAPTER 1: INTRODUCTION**

According to the Centers for Disease Control and Prevention (CDC), almost all new infections in the United States are contracted through the aerosol route from infected patients who are coughing and dispersing infective droplet nuclei into the air.<sup>1</sup> Therefore, the presence and dispersal of aerosols and splatter are a concern in healthcare due to their potential adverse health effects on patients and healthcare workers. The effect of these aerosols on immunocompromised patients is concerning due to their increased susceptibility to the potentially infectious bacteria that are present in these aerosols. Many routine dental procedures produce aerosols and splatter, which may contain infectious material such as blood, saliva, and other organic matter.<sup>2</sup> Published data showed that ultrasonic scalers, as well as other types of dental hand pieces, had a significant effect on the number of colony-forming units (CFU) cultivable from the air when compared to preprocedural levels.<sup>3, 4</sup> The ultrasonic scaler, in particular, has been shown to produce three times the bacterial aerosol contamination as that produced by dental operative equipment.<sup>1, 5</sup> Based on supportive data, the American Dental Association (ADA) has recommended that the high-volume evacuator (HVE) be employed during the use of ultrasonic scalers to minimize the spread of airborne bacterial contamination during ultrasonic scaling.<sup>6, 7</sup> This recommendation creates a unique challenge for dental health care workers, specifically dental hygienists, that traditionally provide clinical care without a dental assistant. Due to the nature of most suction devices, dental hygienists must sacrifice their non-dominant hand, as well as light and indirect vision, while using the ultrasonic scaler.

Further, the HVE can be bulky and difficult to maneuver when an assistant is not available, making the saliva ejector the device of choice to remove excess fluids from the oral cavity. The Isolite™ dryfield illuminator, a product created with “hands-free” dentistry in mind, was introduced to the commercial market in 2005.<sup>8</sup> The primary purpose of this system is to provide isolation and illumination to the oral cavity, but has been purported to have an added benefit of aerosol reduction by as much as 65% when compared to the saliva ejector in a simulated clinical environment.<sup>8,9</sup> There is currently no published data on how the Isolite™ compares to the saliva ejector when used with an ultrasonic scaler in an actual clinical environment. Further, there is no published literature regarding patient acceptance of the Isolite™ in an adult United States population.

After reviewing the literature and developing the protocol for the current study it seemed prudent to attempt to identify an alternative methodology for the collection and quantification of aerosols and splatter. The methods available often included the use of equipment that was either expensive or not readily available and these methods typically did not include both the quantification of aerosols and splatter and identification of specific bacteria collected.

Therefore, the purpose of this study was two-fold:

- (1) Compare the effect of the Isolite™ suction and saliva ejector on aerosols and splatter during ultrasonic scaling, and
- (2) Determine patient acceptance of the Isolite™ in an adult U.S. population.

Additional specific objectives included:

- (3) Develop an alternative methodology to collect and quantify aerosols and splatter, and
- (4) Confirm that recovered bacteria originated from an oral source.

## **CHAPTER 2: A REVIEW OF THE LITERATURE**

### **Introduction**

Since the 1960's, the detrimental effects of aerosols and splatter and their role in the spread of infectious diseases have been extensively researched and documented. The role of aerosols and splatter in the spread of infectious diseases have been linked to outbreaks of influenza, chickenpox, tuberculosis (TB), legionnaire's disease, severe acute respiratory syndrome (SARS), etc.<sup>1,5,6</sup> It is known that the transmission of these diseases occurs via the airborne route from human sources through the inhalation of droplet nuclei that are aerosolized by respiratory secretions such as coughing, sneezing, or talking.<sup>10</sup> Aspiration of pathogenic bacteria from the oral cavity has also been linked to certain systemic infections.<sup>11,12</sup> Pneumonia, an infection of the lungs, has been extensively studied and is most common in an immunocompromised population, such as the elderly.<sup>13</sup>

In order to address the control of potentially infectious aerosols it was necessary to first examine their characteristics and capabilities. Assessment of aerosols and splatter included factors such as generation, particle size and concentration, infectivity and virulence, viability, air flow, environmental sampling, and analysis.<sup>10</sup> These factors have been studied and reported by a range of experts, including those who specialize in aerobiology to dentists who were interested in reducing the presence of aerosols and splatter in the dental office. The spread of aerosols and splatter were first appraised by exploring their history in healthcare and dentistry and their impact on healthcare workers.

This was followed by identifying those procedures known to produce the greatest amounts of aerosols and splatter, which has proven key in the process of sampling and studying the microorganisms that are linked to the spread of infectious diseases. Finally, the mechanisms to control the spread of aerosols and splatter are the final piece to preventing their dissemination in the dental office.

### **Defining Aerosols and Splatter**

Although the Occupational Safety and Health Administration (OSHA) does not explicitly define aerosols according to size, the consensus among the literature states that aerosols can be liquid or solid particles that are approximately 50 $\mu$ m or less in diameter, are suspended in the air, and are capable of penetrating deep into the respiratory system.<sup>3,14,15,17</sup> Previous aerobiology studies have shown that aerosols tend to stay airborne for an extended period of time before they settle on surfaces or enter the respiratory tract.<sup>3,10,17,18</sup> It is the smaller particles of an aerosol (.5 $\mu$ m -10 $\mu$ m in diameter) that are of particular concern because they are thought to carry the greatest potential for transmitting infections due to their ability to penetrate and lodge into the smaller passages of the lungs.<sup>5</sup>

Splatter particles differ from aerosols because they are visible to the naked eye and are considered too large to be inhaled and imposed deep within the lung.<sup>3,16</sup> Splatter particles are known to behave in a ballistic manner, in that they follow an arc trajectory from the oral cavity until they contact a surface.<sup>15</sup> It is because of this trajectory that splatter particles do not remain suspended in the air for long periods of time, making them less likely to transmit disease via the airborne route.

Another important consideration when discussing aerosols and splatter is the viability of the microorganisms within the environment once they have left the host. The viability of the

microorganism is essentially the ability of the microorganism to reproduce. When microorganisms leave their host and are aerosolized, they are potentially injured during the generation process.<sup>10</sup> Previous data have shown that microorganisms could remain viable in the airborne state for long enough to permit their wide dissemination.<sup>19</sup> Factors such as temperature, relative humidity, air flow, and oxygen sensitivity will impact whether or not microorganisms are able to survive outside of the host and replicate.<sup>19</sup> Engineering controls should be in place within healthcare settings to eliminate these microorganisms and limit the exposure of the staff and public to their presence. It is necessary to understand the factors related to aerosol and splatter generation and survival when considering disease transmission. This knowledge is crucial to the task of implementing protocol for the elimination of aerosols and splatter, therefore preventing disease transmission.

## **Aerosols and Healthcare**

With regard to healthcare workers, OSHA stated that the primary routes of infectious disease transmission in U.S. healthcare settings are through contact, droplet, and airborne particles.<sup>1,6</sup> Airborne diseases such as tuberculosis, influenza, measles, chickenpox, and Legionnaires' disease have been well documented as capable of remaining viable and airborne within the indoor environment.<sup>20,21-24</sup> The ability of aerosols to remain suspended in the air and disperse over considerable distances increases the likelihood of cross-contamination.<sup>25</sup> Because of these characteristics, it is not surprising that healthcare workers and patients are at an increased risk of infection.

In cases where measles and tuberculosis have been spread in an indoor environment, airflow studies revealed that droplet nuclei were generated throughout the entire office.<sup>24, 26</sup> Legionnaire's disease, a severe form of pneumonia, is also spread via the aerosol route. This

disease is estimated to infect 10,000 to 15,000 persons per year in the United States, where 9% of cases occur at least six months before or after a hospital outbreak.<sup>23</sup> This disease has become especially concerning in dentistry due to its link to dental unit waterlines (DUWL).<sup>4,19,23</sup> Another systemic infection that has been extensively studied is pneumonia. This infection is especially common in the elderly and can be acquired from cross-contamination or self ingestion.<sup>13</sup> Aspiration pneumonia, a type of pneumonia caused by the inhalation of a substance into the lung, is often associated with anaerobic bacteria.<sup>11</sup> This type of pneumonia is especially likely during dental procedures, which can yield high amounts of bacteria laden aerosols and splatter.

Based on this knowledge, the presence and dispersal of aerosols in indoor environments are a growing concern in epidemiology.<sup>25, 27</sup> Further, special patient populations, such as the immunocompromised patient, have been shown to be at a greater risk of infection than the average healthy patient.<sup>20</sup>

## **Aerosols and Dentistry**

In the dental clinic, aerosols are of particular concern due to the inherent nature of most dental procedures. Studies have demonstrated that many routine dental procedures that incorporate the use of water sprays or rotary instruments artificially generate aerosols that produce significantly greater numbers of bacteria than those activities produced by non-dental related oral activities, such as coughing and sneezing.<sup>17, 28</sup> In a review of the literature by Harrell et al., it was emphasized that saliva and nasopharyngeal secretions may contain pathogenic organisms such as herpes viruses, streptococci, staphylococci, and the SARS virus.<sup>85,29</sup> Further, bloodborne diseases such as Hepatitis and Human Immunodeficiency Virus (HIV) can be transmitted into the air via blood droplets.<sup>5,29</sup>

In addition to the transmission of infectious diseases, aerosols can be a source of irritants, allergens, and other toxic substances, which are a potential source of acute or chronic respiratory disease.<sup>17</sup> A survey of aerosol-related symptoms in dental hygienists who frequently use ultrasonic scalers revealed that symptoms such as nasal irritation, persistent cough, runny eyes, itchy and dry skin, were more common in dental hygienists than in nurses and hospital staff.<sup>30</sup> Dental staff must consider protecting themselves and their patients from common airborne diseases and infectious diseases that are not characteristically airborne and are being transmitted into the air during dental procedures. The inhalation of these substances may not be of concern to the average healthy patient, but special patient populations, such as the immunocompromised patient, can be especially susceptible to the adverse effects of these aerosols. Both the CDC and the ADA have recommended reducing the risk of infection posed by aerosols by the use of rubber dams, high-velocity air evacuation, and proper patient positioning, along with standard precautions.<sup>1</sup> Based on the evidence surrounding the generation of water spray by the ultrasonic scaler, one may infer a relationship between the production of infectious aerosols and treatment techniques. Thus, mechanisms to reduce aerosol spray should be considered during ultrasonic instrumentation.

