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Adequacy of Sterilization Techniques for NOLA Dry Field Retractors

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June 2018

Adequacy of Sterilization Techniques for NOLA Dry Field Retractors

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Adequacy of Sterilization Techniques for NOLA Dry Field Retractors

A THESIS

Presented to the Faculty of

Uniformed Services University of the Health Sciences

In Partial Fulfillment

Of the Requirements

For the Degree of

MASTER OF SCIENCE

By

Katherine Marie Darling Lund, D.D.S.

San Antonio, TX

June 30, 2018

The views expressed in this study are those of the author and do not reflect the official policy of the United States Army, the Department of Defense, or the United States Government. The author does not have any financial interest in the companies whose materials are discussed in this article.

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DEDICATION

This thesis is dedicated to my family. To my mom and dad, for all their encouragement in my educational endeavors, for their support in pursuing my goals, and for their constant faith in me. To my amazing and talented sisters, Kimmie and Amy, for their ability to lift my spirits and inspire me to achieve more. Finally, to my brilliant and wonderful husband, for his constant support, devotion and love, and for always inspiring and encouraging me to follow my dreams.

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ABSTRACT

Since its development in the mid-1980s, the NOLA Dry Field System (NOLA) has provided many orthodontists the means to achieve more optimal moisture isolation for orthodontic bonding procedures. However, while the small diameter suction tubing is desirable from a patient comfort standpoint, it is likely the most challenging component to effectively sterilize because of its narrow lumen. We hypothesized that there would be no bacteria present in the NOLA Dry Field suction tubing following mechanical rinsing with water and steam sterilization. Four test groups (N=10) including a positive control were inoculated with a 1.5 x 10^7 CFU/mL solution of *Geobacillus stearothermophilus*. One group was not inoculated to serve as a negative control. All groups, excluding the positive control, were subjected to varying levels of mechanical cleansing prior to sterilization with a steam autoclave. Swipe tests were also collected from NOLA Dry Field cheek retractors and tongue guards to check for microbial contamination at the time of removal from their original packaging. Results showed no bacterial growth of any of the samples (groups 2-5) that were processed in the steam autoclave. Similarly, no bacterial growth was observed from the swipe samples collected from the NOLA systems taken immediately after removal from the packaging. Within the scope of this study, it appears that the NOLA Dry Field System can be successfully sterilized after bacterial contamination. This finding lends support to the claims by Great Lakes Orthodontics that it can be reprocessed for repeated use. Our swipe test results provide reassurance that the NOLA is free of microbial contamination

immediately upon removal from the packaging. However, the author would still recommend following the manufacturer's recommendations for processing prior to use.

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I. INTRODUCTION

Since its development in the mid-1980's, the NOLA Dry Field System (NOLA) (Figure 1) has provided many orthodontists the means to achieve more optimal moisture isolation for orthodontic bonding procedures. The NOLA's combination of retraction and saliva evacuation, as well as its compact design, make it a desirable item for the orthodontic armamentarium. However, with ever increasing knowledge and improvements in infection control protocols, the NOLA's design also raises some questions as to whether it can be effectively sterilized. While the small-diameter suction tubing is desirable from a patient comfort standpoint, it is likely the most challenging component to effectively sterilize because of its narrow lumen.

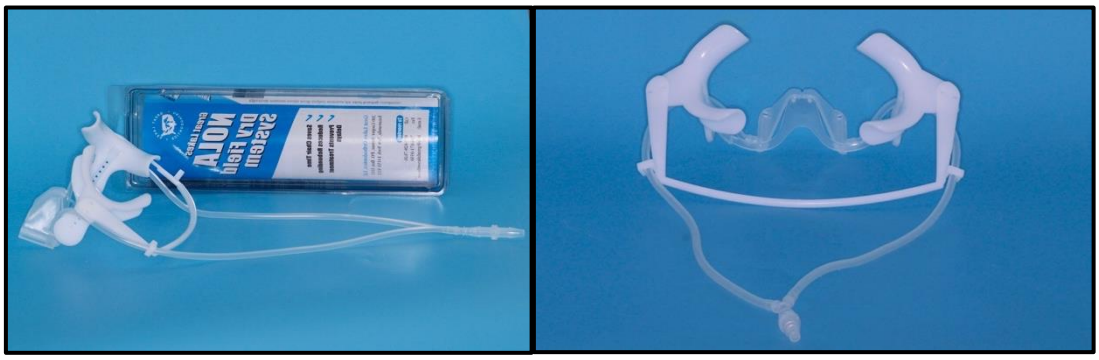

Figure 1. Images of the NOLA Dry Field System and original packaging.

As more research is conducted regarding dental infection control procedures, some members of the dental profession have begun to doubt whether commonly accepted methods actually result in sterility. Using autoclave procedures to achieve sterility of reusable dental burs and endodontic files is among those methods that have been called into question. Morrison and Conrad (2009) found that test groups of reusable burs and files had contamination rates of up to 15%

and 58%, respectively, after autoclave sterilization. These findings led them to conclude that these instruments should be considered single-use items.

Great Lakes Orthodontics, the manufacturer of the NOLA, maintains that all NOLA components can be sterilized for repeated use in patient care. Alternatively, they state that the NOLA may also be processed by cold sterilization with glutaraldehyde or an equivalent solution. They recommend that the device be sterilized on a “plastics” cycle in a steam autoclave for 30 minutes (if packaged) at a constant temperature of 250^{o}F (121^{o}C) with 15 psi of pressure. They advise that, prior to sterilization, the tubing should be flushed with water using the Great Lakes NOLA Purging Syringe (Figure 2) to remove saliva and debris.

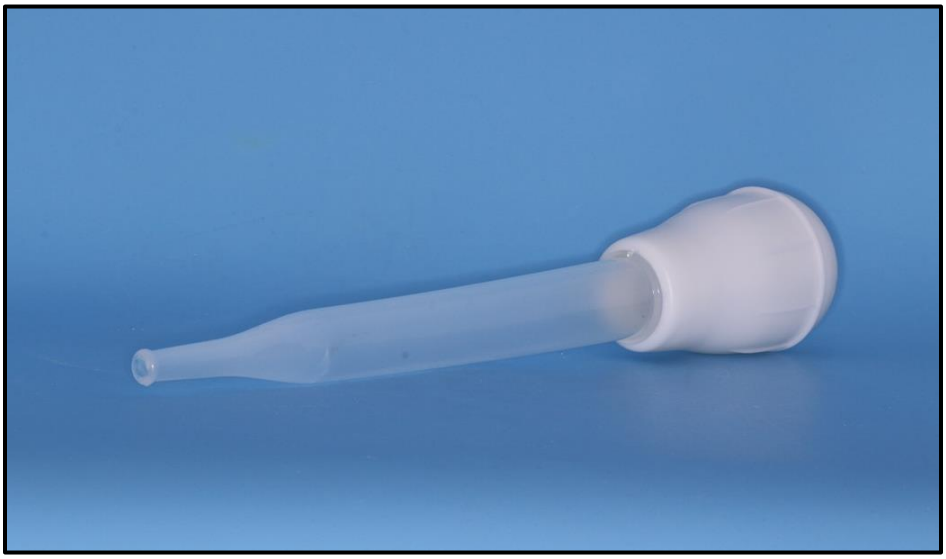

Figure 2. Great Lakes NOLA Purging Syringe used to flush tubing prior to processing in autoclave.

Steam sterilization cycles that include a vacuum purge are necessary for instruments with narrow lumina in order to remove entrapped air, so that the

steam will contact these internal surfaces. Then, in theory, the heat transfer from the condensation renders the instrument sterile. For this reason, the dry-heat sterilization used for many orthodontic instruments is insufficient for instruments with lumina (Sildve, 2012).

The problem for many instruments with lumina is ensuring that debris won't interfere with the condensation heat transfer necessary to kill microorganisms. Avoiding interference has proved difficult in the case of dental hand pieces that require oil lubrication. In this instance, the oil can impede access for steam to contact the internal surfaces (Kudhail, 2013). Other than visible debris, biofilms present are arguably the greatest challenge to fully sterilizing instruments with lumina. Biofilms are not cleared as easily as planktonic bacteria and are more resistant to biocides, which makes them particularly difficult to eradicate from tubing and instrument lumina. In a study examining contamination of dental syringes, more than 10^4 bacteria cells per milliliter were present after 6 minutes of flushing with water. In addition, sterilization of the entire syringe failed to render it free of bacterial contamination (Mayo, 1990).

In the case of sterilization of instruments with lumina, time may also be an important factor. Vickery et al. (2000) investigated whether dental syringes contaminated with duck Hepatitis B virus could still infect ducklings following sterilization procedures. Using a scanning electron microscope, they observed the presence of bacterial biofilm within the cannula lumen into which the needle hub is inserted. Syringes that had been autoclaved with steam sterilization at 134^{o}C for 3 minutes still transmitted the virus to 1 in 16 ducklings. However,

syringes that had been sterilized at 121^{o}F for 15 min didn't cause any transmission.