### **The Ultrasonic Scaler**

Those dental procedures shown to create high amounts of aerosols and splatter are of particular concern in oral epidemiology. Hand scaling, for example, has been shown to create negligible amounts of aerosol and splatter.<sup>2</sup> Ultrasonic scalers and high-speed hand pieces have been studied extensively and were shown to produce measurable amounts of aerosols and splatter.<sup>2, 6,16,17,28,31,32,42</sup> Since the water spray emitted from the working tip of an ultrasonic scaling hand piece bears a strong physical resemblance to the spray of high-speed dental hand

piece, many studies have advised the same type of aerosol reduction device.<sup>33</sup> A dental hygienist commonly uses ultrasonic scalers during periodontal instrumentation and routine prophylaxis. These devices utilize high-frequency vibrations and water as a medium of ultrasonic energy to remove calculus deposits and have been labeled as one of the major sources of potential aerosol contamination in the dental setting due to the large amount of aerosols expelled into the air during their use.<sup>9, 33</sup>

### **Reduction of Aerosols and Splatter**

Abundant research is available regarding the presence and dispersal of aerosols and various devices have been evaluated regarding their effectiveness.<sup>2-5, 16,20,31,33-36</sup> Traditional methods that reduce potentially infectious aerosols during dental procedures include the low and high-volume evacuator, dental dam, pre-procedural rinses, and various air quality devices.<sup>2, 8,16,31,35-37</sup> A landmark study conducted by Micik et al. in 1969 was published regarding the reduction of aerosols during routine dental procedures and found that the HVE demonstrated the highest efficiency.<sup>15</sup> Since then, various studies have shown that when compared to no suction or the saliva ejector the HVE has proven to be the most effective at reducing aerosols created during dental procedures by as much as 90%.<sup>14,15,34-36</sup> The use of a rubber dam has also been shown to eliminate almost all contamination that arises from saliva or blood, but this type of device is not feasible for most periodontal and dental hygiene procedures.<sup>32,37,38</sup> During most dental procedures, it is the assistant who manipulates the HVE due to the manner in which it must be used to properly control aerosol and splatter. The HVE can be cumbersome and uncomfortable to the patient and clinician if not used correctly. These actualities make the HVE difficult to use as a single clinician, which is often the case during procedures rendered by a dental hygienist.

In 1996, Harrel et al. published an in vitro study, which primarily investigated the reduction of aerosols and splatter with the use of an HVE attachment compared to no suction during use of the ultrasonic scaler.<sup>34</sup> Recognizing the limitations of the HVE, a sheath was engineered to connect the HVE to the ultrasonic scaler. To assess aerosol reduction, a plastic enclosure with one centimeter square gridlines was assembled to enclose around a dentoform model that was mock scaled for one minute with an ultrasonic scaler. Instead of water, red erythrosine solution was used to represent contamination. Each square containing at least one erythrosine spot was considered contaminated and squares were counted twice following the exposure by an evaluator. The scaling procedure was repeated ten times by two operators, resulting in a total of twenty trials. Mean numbers of contaminated squares were calculated and results indicated gross differences based on the operator, making the findings variable based on practices of the clinician. Overall, the study found that the HVE attachment device greatly reduced detectable aerosol and splatter contamination by as much as 100% in a single trial. These results represent a greater than 93% reduction in the average amount of contamination produced by the ultrasonic scaler with the HVE attachment when compared to no suction device.<sup>34</sup> A potential limitation of this study is that it was a small sample size and was completed in vitro, making the results difficult to generalize and apply to a clinical environment.

As an extension of the Harrel et al. study, King et al. published in 1997 an in vivo study regarding the reduction of aerosols with an ultrasonic scaler utilizing the same type of engineered HVE attachment to the ultrasonic scaler.<sup>33</sup> Twelve subjects were enrolled and each subject served as his/her own control. Each subject was scaled with an identical ultrasonic unit, insert, power, and water setting. Three blood agar plates were placed at a 50° angle and 6-inches from the subject's mouth to collect aerosols. In separate closed-door rooms, ultrasonic instrumentation

was performed for five minutes on each side of the patient's mouth, one side with the HVE attachment and one side without. After being exposed for 25 minutes, the blood agar plates were covered and incubated at 37°C for three days prior to counting CFU.<sup>33</sup> Results were in concurrence with the study completed in 1996 by Harrel et al. and revealed that the use of a HVE attachment significantly reduced aerosols and splatter.<sup>33, 34</sup>

### **The Isolite™ dryfield illuminator**

The Isolite™ is a device designed to provide isolation, suction, illumination, and retraction simultaneously when used by a single operator. The bite-block component of the mouthpiece allows for isolation of the maxillary and mandibular quadrants simultaneously so that the patient can rest open during the entire dental procedure.<sup>8</sup> Due to the relatively new status of this product, little research has evaluated the company's reported benefits of decreased procedure time, increased retention rates for restorations and sealants, and reduction of aerosols in the operatory. Isolite™ Systems (Santa Barbara, CA) specifically purports to reduce airborne aerosols by up to 65% compared to the saliva ejector, which is of particular interest due to the role that aerosols play in the spread of infectious diseases.<sup>8, 35</sup> Because the Isolite™ is designed to attach to the high-volume suction hose and previous research has shown that the ultrasonic scaler produces the greatest amount of aerosols, it would be prudent to determine how effectively the Isolite™ reduces aerosols and splatter while performing ultrasonic scaling in an actual clinical environment.

An unpublished study by Jacks and Pollard in 2007, compared the Isolite™ to the HVE alone and saliva ejector in an independent laboratory trial and measured the amount of aerosol particles that reached the breathing space of the clinician.<sup>35</sup> A total of 21 trials were conducted and involved mock scaling for two minutes with an ultrasonic scaler. Scaling was performed on

all surfaces of all teeth on a DENTOFORM model. The HVE, saliva ejector, and Isolite™ were compared and each trial was divided into two minute sampling periods: pre-exposure, exposure, and post exposure. Aerosols were measured with the DataRAM Real-Time Aerosol Monitor every 10 seconds during all phases. Each group was statistically different when compared. The Isolite™ was shown to reduce aerosols by as much as 66% and the HVE by as much as 76% when both were compared to the saliva ejector. The study's overall recommendation was to alter the design of the Isolite™ mouthpiece to increase airflow, which may deliver a closer reduction amount to that of the HVE.<sup>35</sup> A limitation of this study is that it was performed in a laboratory environment so all of the variables present in a clinical environment were not taken into account.

In 2009, Noro et al. published the first study evaluating the Isolite™ and examined its clinical usefulness in a Japanese population.<sup>37</sup> Volunteer resident dentists in the Department of General Dentistry at Tokyo Dental College Chiba Hospital were utilized as study subjects. Subjects were randomly divided into two groups of 15 and paired with an individual from the opposite group. In each pair, the subject playing the role of the clinician placed the Isolite™ into the oral cavity of the subject playing the role of the patient and used an air turbine hand piece equipped with a dummy bur to simulate tooth preparation for a crown. Following the simulation, all subjects completed a survey composed of nine questions regarding their experience with the device. Based on the mean overall ratings, the subjects playing the role of the surgeon rated the device higher in satisfaction than the patients. The lowest patient ratings were in response to the question regarding how the Isolite™ fits in the mouth, leading to the conclusion that the Isolite™ needed to be altered for a better fit with the Japanese people.<sup>37</sup> A drawback of the study was their exclusive focus on the Japanese population, making these conclusions difficult to apply to other populations.

In 2010, a study by Colette et al. assessed patient acceptance of the Isolite™ during sealant placement compared to cotton roll isolation (CRI) while evaluating sealant application times.<sup>38</sup> A total of 48 children were seen at the pediatric dental clinic at Children's Hospital in Cincinnati, Ohio. Data on patient acceptance were collected via a verbal survey, consisting of nine questions, which asked the patient about his or her experience with both the Isolite™ and CRI. Subjects were randomly assigned to one of two groups, both utilizing the Isolite™ and CRI but alternating which method was used first. The survey was administered following sealant placement. The results of the study revealed discomfort was reported with the Isolite™ for the following reasons: stretching of the cheeks, inducing their gag reflex, and high amounts of noise. The author concluded that because the participants of the study were all under the age of 11 it would be beneficial to utilize older children or adults in future research to further evaluate patient acceptance.<sup>38</sup>

A study published in November 2012, by Dahlke et al. compared the effectiveness of a splatter reduction at an operative site by three groups: the Isolite™ alone, the HVE with dental dam, and the HVE alone in a patient simulated environment.<sup>36</sup> During the study, a total of 72 trials were completed in a closed door operatory and tooth preparation was simulated on a typodont manikin during a benchtop exercise. A high-speed hand piece with a carbide bur was used to create splatter with a fluorescein dye solution, which was added to the water supply. A bulletin board was mounted to surround the typodont head and was used to collect splatter emitted from the hand piece and contaminated squares were counted following the conclusion of each trial. Each type of dry-field technique was used while tooth prep was simulated on tooth numbers 18-20. The control consisted of the HVE alone during simulated tooth preparation. The first experimental group consisted of the Isolite™ set at maximum strength during tooth-

simulated preparation. The second experimental group consisted of the standard 6-inch dental dam with only three holes to isolate the three teeth being prepped. The HVE for the control and experimental groups was oriented in an identical position and the Isolite™ was kept in the same position throughout the entire experiment. The study found no significant difference in the reduction of splatter between the two experimental devices, but splatter was decreased significantly when compared to the HVE alone. The only statistical difference found was that when tooth prep was simulated on a more anterior tooth, the HVE with dental dam reduced splatter slightly more than the Isolite™, likely due to the design of the mouthpiece and the focus of evacuation being in the posterior. The conclusions stated that because the Isolite™ reduced aerosols just as well as the HVE with dental dam during tooth preparation, it may be the preferred device because of the other benefits it offers, specifically illumination, isolation, protection of adjacent soft tissues, assistance in opening the mouth and protecting from accidental aspiration.<sup>36</sup> The observation that the Isolite™ was not as effective in the anterior was beneficial when identifying possible confounding variables for the current study.

Bacterial contamination from ultrasonic scaler aerosol has been well documented in the past.<sup>3-5, 8,16,31,32,34,35</sup> Developing protocol for the reduction of aerosols during the treatment of immunocompromised patients can help protect these patients during dental procedures, such as ultrasonic scaling, that create high amounts of potentially infectious aerosols. Presently, there is no known research regarding the Isolite™'s ability to reduce aerosols or its performance compared to other suction devices in an actual clinical environment during use of the ultrasonic scaler. Based on the findings of the study, if the Isolite™ reduces aerosols more effectively during ultrasonic scaling than the saliva ejector alone then it may be recommended as a standard of care, especially during the treatment of immunocompromised patients.