In addition to time, the diameter of the lumen may also be an important factor. In a study testing the effectiveness of sterilization of various 9.4 cm-long dead-end tubes of various lumen diameters (0.4-1.7 cm) contaminated with *Bacillus stearothermophilus*, investigators found that smaller diameter tubes required longer cycles. The researchers' main challenge was effectively removing all the air from the smaller diameter tubes. For the smallest diameter tubes, sterilization was not fully achieved even after a 2-hour cycle (Young, 1993).

While these studies provide insights for processing the NOLA, the author is unaware of any available literature to substantiate whether Great Lakes Orthodontics' recommendations effectively sterilize the NOLA suction tubing. Some of the most relevant research studies that may provide insight into NOLA sterilization are those of laryngoscope, endoscope and colonoscope processing. These instruments also have lumina that pose challenges in eradicating bacterial contamination after procedures. The endoscope is particularly applicable because, like the NOLA, the main source of contamination is from patient saliva.

The challenges of disinfecting endoscopes and colonoscopes were well studied throughout the 1990s and early 2000s. Foliente et al. (2001) were able to successfully use glutaraldehyde and manual cleaning to disinfect colonoscopes and duodenoscopes inoculated with 9×10^{7} colony forming units (CFU) of *Mycobacterium chelonae* per the FDA definition of high-level disinfection (6-log reduction of mycobacteria). In another study, 6 flexible laryngoscopes were

examined at the beginning, middle and end of the workday. Experimental groups consisted of laryngoscopes that had been contaminated with patient saliva. Test groups were compared with control groups after undergoing high-level disinfection. All control groups were positive for bacteria cultures, but all test groups were negative following high-level disinfection (Bhattacharyya and Kepnes, 2004). Furthermore, a literature review in 2003 evaluating present procedures for disinfecting endoscopes concluded that current disinfecting agents could ensure removal of most resilient pathogens, including HIV. However, the authors recommended strict adherence to published guidelines, including meticulous cleaning with a manufacturer-approved enzymatic detergent and thorough rinsing with water, prior to high-level disinfection (Lisgaris, 2003).

In addition to the inherent challenges of reprocessing instruments with lumina, another hindrance to successful disinfection is the skill and knowledge of the team processing the instruments. A review of otorhinolaryngology nursing clinical practices revealed confusion regarding which disinfectant to use and proper manner to use it when reprocessing endoscopes. The article maintained that high-level disinfection was an acceptable, safe and timely method, but that the practitioner had a responsibility to understand and employ proper protocols for this type of processing (McCullagh and Baker, 2000). In another study lasting 9 years, researchers attempted to grow cultures of bacteria from disinfected gastrointestinal endoscopes to measure the effectiveness of reprocessing. The results revealed a correlation between poor mechanical cleansing prior to disinfection and increased positive bacteria cultures. The authors advised that

environmental endoscope cultures be used regularly to monitor reprocessing procedures (Moses and Lee, 2003).

Some physicians have suggested the use of endoscope sheaths to decrease contamination and make sterilization or high-level disinfection more effective. Silberman et al. (1993) demonstrated that the use of prepackaged disposable sterile sheaths could improve disinfection procedures. Some skepticism surrounding these disposable endoscope sheaths involved their potential to develop holes that would still allow for contamination. One study looked at the effectiveness of endoscope sheaths at preventing viral contamination when perforated. A number of holes ranging from 2-30 μm was made in endoscope sheaths before introducing them into a high-titer virus suspension. Although some contamination was found, use of the sheaths in combination with high level disinfection was effective for reprocessing (Baker et al., 1999). However, because the NOLA is specifically designed to evacuate saliva from the patient's mouth, disposable barriers would likely interfere with this function.

Although some endoscope sterilization studies have used chemical cold sterilization, a study in the Journal of Hospital Infection (Ayliffe, 2000) maintained that all heat-tolerant endoscopes should still be reprocessed using vacuum-assisted steam sterilizers. The study concluded that sterilizers without vacuum-assisted air removal were inadequate for instruments with lumina. The authors also reported that submersion in 2% glutaraldehyde was needed to disinfect against mycobacterial species and required 3 hours of contact to kill bacterial spores. Control measures such as gloves, aprons, and eye protection are

needed to protect employees handling this solution. They also recommended that employees be screened for mucosal sensitivity or asthma prior to using this solution (Ayliffe, 2000). Because of the inherent risks of handling high-level disinfectants, steam autoclave sterilization may be the most fitting method for reprocessing of the NOLA in the orthodontic practice.

As previously mentioned, there is a lack of research to substantiate the current recommendations for sterilization of the NOLA System. However, one study by Barnes et al. (1994) investigating the effectiveness of sterilization procedures to eliminate contamination in the internal components of prophylaxis angles provided some helpful insights into the experimental design for the present study. Test groups included both oiled and non-oiled prophylaxis angles that were processed either assembled or disassembled. *Bacillus stearothermophilus* spore concentrations of 1.15×10^6 CFU/mL were used to create a bioburden much higher than likely found in a typical oral environment. The spores were also combined with 20% bovine serum albumin to simulate whole blood, in order to assess whether organic debris would inhibit sterilization effectiveness. Steam autoclave procedures rendered the prophylaxis angles sterile in all test groups.

Geobacillus stearothermophilus (formerly *Bacillus stearothermophilus*) is a gram-positive, rod-shaped bacterium that grows within a temperature range of 30-75^{o}C. It is a common cause of food spoilage and is routinely used as a biological indicator in sterilization due to its highly heat-resistant endospores. Dormant bacterial endospores are highly resistant to many physical and chemical

treatments, including heat, drying, radiation and chemical disinfectants such as Cavicide, hydrogen peroxide, ethanol and glutaraldehyde. The most effective way of eliminating bacterial endospores is through the sterilization process. Steam sterilization involves moist heat at 121°C or higher temperatures at 30 psi for 30 minutes, which destroys most forms of life, especially microorganisms (including bacterial spores). The *Geobacillus stearothermophilus* spore is one of the most heat-resistant spores of aerobic microorganisms (Albert et al., 1998).

Both Type 5 indicators (e.g. Figure 3) and biological indicators are used during the sterilization process to provide direct evidence that conditions are sufficient to kill bacterial spores. In most steam autoclaves, the biological indicator used is *Geobacillus stearothermophilus* spores due to their resistance to heat and pressure (Watanabe et al., 2003). If these bacterial endospores have been eliminated, then all less virulent microorganisms are eliminated, and the lethality of the sterilization process is assured. Biological indicators can detect even marginal sterilization failures that result from inability to reach temperature, inadequate air removal or insufficient superheated steam. They are considered the "gold standard" of load-sterilization monitoring (Albert et al., 1998).

Figure 3. 3M Comply SteriGage class 5 biological indicator strips; unprocessed (left) and post-autoclave processing indicating successful sterilization (right).

A self-contained biological indicator consists of a spore strip (spores that are coated on a paper strip) enclosed in a plastic vial along with growth medium contained in a crushable glass ampoule (Figure 4). The cap is designed to allow steam to penetrate into the plastic vial, killing the spores and demonstrating that sterilization conditions were met. After exposure to the sterilization process, the spores must be incubated to determine if any survived. Live spores start to grow when they come in contact with the nutrients contained in the growth medium and when incubated at the correct temperature. A color and/or turbidity change is used to denote success or failure of the sterilization process. No color change indicates that the sterilization conditions were achieved, while change indicates spore growth and, thus, a lack of sterility (Albert et al., 1998).

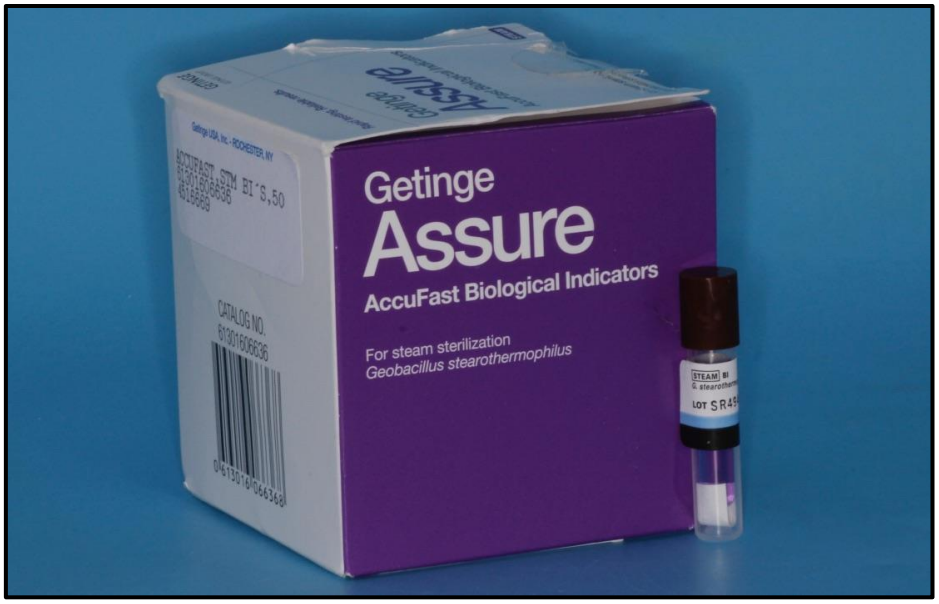

Figure 4. Getinge Assure AccuFast Biological Indicator *Geobacillus stearothermophilus* spore test kit.