## **Previous Research Methods to Collect and Quantify Aerosols and Splatter**

A variety of methods have been employed in research to collect and quantify dental aerosols and splatter. Historically, these methods have depended on the type of particle being studied (aerosol vs. splatter) and/or the type of microbe in questions (anaerobic vs. aerobic). Techniques for aerosol and/or splatter collection have ranged from benchtop exercises with fluorescein dye to elaborate air sampler devices.

The most basic approach to evaluate aerosols and splatter has been the use of benchtop studies where dental procedures were simulated and no actual microbes were created or measured.<sup>5, 21,29,34</sup> In these cases, the primary objective was the testing of aerosol and splatter reduction devices instead of collecting and quantifying actual microbes. These studies have helped to identify devices that produce as well as reduce the greatest amounts of aerosols and splatter.<sup>5, 21,29,34</sup> In two separate studies by Harrel et al., aerosol production and reduction were evaluated.<sup>29, 34</sup> The first of the two studies examined aerosol reduction with an HVE attachment to an ultrasonic scaler<sup>29</sup> while the subsequent study identified differences in aerosol production between hand scaling and various ultrasonic inserts.<sup>34</sup> In both studies the coolant water for the ultrasonic scaler was replaced with a fluorescein solution and a grid containing one centimeter squares surrounded a dentoform model. Squares containing a drop of the fluorescein solution were considered contaminated and were counted and recorded.<sup>29, 34</sup> A more recent study, which used this method while evaluating the Isolite™ was conducted by Dahlke et al., and compared the splatter reduction of the Isolite™ to the HVE with the rubber dam and HVE alone.<sup>36</sup> The methods used were similar in that an overlay grid was used to show contaminated squares of fluorescein dye. Similar to previous studies, this method proved appropriate for their aims but was limited in its inability to collect, identify, and quantify actual microbes.

Some of the earlier studies in the 1940's and 1950's were critiqued because of their inability to differentiate between viable and non-viable aerosols.<sup>39</sup> The earliest published study to identify an instrument that was able to collect and count viable airborne particles was in 1958, by Ariel Andersen.<sup>39</sup> This device is known as the Andersen sampler, consisting of six-stages through which the air or aerosol is drawn in by air flow, at a rate of one cubic foot per minute, through perforated (400) holes into a Petri dish filled with agar medium to collect the microbes. Petri dishes were removed and incubated for an undisclosed amount of time. The way in which colonies were counted were not specified but stated as being quantified "in a usual manner" as defined in microbiology and in the case of heavily loaded plates, by a dissecting type microscope before the colonies were able to merge.<sup>39</sup> Although groundbreaking at the time, this method proved problematic due to its inability to include splatter as well as its tendency to also include dust, molds, yeast, and other particles present in the environment at the time of sampling. This study was followed by a very similar study by Larato et al., in 1967, which utilized a similar device; the Reyner air sampler.<sup>27</sup> An advantage of this study was the specific identification of multiple types of bacteria sampled from the air. It was determined that most of the bacteria collected were mold or yeast that were either often found in the air or water. The exception to this was the identification of alpha streptococcus, which is present in large numbers in the oral cavity. This device faced the same drawbacks as the Andersen air sampler with its inability to account for splatter and its tendency to include airborne particles such as mold and yeast because of the focused airflow that draws particles into the machine.<sup>27</sup>

As an attempt to address these problems, Micik and Miller et al. in 1969 implemented a two-part dental aerobiology study to examine characteristics of bacterial aerosols generated from a patient's mouth during dental procedures.<sup>15</sup> These studies utilized a human aerosol test

chamber which enclosed the patient's head and used sealed slots allowing for entry of hands and equipment needed to conduct various dental procedures such as hand scaling and cavity preparation. Four Anderson six-stage sieve samplers used to collect aerosols and Petri dishes containing heart infused agar were incubated for 48 hours at 37°C. Only aerobic bacteria capable of growing on heart infusion agar were counted according to the Andersen method and were expressed as CFU/min. A noted limitation of the study was the inability to include splatter and anaerobic bacteria. Therefore, in 1971 a subsequent study by Micik and Miller et al. utilized a different technique to collect and quantify aerosols.<sup>2</sup> This time, the aim was to target splatter and a system was engineered to rapidly open and close strategically placed Petri dishes. An apparatus was built and installed out of wood battens three feet above the floor radiating one foot below the patient's mouth with the sides extending to the end of the operatory (8 x 10 x 7.5 feet). Petri dishes containing heart infused agar were fixed with suction cups to each wooden batten and were rotated 360°, opened for exposure, and then closed immediately by rotating the battens in the opposite direction. Test dental procedures were performed for 30 seconds to create splatter. Once plates had been exposed and closed they were incubated at 48 hours at 37°C and colonies were counted. CFU were computed and expressed as CFU/foot.<sup>2</sup> A benefit of this study was the ability to differentiate from aerosols and splatter. But because of elaborate study design, it would be difficult to replicate and reproduce these results.

A more common method to collect and quantify aerosols and splatter has been the placement of blood agar plates in the vicinity of where aerosols and splatter are being produced. This method has shown success in multiple studies in the collection, quantification, and identification of specific microbes sampled from the air.<sup>13,31-33,30</sup> This approach was used by King et al. in 1997 during an attempt to evaluate the effectiveness of an HVE attachment to the

ultrasonic scaler at reducing aerosols.<sup>33</sup> Prior to initiation of the study, a pilot study was conducted and results showed that it took approximately 30 minutes for aerosolized bacteria to return to baseline levels. Three blood agar plates were mounted at a 50° angle six-inches from the subject's oral cavity and exposed to the ultrasonic for five minutes and then left open for 25 minutes following scaling. Plates were then incubated at 37°C for 72 hours and recorded. There was no noted attempt to culture or identify types of bacteria in this study. Instead, the primary outcome was assessed by the quantification of CFU in order to verify the findings of the in-vivo Harrel et al. study.<sup>33</sup>

A study conducted in 2006 by Rautemaa et al. took this process a step further and used Gram stain to classify aerosolized bacteria.<sup>40</sup> In this particular study, aerosol samples were collected using horse blood chocolate agar plates. Plates were strategically placed, a set of two in six different areas of the operatory, ranging from 0.5 to 2m from the patient's oral cavity. Each plate was opened when treatment was initiated and one plate from each group was closed after 1.5 hours and the other closed after three hours. The plates were incubated at 37°C for 48 hours followed by counting and classifying with Gram stain using a light microscope with 1000x magnification. The most common types of bacteria identified were Gram-positive cocci, namely viridians streptococci and staphylococci.<sup>40</sup> Authors did not state whether plates were incubated in an aerobic or anaerobic environment.

In 2001, Klyn et al. sought to identify methods to reduce bacteria-containing spray during ultrasonic scaling.<sup>32</sup> To collect aerosols during ultrasonic scaling, three blood agar plates were placed six inches from the oral cavity and one plate was placed two feet from the oral cavity. Plates were kept covered until testing and were left open for five additional minutes following exposure to the ultrasonic scaler. Plates were immediately incubated at 37°C for 72 hours before

being recorded. The organisms collected were aerobic and were recognized as mostly staphylococci, which are not considered pathogenic due to their tendency to be found in the saliva of healthy adults. The results of the study were generalized to include pathogenic organisms because literature “supports the potential presence of these organisms in aerosols and splatter.”<sup>32</sup> It would have been beneficial to culture and identify anaerobic bacteria to confirm their presence, which would be constructive when discussing aerosols and the spread of infectious disease.

As outlined in the above-mentioned studies, there are various methods to collect and quantify aerosols and splatter. Those methods that were able to collect bacteria-laden aerosols and identify specific microbes were most telling when considering the potential to spread infectious diseases in a dental setting. A major downfall of studies that utilized blood agar to directly collect splatter and aerosols without the use of an air sampling device was the inability to differentiate CFU when there were high counts of aerosols and splatter present. On the other hand, studies that did use air-sampling devices were not able to take splatter into account, which can be a major source of contamination in the dental office. It seemed prudent to consider an alternative method to include both aerosols and splatter while uniformly dispersing the microbes so that they could be counted and quantified in a manner that would be as comprehensive as possible.

## **CHAPTER 3: MATERIALS AND METHODS**

### **Test Device**

The test device utilized for the study was the Isolite™ dryfield illuminator (Isolite™ Systems, Santa Barbara, CA) (Appendix 1). This system was designed to retro-fit onto the high volume suction hose and consists of an autoclavable control head with built in LED light and disposable mouthpiece. The control head portion of the system can be removed between patients. The mouthpiece portion has an integrated bite-block that is continuous with a piece of malleable plastic (tongue and cheek shield) that fits in the vestibule and oropharynx area. The tongue/cheek shield assists with suction, retraction, and blockage of the throat to help prevent aspiration.

### **Control Device**

The positive control for the study was the saliva ejector, a disposable attachment to the low-volume suction hose. This device consists of a straight tube of plastic with a standard 4mm slot attachment and assists with suction and retraction.

### **Ultrasonic Scaling Equipment**

The ultrasonic unit for the study was a 30KHz Cavitron SPS ultrasonic scaler and a Dentsply 30K slimline scaling tip (Dentsply Preventive Care, York, PA).

### **Lab Equipment**

The liquid medium used to collect aerosols and splatter was Dulbecco's phosphate buffered saline (DPBS) (GIBCO® DPBS, pH 7.4 from invitrogen™ Grand Island, NY 14072).

Pre-gassed Brucella agar with 5% sheep Brucella enriched with hemin and vitamin K (BRU by Anaerobe Systems, Morgan Hill, CA 95037) was used for the growth of anaerobes. To assess DUWL contamination prior to initiation of the study, R2A Agar (BBL® R2A Agar, from Becton Dickinson and Company, Cockeysville, MD 21030) was utilized. A Model D Spiral Plater (Spiral System™ by Microbiology International, Frederick, MD 21701) was utilized to plate replicate aliquots of the dispersed suspension onto Brucella agar for the quantitation of CFU. The inoculated Brucella agar was incubated at 37°C in a Coy anaerobic chamber with an atmosphere of 5% CO<sub>2</sub> / 10% H<sub>2</sub> / 85% N<sub>2</sub> for up to seven days. A ProtoCOL automated CFU counter (ProtoCOL RGB, Model No. 9000, Synoptics Ltd, UK) was utilized to calibrate the hand counted CFU by the principal investigator.

## **Methods**

All dental cleanings were conducted in the same enclosed dental operatory, fully equipped with high and low volume suction hoses, air/water syringe, an Isolite™ dryfield illuminator, and a Dentsply™ Cavitron Jet. The air in the operatory was set to change over at a rate of six to eight times per hour. Each patient was seated in a supine position during their cleaning and was treated by the same clinician. The clinician was a licensed dental hygienist with five years of clinical experience and three years of experience with the test device. Each subject was asked to refrain from oral hygiene care, such as brushing, flossing or rinsing for 12 hours prior to his or her appointment.

Participants were English-speaking adults, 18 years of age or older, receiving treatment in the General and Oral (GO) Systemic Health clinic located within the School of Dentistry at the University of North Carolina Chapel Hill. Participants were recruited by contacting individuals of previous Institutional Review Board (IRB) approved studies who still attend the GO Health

clinic for a dental hygiene services. Subjects' rights have been protected by the IRB and written informed consent was granted from all subjects.

To be included in the study, subjects met the following criteria: (1) had not received dental scaling, root planing, or prophylaxis in the last three months (2) absence of tooth sensitivity that would prevent use of the ultrasonic scaler, and (3) willing to refrain from oral hygiene practices for 12 hours prior to the appointment. Subjects were excluded from the study if they presented with the following: (1) presence of a respiratory infection (2) presence of a cardiac pacemaker (3) chronic disease with oral manifestations (4) exhibited gross oral pathology (5) currently taking antibiotics or steroids, and (6) presence of active infectious diseases such as HIV, tuberculosis or Hepatitis B.

A telephone script was provided to the scheduling coordinator at the GO Health clinic (Appendix 2). As an incentive, participants were offered an oral prophylaxis at no charge. Participants who met the inclusion/exclusion criteria were scheduled with the principal investigator for a dental cleaning.

Prior to initiation of the study methods were tested on a volunteer to establish a protocol for the following: placement of Petri dishes, type of medium for microbiological collection, and lab procedures for the sampling and quantification of CFU. Two different types of media were tested, Brucella agar (solid) and 20 ml of sterile DPBS (liquid). Three separate plate groups, one to the left, right, and center, were placed six inches from the subject's oral cavity (Appendix 3). Each plate group contained one Brucella agar and one plate containing 20ml of DPBS. Following the manufacturer's directions, water lines were flushed prior to the initiation of scaling. Each plate was opened to the operatory atmosphere during the entire ultrasonic scaling procedure and then closed immediately once scaling was completed. Time spent scaling was

recorded as the during exposure period. Each plate was then replaced with a fresh plate for the post exposure time period of 35 minutes. Hand scaling was initiated following ultrasonic scaling and no other procedures that would create aerosols or splatter were completed (e.g. polishing with a prophylaxis cup or air powder polishing). Following the final collection period, Brucella agar plates were immediately incubated while the plates containing DPBS were spiral plated onto Brucella agar, as detailed in the study procedures. Both types of plates were incubated and CFU were counted after 7 days. After quantifying the CFU on each type of plate, those containing the DPBS and plates centered in front of the subject's oral cavity were found to produce consistently higher CFU, providing a more complete representation of the actual infectious load.

To ensure that CFU collected originated from an oral source an atmospheric baseline of aerosols was obtained as well as a baseline of the DUWL bacteria. To obtain an atmospheric baseline, a single Petri dish containing Brucella agar was placed in the center of the closed door operatory and uncovered for thirty minutes. The Brucella agar plate was not inoculated and instead kept at 37°C and checked after 48 hours, and did not show any growth, indicating a negative atmospheric baseline for aerosols. Further, DUWL were tested for the presence of bacterial contamination by plating water samples onto R2A agar, which was incubated at room temperature and was not enriched. Oral bacteria are unable to grow on this medium, but the bacteria characteristically associated with DUWL contamination can. This test was also negative for the presence of bacteria. Therefore, it was inferred that any CFU collected originated from the treatment subject.

Each patient was instructed to refrain from any oral hygiene care for 12 hours prior to the study. Upon arrival to their appointment, subjects were given an IRB and Health Insurance Portability and Accountability Act (HIPAA) consent form. Once consented, subjects were

randomized with the flip of a coin into one of two treatment groups (test device or positive control) and the appointment for the oral prophylaxis was initiated. The medical history was updated, an oral cancer screening was performed, and a plaque index recorded (Appendix 4). To determine the extent of plaque a Modified Greene and Vermilion plaque index was performed. Each tooth was given a single score for the facial surface only based on the following criteria:

<b>Score</b>	<b>Amount of plaque</b>
0	No plaque present
1	Plaque covering not more than one third of the tooth surface
2	Plaque covering more than one third but less than two thirds of the tooth surface
3	Plaque covering more than two thirds of the tooth surface

Once all teeth were scored a sum was calculated and divided by the total number of teeth present to give a number ranging from 0-3, with zero being the lowest possible score and three being the highest possible score.

Prior to ultrasonic instrumentation, a single Petri dish containing 20ml of DPBS was centrally placed six inches from the oral cavity (Appendix 5). At the onset of ultrasonic instrumentation the lid to the Petri dish was removed for the duration of ultrasonic scaling and exposure time was recorded. On completion of the ultrasonic scaling procedure, the Petri dish was re-capped and replaced with a new Petri dish containing fresh 20ml of sterile DPBS. The second Petri dish remained open to operatory air for 35 minutes to collect aerosols for the post-exposure period and was then re-capped. The purpose of keeping the plate open for 35 minutes following use of the ultrasonic scaler is based on previous studies which have shown that

aerosols settle back to baseline an average of 35 minutes after use of a high speed drill or ultrasonic scaler.<sup>27, 31,32,41</sup> The remainder of the subjects' cleaning proceeded without the use of any devices that would create aerosols or splatter such as coronal polishing or use of an air powder polisher. To prevent cross-contamination of aerosols, only one subject was scheduled per day. The operator position was set at 11 o'clock during the entire procedure. In addition, the amount of water dispensed and the power settings on the ultrasonic unit were identical for each subject, at 50% power and lavage. The dual vacuum levers, which control the suction strength on the test device, were kept at 75% for the maxillary and mandibular arches while in use. At the completion of their appointment, subjects were asked to fill out a nine item survey with questions regarding the suction device used during their appointment to assess patient acceptance (Appendix 6). Each subject received the same survey regardless of the device used. Each survey question was related to the patient's comfort and experience with the device used during their cleaning. A section for comments was provided for feedback regarding the device used.

At the end of each collection period, the exposed DPBS samples were aseptically transferred to a sterile disposable 50ml centrifuge tube and then transferred to the lab within fifteen minutes following the final collection period. Once in the lab the CFU in the solution were dispersed by vortexing and then spiral plated to fresh anaerobic Brucella blood agar. The spiral plater delivered a spiral gradient at a total volume of 0.049 ml sampled from the 20 ml volume of the dispersed inoculum. Recovered CFU/ml of liquid were quantified after incubation. All Brucella agar plates were pre-gassed from the manufacturer and kept packaged until ready for use. The inoculated Brucella agar was incubated at 37°C in a Coy anaerobic chamber for seven days. These conditions permit bacterial species to grow that could only have come from the oral cavity such as  $\alpha$ -hemolytic streptococci, actinomycetes and strict oral anaerobes. Most

environmental bacteria and mold will not grow in this atmosphere or at this temperature. Following incubation, Brucella agar plates were counted by the principal investigator and recorded onto a data collection form (Appendix 7). Each sample was marked with a number so that the principal investigator was blinded as to the device used for each sample when recording CFU.

Extent of aerosol contamination was determined by counting CFU, which were recorded by the principal investigator after plates had incubated for the requisite times. CFU were recorded by plate number only and were not linked with participants. The total CFU per surface area were determined by multiplying by 20 (volume of liquid) and the inverse of the counted dilution. This number would be comparable to the total number of CFU landing on the surface of the agar plate without consideration for aggregates. The CFU were counted by the principal investigator using a counting grid designed to center over the spiral plate and were compared to counts determined by the ProtoCOL automated counter. The grid used by the principal investigator consisted of five concentric circles and eight radial lines, which create annular segments. These segments are further divided creating a number of marked areas. Each and every area marked on the grid corresponds to a known, constant volume of sample deposited on the spiral plate. The number of CFU was then divided by the corresponding volume for that marked area. Following incubation, plates were inspected by the naked eye and by microscope for the following: alpha and beta hemolysis, pigmentation, and morphology.

All sample counts were expressed as colonies of bacteria per milliliter (CFU/ml) and were then transformed to  $\log_{10}$  for normalization. All statistical tests were given an alpha level of significance of 0.05. To determine if a difference existed in aerosols and splatter reduction between the two device groups a Student t-test was utilized to compare the average  $\log_{10}$  CFU

collected during ultrasonic scaling in each device group. A Student t-test was also performed to assess aerosol and splatter reduction within each device group (from during to post exposure) and to determine if there was a significant difference between the two device groups in terms of average time spent ultrasonic scaling. Spearman's correlation coefficient was used to measure the relationship between full mouth plaque extent and CFU, as well as location of plaque (anterior vs. posterior) and CFU. Survey responses were entered into an Excel spreadsheet and analyzed for frequencies following completion of the survey.

## **CHAPTER 4: RESULTS**

A total of fifty-two subjects were enrolled in the study. Data on two subjects were excluded from the data analysis due to an incorrect dilution of the DPBS in their samples. Twenty-six subjects were randomized into each treatment group. A majority of subjects in each group were female, with 77% in the test group and 76% in the control group. The average age of subjects in the test group was 40 compared to 45 in the control group. Maximum, median, and minimum tooth counts were also calculated for each group, revealing a maximum of 32 teeth and a median of 28 teeth in each group, and a minimum of 24 teeth in the test group and 21 teeth in the control group. Subjects within each group represented a range of plaque extent scores, which were not statistically different between groups (Table 4.1).

To assess for bias, time spent ultrasonic scaling and plaque extent scores were assessed between the two groups. A Wilcoxon Rank Sums test revealed that there was not a statistically significant difference between the two groups in the average time spent ultrasonic scaling during the procedure ( $P = 0.68$ ). Similarly, a Student t-test revealed there was not a significant difference in the average full-mouth plaque extent scores between the two groups ( $p = 0.56$ ).

### **Primary Objective**

There was not a statistically significant difference in aerosol and splatter reduction during ultrasonic scaling between the test and control group ( $p = 0.25$ ). Descriptive statistics of aerosols

and splatter collected in both exposure periods are displayed in Table 4.2. The range of aerosols and splatter collected during ultrasonic scaling in each group can be compared in Figure 4.1. As shown in this figure, the average number of aerosols and splatter collected in the control group was approximately  $\log_{10} 3.6$  CFU/ml (4,000 CFU/ml) compared to  $\log_{10} 3.3$  CFU/ml (2,000 CFU/ml) in the test group. The range of aerosols and splatter collected in the 35-minute post exposure period in each group is displayed in Figure 4.2. As shown in this figure, the average number of aerosols and splatter collected in the control group and test groups in the post exposure period was approximately  $\log_{10} 2.0$  CFU/ml (100 CFU/ml) and  $\log_{10} 1.6$  CFU/ml (45 CFU/ml) respectively.