II. OBJECTIVES

A. Overall Objective

The aim of this present study is to determine whether the recommended autoclave procedures, as outlined by Great Lakes Orthodontics, are effective in sterilizing the suction-tubing lumen of the NOLA Dry Field System after bacterial contamination.

B. Specific Hypothesis

There will be no bacteria present in the NOLA Dry Field suction tubing following mechanical rinsing with water and steam sterilization.

C. Null Hypothesis

There is no difference in the amount of bacteria present in the NOLA Dry Field tubing before and after mechanical rinsing and steam sterilization.

III. MATERIALS AND METHODS

This experiment was designed to determine whether conventional sterilization with saturated steam autoclaving adequately sterilizes the bilateral silicone tubing and the “Y” connector in the Great Lakes NOLA Dry Field System. Five groups, each composed of ten sets of tubing and “Y” connectors, were used in this study. Prior to inoculation with bacteria, the tubing and “Y” connectors were sterilized using a Steris steam sterilizer at 250°F (121°C) at 15 psi for 30 minutes.

Geobacillus stearothermophilus ATCC 7953 was grown on nutrient agar (Remel™, R01640) and incubated at 50°C ambient air for 3 days. This bacterial strain is used for sterilization validation studies and periodic validation of sterilization cycles. Bacterial suspension of *Geobacillus stearothermophilus* was prepared by harvesting growth of the bacteria from nutrient agar and suspending it in sterile saline to a turbidity meeting the 0.5 McFarland turbidity standard (approximately 1.5×10^8 CFU/mL). The suspension was then diluted 1:10, resulting in a bacterial suspension of approximately 1.5×10^7 CFU/mL.

The tubing and “Y” connector were inoculated (Figure 5) with 2.5 mL of bacterial suspension using a 5 mL sterile syringe (B-D, 301604). Green clamps (Terumo BCT, 1BB*CLIPS) were used to retain the bacterial suspension in the tubing for 90 minutes to simulate the time a NOLA Dry Field retractor would be in a patient’s mouth. After the bacterial suspension was drained from the tubing, the tubing remained at room temperature for 2 hours (Figure 6) to simulate the potential delay before tubing is sterilized.

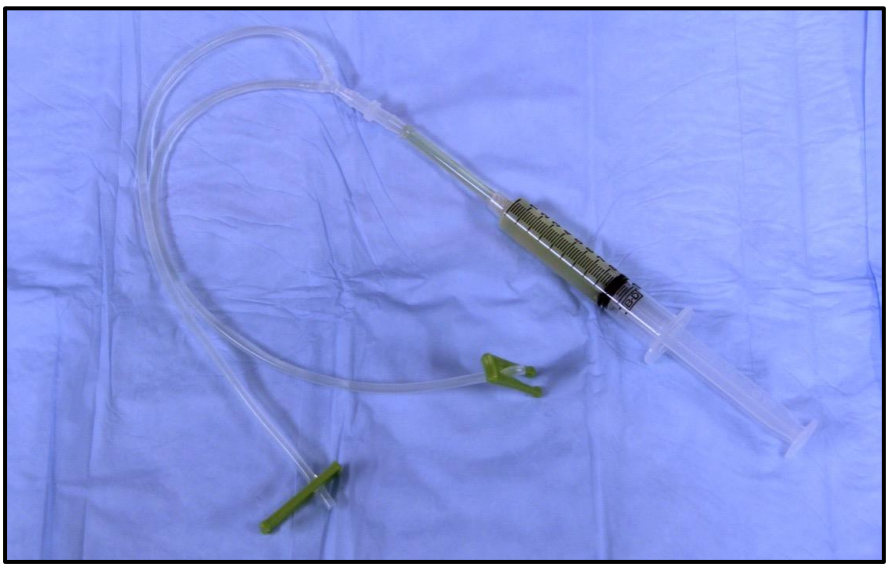

Figure 5. Inoculation of the NOLA Dry Field "Y" connector and evacuation tubing with *Geobacillus stearothermophilus* suspension.

Figure 6. NOLA tubing samples on sterile drapes following inoculation during 2-hour delay period prior to processing for sterilization.

The following pre-sterilization steps were performed for each experimental group:

Group 1: Not flushed or sterilized after inoculation with bacterial suspension (Positive Control).

Group 2: Not inoculated with bacteria, injected with sterile saline (Negative Control).

Group 3: Inoculated and flushed with 5 mL deionized water using Great Lakes NOLA purging syringe.