When looking within each device group, there was a significantly sharp decline in aerosols and splatter following ultrasonic scaling ( $p < 0.0001$ ), as displayed in Figures 4.3 and 4.4. Overall, each group exhibited a maximum amount of aerosol and splatter contamination of approximately  $\log_{10} 5.0$  CFU/ml (100,000 CFU/ml) during ultrasonic scaling with plates exhibiting no growth in the post exposure period. Within the control group (Figure 4.3) the average number of aerosols and splatter declined from  $\log_{10} 3.6$  CFU/ml (4,000 CFU/ml) to  $\log_{10} 2.0$  CFU/ml (100 CFU/ml) representing an almost 98% reduction. A significant decline was also found in the test group ( $p < 0.0001$ ) where the average number of aerosols and splatter declined from  $\log_{10} 3.3$  CFU/ml (2,000 CFU/ml) to  $\log_{10} 1.6$  CFU/ml (45 CFU/ml) representing an almost 98% reduction, displayed in Figure 4.4.

To assess for potential bias, Spearman correlations were performed to assess the relationship between plaque extent and aerosols and splatter collected in each device group. Within the control group there was a significant positive correlation ( $p = 0.003$ ) between aerosols and splatter collected during ultrasonic scaling and full-mouth plaque extent with an  $R^2$  value of

0.27, displayed in Figure 4.5. When this relationship was assessed in the anterior region a significant positive correlation ( $p < 0.0001$ ) was also found with an  $R^2$  value of 0.43, displayed in Figure 4.6. However, no significant relationship ( $p = 0.201$ ) was found in the posterior region, displayed in Figure 4.7. These same relationships were also examined in the test group. When assessing full-mouth extent a significant relationship was not found ( $p = 0.087$ ) displayed in Figure 4.8. In contrast to the control group, a significant positive correlation was not found in the anterior region ( $p = 0.105$ ) with an  $R^2$  value of 0.18, displayed in Figure 4.9. In the posterior region no correlation was found ( $p = 0.290$ ) with an  $R^2$  value of 0.04, displayed in Figure 4.10.

All samples were assessed for the presence of oral bacteria. The types and frequencies of identified bacteria types are shown in Table 4.3. The most prominent type of bacteria present was *alpha hemolytic streptococcus*, present in 100% of the samples, followed by: *Fusiform* (64%), black pigmented (26%), beta hemolytic bacteria (20%), *Eikenella corrodens* (12%), *Prevotella intermedia* (10%), *Tannerella forsythia* (4%), and *Porphyromonas gingivalis* (2%).

### **Secondary Objective**

Survey responses indicated that the test device was not well liked. When subjects were asked, “Would you like to have this device used during your next cleaning?” 92% of those in the control group said “yes” where only 64% of those in the test group said “yes.” When asked if the device made them feel as if they were going to gag, or stretched their cheek and lips, there were no subjects that answered “yes” in the control group where a range of 12-20% said “yes” in the test group. Table 4.4 displays the percentage of “yes”, “no”, and “don’t know” responses of each question, bolding those questions with considerable differences in responses.

When examining comments related to their experience 24% in the test group and 28% in the control group reported a “good” experience. A more common theme among comments from

subjects was related to the size and design of the test device mouthpiece. Specific emic expressions regarding the test device related to this theme include:

- “It does hurt being inserted but once there it’s comfortable.”
- “It felt only a little bit uncomfortable initially and only made me feel like I was going to gag at first.”
- “Kept the back of my mouth dry, but didn’t really help with the fluids in front. Had more dribble down my face and neck than previous traditional suction devices and the same sonic cleaner.”
- “My only negative issue was slight buildup of water at the back of my throat.”
- “I have a small mouth so it was a lot in my mouth....”
- “.....I felt a little stretch to the cheeks and lips but this may be normal for inserting for comfort/fit....”
- “Device made me feel as if not getting enough air (intermittent), [I] needed to take a deep breath occasionally.”

## **CHAPTER 5: DISCUSSION**

### **Introduction**

Due to the amount of contamination that takes place during most dental procedures, it is of great importance to minimize the presence of potentially infectious aerosols and splatter as much as possible. Minimizing aerosols and splatter during treatment may be one mechanism for achieving this goal.

### **Key Findings**

The purpose of this study was to compare the effect of the Isolite™ suction and saliva ejector on aerosols and splatter during ultrasonic scaling. Results indicated that there was not a significant difference in the average number of aerosols and splatter collected during ultrasonic scaling between the two groups tested ( $p=0.25$ ). However, the amount of contamination taking place during ultrasonic scaling, as indicated by high counts (approximately  $\log_{10}$  5.0 CFU/ml or 100,000  $\log_{10}$  CFU/ml) in both groups, is concerning. When considering the saliva ejector only, these findings were in agreement with previous studies and confirmed that the saliva ejector was not effective at removing aerosols and splatter created during ultrasonic scaling.<sup>27, 31, 33</sup> It was unexpected to find similar amounts of aerosols and splatter collected during ultrasonic scaling in the test group due its design to attach to the high-volume suction hose. These findings conflicted with the findings of the 2007 Jacks and Pollard study and the 2012 Dahlke et al. study.<sup>35, 36</sup> Each of these studies detected a significant reduction of aerosols when the test device was compared to the saliva ejector<sup>35</sup> and HVE alone.<sup>36</sup> Further, the Dahlke et al. study found the test device to be

comparable to the HVE with rubber dam at aerosol reduction, except when in the anterior region.<sup>36</sup> Because the current study was the first of its kind to evaluate the Isolite™ in regards to aerosol/splatter reduction in an actual clinical environment, it was presumed that the addition of other variables (e.g. plaque, saliva, patient/operator positioning) contributed to dissimilar findings. Therefore, it can be concluded from these studies that although attempts can be made to create an environment identical to a clinical environment, it is difficult through bench-top studies to capture all of the variables involved in actual clinical studies.

When aerosol and splatter reduction within each group was assessed, the reduction was significant ( $p < 0.0001$ ). A reduction of approximately 98% was seen in both device groups, with some samples in the during exposure period containing as much as  $\log_{10}$  5.0 CFU/ml (100,000 CFU/ml) and some samples in the post exposure period containing no growth at all. However, this sharp decline of aerosols and splatter cannot be attributed to any properties of the suction devices because both suction devices were turned off immediately following ultrasonic scaling. This significant decline was thought to be directly related to the air clearance by the air handling system of the operatory. Due to the use of a closed-door operatory for the current study, it may be beneficial to examine this effect in an open-bay clinical environment.

The significant relationship between anterior location of plaque and higher counts of aerosols and splatter in the control group can likely be explained by the inherent nature of the saliva ejector (i.e. to remove saliva not aerosols). Because the saliva ejector sits in the floor of the mouth and suctions out pooled saliva, the aerosols were allowed to escape into the environment. In contrast, the Isolite™ did not show a significant relationship between anterior location of plaque and higher counts of aerosols and splatter. This finding can be explained by the design of the Isolite™ mouthpiece, which is able to provide suction in multiple areas of the

mouth, allowing it to target aerosols and splatter instead of just saliva alone. This finding is not in agreement with the Dahlke et al. study, which found a significant positive correlation between increased aerosol contaminations in the anterior region during use of the Isolite™.<sup>36</sup>

### **Findings related to microbiological methodology**

The presence of strict oral bacteria in all samples (e.g. *alpha hemolytic streptococci*) confirmed that the bacteria collected had originated from the oral cavity (saliva and/or plaque) of the host and not from the skin or the environment. As detailed in the methods, each sample was collected in a liquid medium (DPBS) and spiral plated onto Brucella agar. Utilizing a liquid medium instead of a solid medium and vortexing all samples before spiral plating allowed for a more complete representation of the actual infectious load. Further, the use of Brucella agar enriched with hemin and Vitamin K incubated in an anaerobic chamber excluded the growth of bacteria not typically associated with the oral cavity.

Previously, the most basic approach to evaluate aerosol and splatter reduction devices has been in a simulated clinical environment where no actual bacteria were involved.<sup>27, 29,34,38</sup> These methods eventually progressed to the collection of aerosols by use of an air sampling machine<sup>9,15,39</sup> or placement of blood agar plates in the vicinity of the patient's oral cavity during dental treatment.<sup>14,31-33,40</sup> A downfall of air sampling devices were their inclusion of environmental contaminants and their inability to evaluate splatter, which eventually led to the use of blood agar plates. Blood agar plates, although useful for collecting aerosols and splatter, can make it difficult to differentiate individual microbial colonies when high amounts of aerosols and splatter are collected during a single collection period. Further, a majority of studies that utilized blood agar plates for collection only allowed incubation to occur for 48-72 hours, which did not give some periodontal pathogens long enough to grow. A seven-day incubation period in the current study allowed for the growth of black-pigmented bacteria, which were inspected and identified as

having morphology consistent with periodontal pathogens (i.e. *P.gingivalis* and *P.intermedia*). The presence of these anaerobic pathogens is disconcerting due their link to acute bacterial endocarditis<sup>12</sup> and more commonly, aspiration pneumonia.<sup>11</sup> The process of ultrasonic scaling almost always involves working sub-gingivally, which disrupts the microbes present in the periodontal pocket and aerosolizes it. A benefit not offered by the saliva ejector but reported by the Isolite<sup>TM</sup> is the protection against aspiration. The Isolite<sup>TM</sup>'s mouthpiece provides this benefit by wrapping around the back of the mouth and blocking the oropharynx.<sup>8</sup> This advantage should be considered, especially when working with an immunocompromised population who are more susceptible to these infections.

### **Secondary Objective**

When assessing patient acceptance of the test device it was determined that the mouthpiece should be modified so that it is less likely to stretch the cheeks and lips during insertion and cause gagging. Leading factors to this conclusion were based on the frequency of “yes” responses to survey question #'s 2-4 which asked subjects if the suction device used during their cleaning made them feel as if they were going to gag and stretched their cheeks and/or lips. Moreover, when subjects were asked if they wanted to have that suction device used during their cleaning, 92% said “yes” in the control group where only 64% said “yes” in the test group. These findings are in direct agreement with two previous studies which evaluated patient acceptance of the test device.<sup>37,38</sup> It was recommended by Colette et al. to modify and trim the flange portion of the mouthpiece to decrease gagging.<sup>38</sup> This recommendation along with decreasing the width of the bite-block portion may better increase patient acceptance of the test device.