Group 4: Inoculated and flushed by suctioning 5 mL deionized water using a sterile syringe.

Group 5: Inoculated and not flushed after inoculation with bacteria.

Groups 2 through 5 were then steam sterilized. Following sterilization, the retractor tubing was injected with 2.5 mL of sterile saline. The recovered saline was serially diluted and plated on nutrient agar and incubated at 50°C ambient air for 3 days (Figure 7). The number of colony forming units (CFU) on the plates were counted, and CFU/mL recovered was calculated. One mL of the recovered saline was also inoculated into Trypticase Soy Broth (BBL™, 221716) and incubated at 50°C ambient air for 7 days. The samples were microscopically evaluated on a hemocytometer to observe growth of *Geobacillus stearothermophilus* spores.

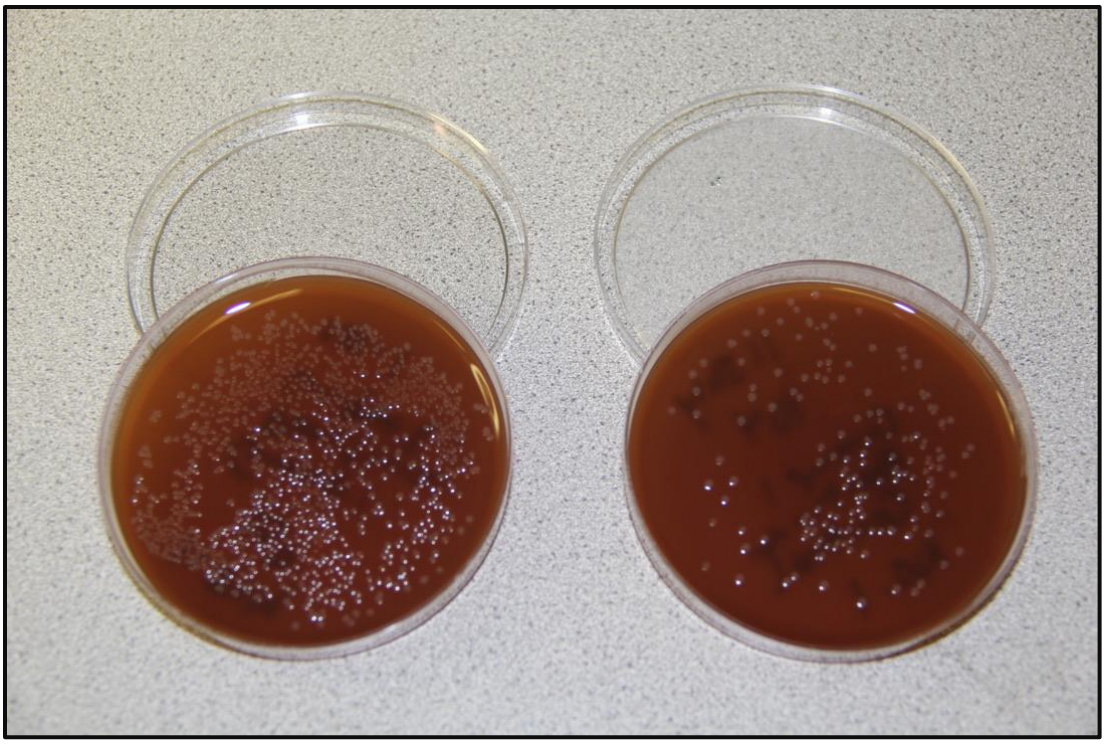

Figure 7. Positive control samples indicating growth of *Geobacillus stearothermophilus* on nutrient agar (incubated at 50°C ambient air for 3 days).

Swipe testing was performed on ten sets of tongue guards and cheek retractors to evaluate sterility of the NOLA Dry Field System directly upon removal from the original packaging. In addition, swipe testing was performed on the inside of ten saliva ejectors (Henry Schein®, 1004092) in order to determine whether the NOLA and saliva ejectors would have contamination directly out of the packaging. For each, a sterile swab moistened with sterile water was plated on trypticase soy agar (TSA) II and incubated at 35$^{\pm}$2°C ambient air for 24 hours. After incubation, the plates were read and results reported as “growth” or “no growth” for bacteria.

IV. RESULTS

In this study, colony forming units per milliliter (CFU/mL) of bacterial growth were analyzed for each of the test groups in order to ascertain the effectiveness of the sterilization procedures employed for the NOLA tubing. A One-Way Analysis of Variance (ANOVA) was used to detect significant differences in mean growth values among the test groups. Group 1 (positive control – not sterilized) was the only group that displayed any measurable growth and had a statistically significantly different mean from the other 4 test groups: $F(4,45) = 77.505$, $p < 0.0001$. Analysis was performed in SPSS v.22 (IBM Corp).

The mean growth for Group 1 (positive control) was 1,028,000 CFU/ml (Std. Dev. = 369,257.513; 95% confidence interval: 763,849.09 ±1,292,150.91) (Figure 8). All of the other test groups (groups 2-5), which underwent sterilization procedures, displayed no growth. No significant differences were found among any of the sterilized test groups (groups 2-5).

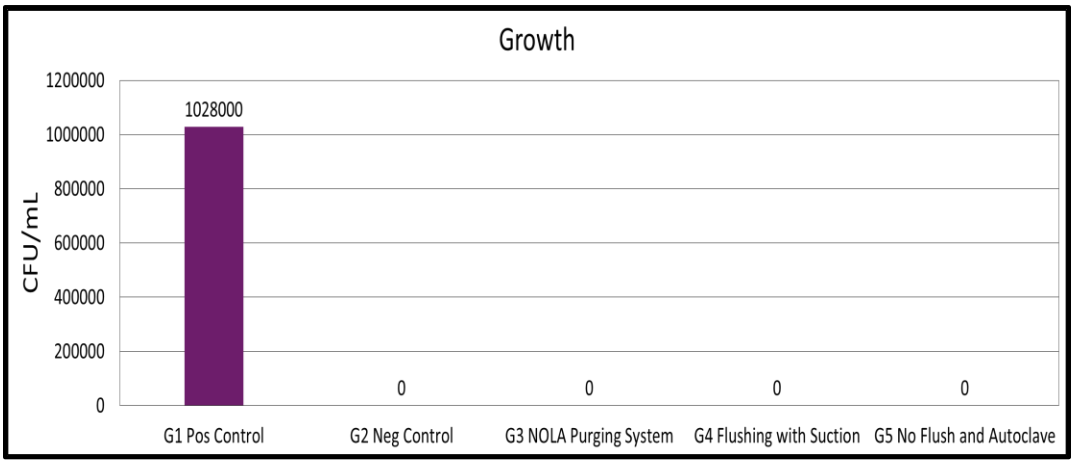

Figure 8. Mean growth values (CFU/mL) for each of the 5 test groups (N=10).

Incubation of the swabs from 10 NOLA retractors taken straight out of the original packaging yielded no bacterial growth. No bacteria such as *Micrococcus*, S*taphylococcus*, *Bacillus* or *Pseudomonas* species commonly found in businesses and homes were seen on the TSA II media. Similarly, no growth was observed from the plated swabs taken from the saliva ejectors. In all cases, the growth media had no bacteria after incubation at 35±2^{o}C for 24 hrs.

V. DISCUSSION

The NOLA system has been available to dentists and used in clinical practice for multiple decades. Those reluctant to use this system have expressed concerns about the efficacy of sterilization techniques in ensuring safe reuse of instruments with such narrow lumen. Thus, it seemed important to validate the sterilization procedures recommended by the manufacturer. In this study, the NOLA tubing was contaminated with *Geobacillus stearothermophilus* because it is used in biological indicators to validate whether sterility is achieved with steam autoclaves in clinical practice. A *Geobacillus stearothermophilus* endospore is one of the most resilient among bacteria and other pathogens. A lack of growth from the samples taken from NOLA tubing contaminated with these bacteria is considered a reliable test of sterility.

The challenge of sterilizing instruments with narrow lumina has been investigated by many in the medical community. However, a literature search revealed no studies that have investigated the effectiveness of sterilization procedures for the NOLA Dry Field System. In fact, it was difficult to find infection-control research in the dental community that addressed similar challenges involving sterilizing narrow lumina. Therefore, medical literature was reviewed about sterilization procedures for endoscopes and colonoscopes. While many of those studies are somewhat dated, they helped frame the issue of why instruments with narrow lumina are so difficult to sterilize. They also provided insight into how these challenges have been addressed with various sterilization protocols.

One challenge for the present invitro study was to develop a research design that would provide relevant insights for clinical practice. One study by Barnes et al. (1994) that examined sterilization procedures for the internal components of prophylaxis angles was very helpful in the research design. In this study, the investigators used a $1.15x10^6$ CFU/mL solution of *Bacillus stearothermophilus* to inoculate their prophylaxis angles. They maintained that the concentration they used was likely much higher than bacteria found in the oral environment. Multiple studies examining concentrations of *Streptococcus mutans* and *Lactobacillis* have found average oral concentrations to be in the range of 10^4-10^6 CFU/mL of test subjects (Van Houte and Green, 1974; Pannu et al., 2013). In this study, we used an inoculum of 1.5×10^7 CFU/mL based on a 1:10 dilution of the 0.5 McFarland turbidity standard, so that our inoculum would definitely be higher than average concentrations of oral bacteria.