## CHAPTER 6: CONCLUSIONS

It remains, as recommended by the ADA and CDC, that the HVE, along with proper patient positioning, should continue to be used during dental procedures that yield high amounts of aerosols and splatter.<sup>6, 7</sup> However, due to its design, the test device has been reported to help prevent aspiration<sup>8</sup> which should be taken into account, especially when treating immunocompromised patients. The test device also has various benefits not related to aerosol reduction (i.e. isolation, illumination, etc.) and if being used for those reasons it is recommended that additional measures be taken to further reduce aerosols and splatter. For example, it would be beneficial to follow the ultrasonic scaler with an additional suction device in the anterior region while the test device is being used. Further, preprocedural mouth rinses have been shown to reduce bacterial laden aerosols and splatter prior to use of aerosol creating devices<sup>42</sup> and should therefore be used in instances where aerosols creating devices, such as the ultrasonic scaler, will be utilized. In addition to this, removal of gross biofilm would be helpful in reducing the patient's plaque load by having the patient brush before initiating ultrasonic scaling. It is also recommended that dental offices and institutional settings consider air clearance as a way to further reduce cross-contamination, especially in open-bay clinic environments where aerosols have the opportunity to spread much further. Patient positioning should also be considered when optimizing aerosol reduction. A supine patient position was utilized in the current study and proper patient positioning should be used whenever possible during aerosol producing procedures, as outlined by the ADA and CDC.<sup>6, 7</sup>

**Limitations**

A limitation of the current study is the small number of participants in each group when analyzing by device. An increased sample size would be beneficial in future studies.

**Future Studies**

Future research conducted in this area should be designed to allow comparison of the Isolite™ and HVE in a randomized clinical trial utilizing the aerosol and splatter collection methods of the current study.

## TABLES

**Table 4.1**

Descriptive Statistics of Plaque Extent Scores by Group

Group	Anterior	Posterior	Full Mouth
<b>Control</b> n=25			
Mean $\pm$ s.d.	0.94 $\pm$ 0.63	0.80 $\pm$ 0.43	0.87 $\pm$ 0.48
Median (Min-Max)	0.80 (0-2.33)	0.75 (0-1.62)	0.68 (0.21-1.93)
<b>Test</b> n=25			
Mean $\pm$ s.d.	0.80 $\pm$ 0.53	0.79 $\pm$ 0.41	0.80 $\pm$ 0.40
Median (Min-Max)	0.67 (0-2.17)	0.75 (0.25-1.80)	0.68 (0.12-1.59)
<b>p-Value*</b>	0.39	0.32	0.56

\*Statistical analysis derived from Student t-test (p<.05)

**Table 4.2**

Descriptive Statistics of log<sub>10</sub> CFU/ml by Group

Group	During Exposure log <sub>10</sub> CFU/ml Mean $\pm$ s.d. (95% CL)	Post Exposure log <sub>10</sub> CFU/ml Mean $\pm$ s.d. (95% CL)
<b>Control</b> (n=25)	3.61 $\pm$ 0.95 (0.74, 3.21)	2.00 $\pm$ 1.17 (0.91, 1.52)
<b>Test</b> (n=25)	3.30 $\pm$ 0.88 (0.68, 2.94)	1.65 $\pm$ 1.15 (0.90, 1.17)
<b>p-value*</b>	0.25	0.29

\*Statistical analysis derived from Student t-test (p<.05)

**Table 4.3**

Type and Percentage of Bacteria Present Among all Samples

<b>Bacteria</b>	<b>Total Present (n=50)</b>	<b>Percent (%)</b>
Alpha Hemolytic Streptococcus	50	100
Fusiform	32	64
Black Pigmented	13	26
Beta Hemolytic	10	20
E. corrodens	6	12
P. intermedia	5	10
T. forsythia	2	4
<u>P. gingivalis</u>	1	2

**Table 4.4**

## Survey Questions and Responses

<b>Question:</b>	<b>Test (n=25)</b>			<b>Control (n=25)</b>		
<b>Response</b>	<b>Y %</b>	<b>N %</b>	<b>DK %</b>	<b>Y %</b>	<b>N %</b>	<b>DK %</b>
1. Was the suction device used during your cleaning today comfortable?	84	16	0	96	4	0
<b>2. Did the suction device make you feel as if you were going to gag?</b>	<b>20</b>	80	0	<b>0</b>	100	0
<b>3. Did the suction device stretch your cheeks?</b>	<b>12</b>	88	0	<b>0</b>	100	0
<b>4. Did the suction device stretch your lip(s)?</b>	<b>20</b>	80	0	<b>0</b>	96	4
<b>5. Did the suction device cause discomfort during your cleaning today?</b>	<b>16</b>	84	0	<b>4</b>	96	0
6. Did the suction device make you feel as if you were drowning during your cleaning?	4	96	0	4	96	0
7. Did the suction device help to keep fluids or debris from your face and/or your body during your cleaning today?	60	28	12	76	24	0
8. Did you feel that the suction device created a lot of noise?	24	76	0	20	80	0
<b>9. Would you like to have this suction device used during your next cleaning?</b>	<b>64</b>	24	12	<b>92</b>	0	8

## FIGURES

FIGURE 4.1

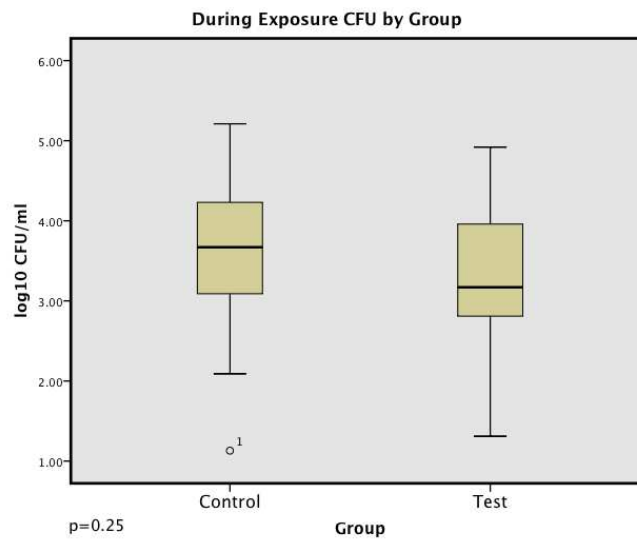
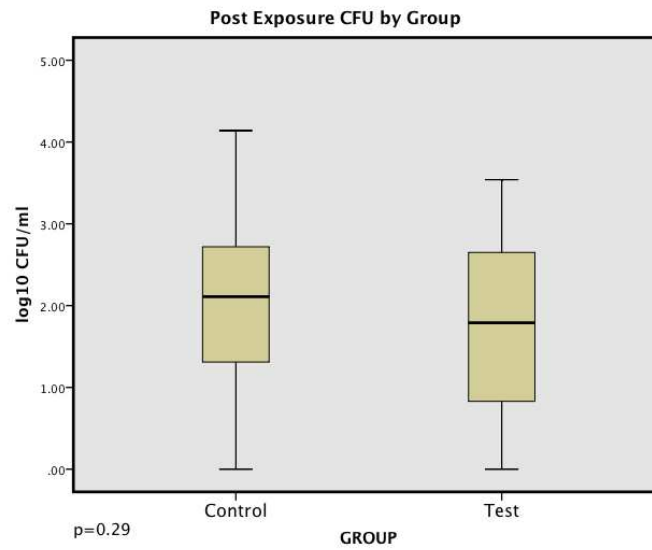
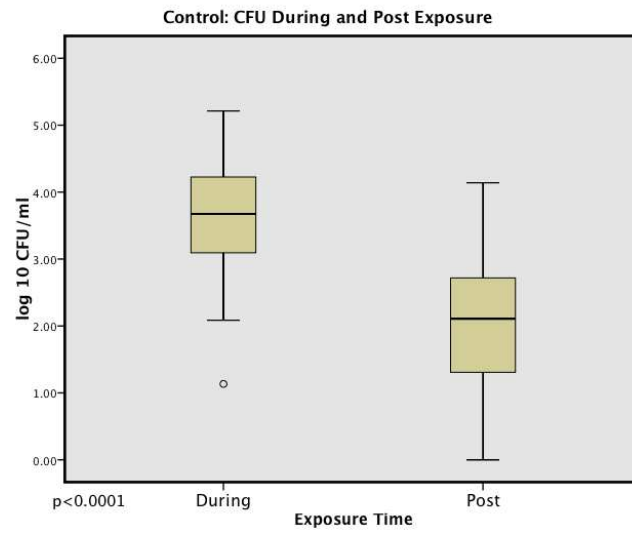


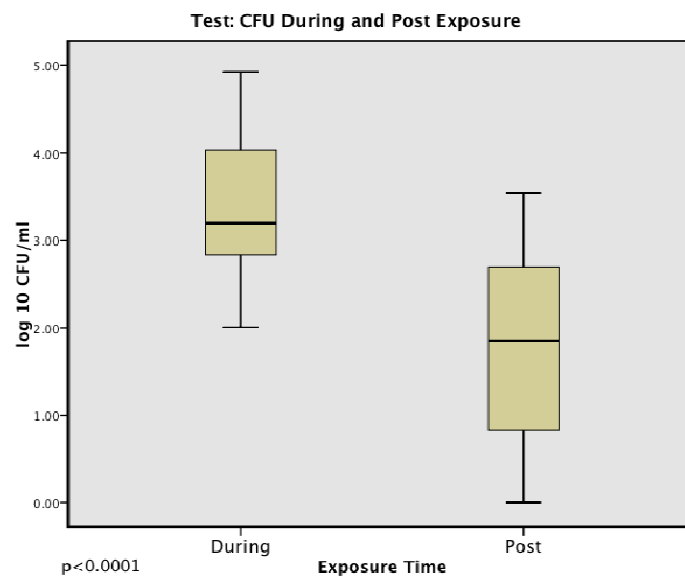
FIGURE 4.2



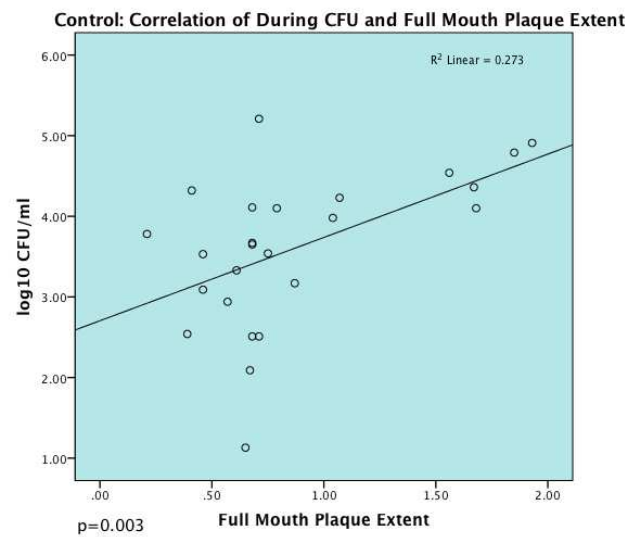
**Figure 4.3**



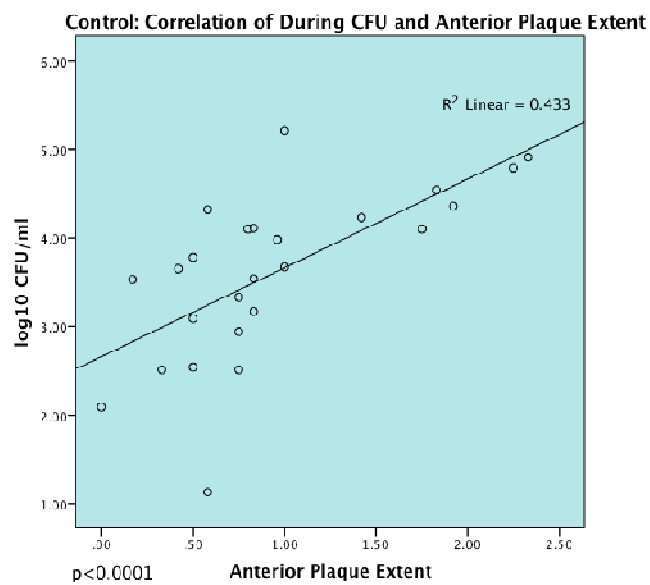
**Figure 4.4**



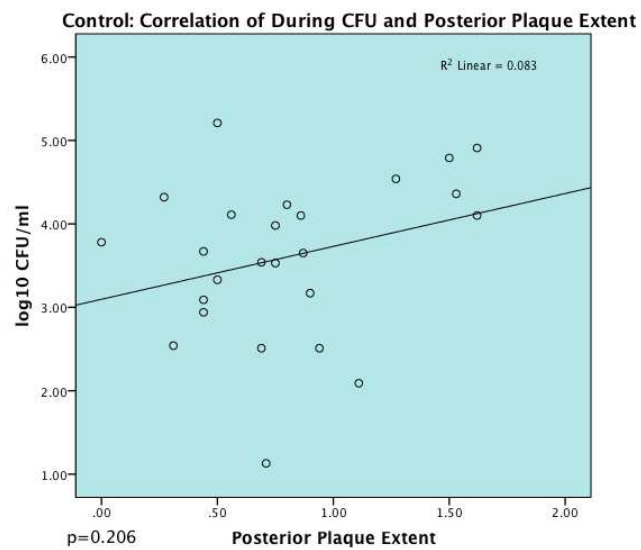
**Figure 4.5**



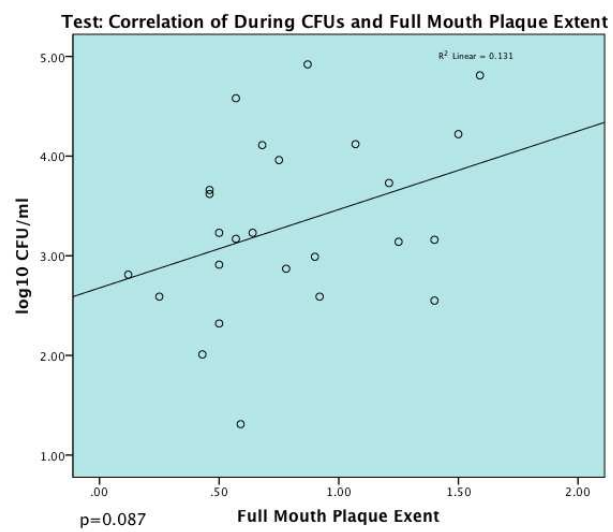
**Figure 4.6**



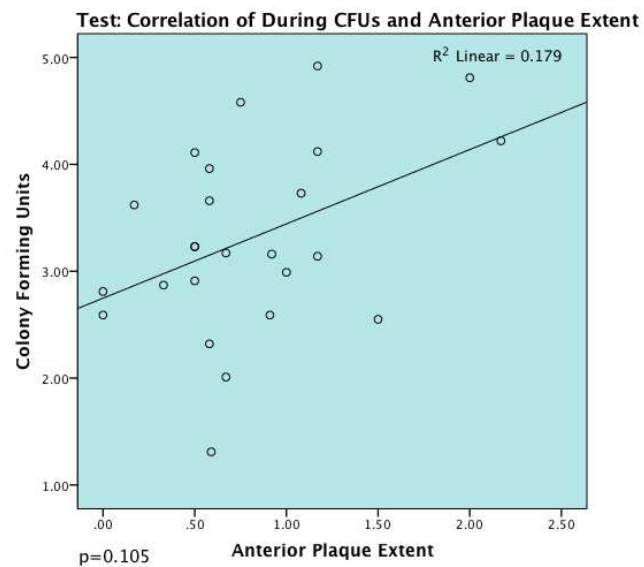
**Figure 4.7**



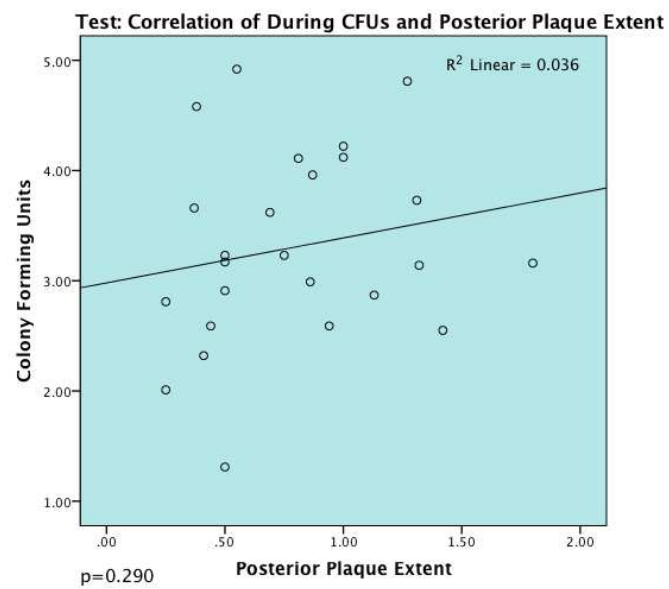
**Figure 4.8**



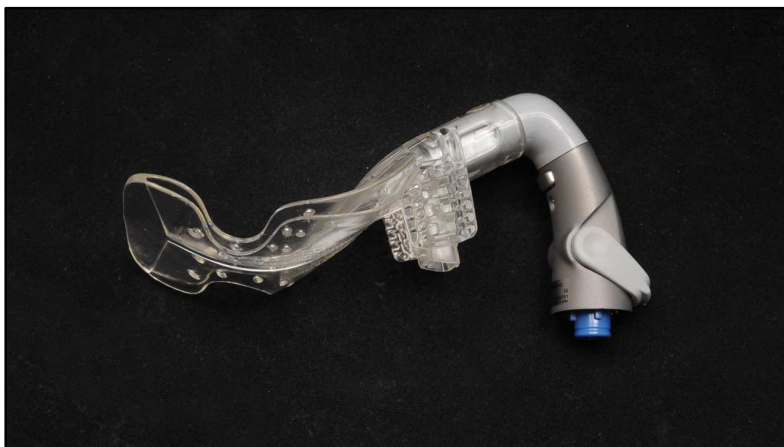
**Figure 4.9**



**Figure 4.10**



## APPENDIX 1: TEST DEVICE



Courtesy of Isolite™ Systems, Santa Barbara, California

## **APPENDIX 2: IRB APPROVED TELEPHONE SCRIPT**

Hi, this is     (name)     calling from the GO Health clinic at the UNC Dental School. I am calling because you are due for your cleaning and you indicated that you would like to be contacted for any upcoming appropriate studies. We have a study that will be beginning in January. The purpose of the study is to determine if aerosols produced during dental cleanings can be reduced when using either a high volume suction or low volume suction. Your participation in the study will involve having your teeth cleaned while aerosol samples are collected in a Petri dish (laboratory saucer) filled with a salt-type solution (saline buffer). At the end of the cleaning, you will be asked to fill out a 10 question survey on the use of the suction device. Participation in the study will take approximately 90 minutes and there is no charge for participating. If you choose NOT to participate in the study, this will not affect you getting your teeth cleaned in the GO health clinic as a regular patient. If you would like to participate in the study, then I would like to ask you three questions to determine if you are eligible to participate.

1. Have you taken an antibiotic or steroid within the past week? (If no, then proceed to next question. If yes, then they are not eligible to participate in the study).
2. Are you currently undergoing treatment for a respiratory infection? (If no, then proceed to next question. If yes, then they are not eligible to participate in the study).
3. Do you currently have a cardiac pacemaker? (If no, then proceed to next question. If yes, then they are not eligible to participate in the study).
4. Are you able to tolerate the use of the ultrasonic scaler while getting your teeth cleaned? (If yes, then proceed to the next section. If no, then they are not eligible to participate in the study).

Based on your answers to these questions, we will be able to schedule your cleaning with the dental hygienist. We do ask that you refrain from all oral hygiene practices, such as brushing and flossing for 12 hours prior to your appointment.

Thank you for your time.

### APPENDIX 3: STUDY DESIGN PHASE



#### APPENDIX 4: PLAQUE EXTENT DATA FORM

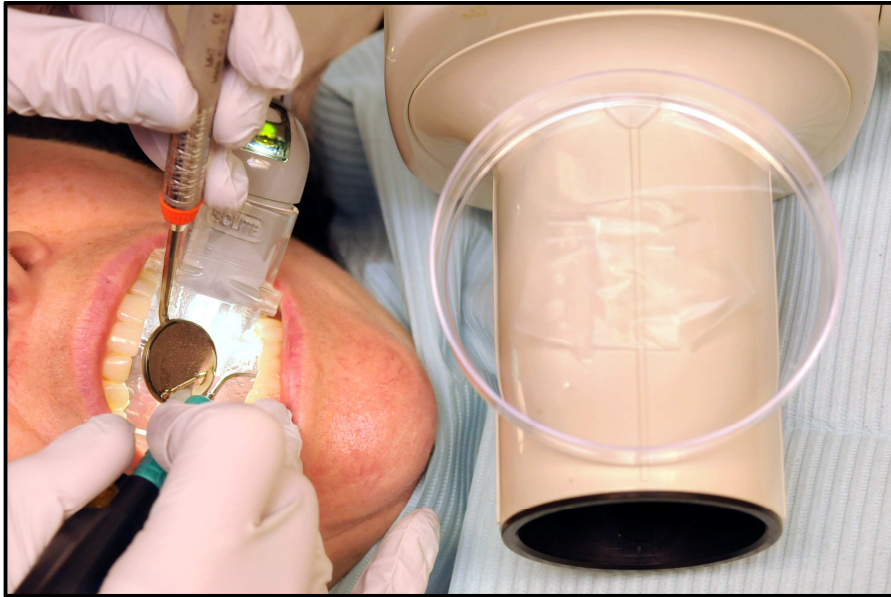
Subject # \_\_\_\_\_

Start Time:

End Time:

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
32	31	30	29	28	27	26	25	24	23	22	21	20	19	18	17

## APPENDIX 5: PLACEMENT OF PETRI DISH



## APPENDIX 6: SURVEY INSTRUMENT

Subject Number \_\_\_\_\_

### Study on the Effect of Suction Devices on Aerosol Production Post-treatment Subject Survey

Directions: Please circle the answer that best describes your experience today.

- |   |     |    |            |
|---|-----|----|------------|
| 1. Was the suction device used during your cleaning today comfortable?  | Yes | No | Don't Know |
| 2. Did the suction device make you feel as if you were going to gag?  | Yes | No | Don't Know |
| 3. Did the suction device stretch your cheeks?  | Yes | No | Don't Know |
| 4. Did the suction device stretch your lip(s)?  | Yes | No | Don't Know |
| 5. Did the suction device cause discomfort during your cleaning?  | Yes | No | Don't Know |
| 6. Did the suction device make you feel as if you were drowning?  | Yes | No | Don't Know |
| 7. Did the suction device help to keep fluids or debris from your face and/or your body during your cleaning today? | Yes | No | Don't Know |
| 8. Did you feel that the suction device created a lot of noise?   | Yes | No | Don't Know |
| 9. Would you like to have this suction device used during your next cleaning?                                       | Yes | No | Don't Know |
| 10. Please provide any additional feedback or comments regarding the device.  |     |    |            |

## APPENDIX 7: LAB CFU RECORDING FORM

SUBJECT # \_\_\_\_\_

Bacteria Present:

CFU COUNTS – During use of ultrasonic				
	Plate #1	Plate #2	Plate #3	Average:
@ 24-48 hrs method:				
@ 72-96 hrs method:				
@ 5-7 days method:				

CFU COUNTS – Post use of Ultrasonic				
	Plate #1	Plate #2	Plate #3	Average:
@ 24-48 hrs method:				
@ 72-96 hrs method:				
@ 5-7 days method:				

## REFERENCES

1. Snider D (1992). *Occupational Safety Health Report- Centers for Disease Control and Prevention*, 1125.
2. Miller RL, Micik RE, Able C, Ryge G (1971). Studies of dental aerobiology: II. microbial splatter discharge from the oral cavity of dental patients. *Journal of Dental Research* 50:621-625.
3. Miller RL, Micik RE (1978). Air pollution and its control in the dental office. *Dental Clinics of North America Journal* 22:453-476.
4. Kohn WG, Collins AS, Clebeland JL, Harte JA, Eklund KJ, Malvitz DM (2003). Centers for Disease Control and Prevention (CDC) Guidelines for infection control in dental health-care setting. *Morbidity and Mortality Weekly Report*, 52(17):16-70.
5. Harrell SK (2004). Airborne spread of disease- implication for dentistry. *Journal of the California Dental Association* 32:901-906.
6. Centers for Disease Control and Prevention. Recommended infection-control practices for dentistry. MMWR (1993) (accessed 2012 Sept 12]; 42(No. RR-8). Available from <http://www.cdc.gov/mmwr/preview/mmwrhtml/00021095.htm>
7. American Dental Association 2012 Current Policies. [Internet] 2012 [cited 2012 Sept]; Available from: <http://www.ada.org/currentpolicies.aspx>
8. Isolite™ Systems, Santa Barbara, California
9. Jacks, ME (2002). A laboratory comparison of evacuation devices on aerosol reduction. *Journal of Dental Hygiene* 76(3):202-206.
10. Cole EG, Cook CE (1998). Characterization of infectious aerosols in health care facilities: An aid to effective engineering controls and preventive strategies. *American Journal of Infection Control* 26(4):453-464.
11. Finegold SM (1991). Aspiration pneumonia. *Review of Infectious Disease*. 13:S737-S742.
12. Siegman-Igra Y, Schwartz D, Ophir O, Konforti N. (1984). Endocarditis caused by *Actinobacillus actinomycetemcomitans*. *European Journal of Clinical Microbiology* 3:556-559.
13. Limeback, H (1998). Implications of oral infections on systemic disease in the institutionalized elderly with a special focus on pneumonia. *Annals of Periodontology*. 3(1): 262-275.

14. Muir KF, Ross PW, Macphee IT, Holbrook WP, Kowolik MJ (1978). Reduction of microbial contamination from ultrasonic scalers. *British Dental Journal*. 145:76-78.
15. Micik RE, Miller RL, Mazzarella MA, Ryge G (1969). Studies on dental aerobiology: I. bacterial aerosols generated during procedures. *Journal of Dental Research*, 48:49-56.
16. Micik RE, Miller RL, Leong AC (1971). Studies on dental aerobiology, III: Efficacy of surgical masks in protecting dental personnel from airborne bacterial particles. *Journal of Dental Research*. 50:626-630
17. Abel LC, Miller RL, Micik RE, Ryge G (1971). Studies on dental aerobiology, IV: Bacterial contamination of water delivered by dental units. *Journal of Dental Research*. 50: 1567-1590
18. Sze To GN, Wan MP, Chao C, Wei F, Yu SCT, Kwan JKC. (2008). A methodology for estimating airborne virus exposures in indoor environments using the spatial distribution of expiratory aerosols and virus viability characteristics. *Journal of Indoor Air*. 18:425-438.
19. Wells WF, Stone WR (1934). On air-borne infection. Study III. Viability of droplet nuclei infection. *American Journal of Dental Hygiene*. 20:619-26
20. Wells WF (1955). Airborne contagion and air hygiene. *Cambridge University Press*, 117-122.
21. Eickhoff TC (1994). Airborne nosocomial infection: a contemporary perspective. *Infection Control Hospital Epidemiology*. 15: 663-72.
22. Beck-Sague C, Dooley SW, Hutton MD, Crawford JT, Williams J, Sordillo EM et al. (1992). An outbreak of multidrug resistant tuberculosis among hospitalized patients with the acquired immunodeficiency syndrome. *New England Journal of Medicine*. 326: 1514-21.
23. Joseph CA, Watson JM, Harrison TG, Bartlett CL (1994). Nosocomial legionnaires' diseases in England and Wales, 1980-1992. *Epidemiol Infect* 112: 329-45.
24. Bloch AB, Orenstein WA, Ewing WM, Spain WH, Mallison GF, Herrmann KL, et al. (1985) Measles outbreak in a pediatric practice: airborne transmission in an office setting. *Pediatrics* 75: 676-83.
25. Leggat PA, Kedjaurine U (2001). Bacterial aerosols in the dental clinic: A review. *International Dental Journal* 51(1): 39-44.
26. Centers for Disease Control and Prevention (1992). Transmission of multidrug resistant tuberculosis among immunocompromised persons in a correctional system. Massachusetts Medical Society, Waltham. *MMWR Morbidity and Mortality Weekly Report* July 1992 41(28):507-590.

27. Larato DC, Ruskin P, Martin A (1967). Effect of an ultrasonic scaler on bacterial counts in air. *Journal of Periodontology* 38:550-554.
28. Williams GH, Pollok NL, Shay De Barr CE (1970). Laminar air purge of microorganisms in dental aerosols: Prophylactic procedures with the ultrasonic scaler. *Journal of Dental Research* 49: 1498-1504
29. Harrel S, Barnes J, Rivera-Hidalgo F (1998). Aerosol and splatter contamination from the operative site during ultrasonic scaling. *Journal of the American Dental Association* 129: 1241-1249.
30. Basu MK, Browne RM, Potts AJC. (1988). A survey of aerosol related symptoms in dental hygienists. *J Soc Occup Med* 38: 23-35.
31. Bentley CD, Burkhart NW, Crawford JJ (1994). Evaluating splatter and aerosol contamination during dental procedures. *Journal of the American Dental Association* 125: 579-584
32. Klyn SL, Cummings DE, Richardson BW, Davis RD (2001). Reduction of bacteria-containing spray produced during ultrasonic scaling. *Journal of General Dentistry* 49(6): 648-652
33. King TB, Muzin KB, Berry CW, Ander LM (1997) The effectiveness of an aerosol reduction device for ultrasonic scalers. *Journal of Periodontology* 68:45-49
34. Harrel SK, Barnes JB, Rivera-Hidalgo F (1996). Reduction of aerosols produced by ultrasonic scalers. *Journal of Periodontology* 67(1): 28-32
35. Jacks M, Pollard C (2007). Isolite™ v. High Volume Evacuation: Effects on dental aerosol reduction. *Unpublished*.
36. Dahlke WO, Cottam MR, Herring MC, Leavitt JM, Ditmyer MM, Walker RS (2012). Evaluation of the spatter-reduction effectiveness of two dry-field isolation techniques. *Journal of the American Dental Association* 143(11):1199-1204.
37. Noro A, Kameyama A, Asami M, Sugiyama T, Morinaga K, Kondou Y, Tsunoda M (2009). Clinical usefulness of "Isolite™ plus: For oral environment of Japanese people. *The Bulletin of Tokyo Dental College* 50(3):149-155.
38. Colette J, Wilson S, Sullivan D (2010). A study of the Isolite™ system during sealant placement: Efficacy and patient acceptance. *Journal of American Pediatric Dentistr* 32(2): 146-150.
39. Andersen A (1958) A new sampler for the collection, sizing, and enumeration of viable airborne particles. *J Bact* 76:471-484.

40. Rautemaa R, Nordberg A, Wuolijoki-Saaristo K, Meruman JH (2006). Bacterial aerosols in dental practice – a potential hospital infection problem? *Journal of Hospital Infection* 64:76-81.
41. Grenier D (1995). Quantitative analysis of bacterial aerosols in two different dental clinic environments. *Applied Environmental Microbiology* 61:3165-3168.
42. Feres M, Figueiredo LC, Faveri M (2010). The effectiveness of a preprocedural mouthrinse containing cetylpyridinium chloride in reducing the bacteria in the dental office. *Journal of the American Dental Association*. 141(4):415-422.