Another factor deemed crucial to research design was contact time between the bacterial inoculum and the tubing. We wanted to ensure that we allowed adequate contact time to best simulate a clinical situation. Considering the typical length of a bonding appointment in both the residency setting and private practice, 90 minutes seemed more than adequate. However, we also assumed that the tubing may not be processed immediately after use depending on the office size and availability of staff. For this reason, we allowed the tubing to sit at room temperature for 2 hours following inoculation before initiation of processing for sterilization.

Based on our results, it appears that autoclaving the NOLA Dry Field systems after bacterial contamination rendered them sterile in all cases, independent of any pre-sterilization measures such as flushing with the Great Lakes NOLA purging system. Thus, even with no mechanical cleansing to lessen the bacterial contamination as in group 5, the tubing was still successfully sterilized (eliminating flushing in group 5 was to simulate not purging the NOLA after clinical use). In contrast, the mean growth of bacteria in the positive control (group 1) remained high, with a range of 5.9×10^5 to 1.66×10^6 CFU/mL. In some of the samples, there was less than a log difference from the inoculum solution, which suggests that our inoculation methods were successful. The fact that there was no difference between the negative control (group 2) and the other contaminated test groups (groups 3-5) after autoclaving helps to substantiate the success of the sterilization procedures employed in our study.

In addition to examining sterilization procedures for the NOLA, we were also interested to know whether contamination is present upon removal from its original packaging. Ideally, the NOLA would be sterilized before initial use, but we speculate that some practitioners may use it immediately after opening the packaging. Therefore, it seemed appropriate to know whether contaminants might be present. Our swipe samples tested for bacteria that would be commonly found in homes and businesses, such as *Micrococcus*, *Staphylococcus*, *Bacillus*, and *Pseudomonas* species. All of these bacteria should have grown on TSA II media if present on the swipe samples collected. Our results indicate that

microbial contamination of unopened NOLA Dry Field Systems in the original packaging is unlikely.

Although we were not able to culture any bacteria from the NOLAs immediately after removal from the packaging, they are not necessarily sterile. The manufacture does not claim that they are sterile in their original packaging and recommends that they be autoclaved prior to initial use. Though our findings provide some reassurance that they are likely free of microbial contamination and would be safe for use right out of the packaging, we cannot be certain based on this evidence alone. For this reason, it seems prudent to follow the manufacturer's guidance for processing prior to first use.

The results of our research also provide some reassurance that recommendations for processing the NOLA Dry Field System between patient encounters truly does sterilize it. However, one must also consider the limitations inherent within this study. One limitation to consider is the relatively small sample size for each of the test groups. With 10 per group, power analysis showed 80% power, which could be improved with a greater sample size.

Another possible limitation of this study is the difference between the bacterial inoculum used and patient saliva. Components of the salivary pellicle are known to be important for bacterial adhesion to teeth. Therefore, it may be harder to sterilize the NOLA tubing contaminated with actual patient saliva. Microbial contamination from patient saliva may adhere better to the lumen of the tubing than our inoculum. However, our results demonstrate that mechanical cleansing of the tubing prior to sterilization didn't seem to affect the ability of the autoclave

to render the samples sterile. Another question that remains is whether components of saliva could prevent steam from contacting bacteria to an adequate degree needed to kill it.

It is also worth mentioning that not every orthodontist uses the NOLA Dry Field System in his or her practice. There are many ways to achieve adequate isolation for orthodontic bonding procedures. The isolation method employed often depends on whether a direct or indirect bonding method is used. Conclusions drawn from our research are relevant only to sterilization procedures specifically intended for the NOLA System.

VI. CONCLUSION

Within the limited scope of this study, it appears that the NOLA Dry Field System can be successfully sterilized after bacterial contamination. This finding lends support to the claims by Great Lakes Orthodontics that it can be reprocessed for repeated use. Results supported the specific hypothesis that there will be no bacteria present in the NOLA Dry Field suction tubing following mechanical rinsing with water and steam sterilization. Although our study results indicate that mechanical flushing makes no difference, it is prudent to remove as many contaminants as possible from the lumen of the NOLA prior to processing with an autoclave. Due to inherent differences between patient saliva and our inoculum as discussed above, we would recommend using the Great Lakes NOLA purging syringe prior to processing with an autoclave, as directed by the manufacturer. Our swipe test results provide some reassurance as to the lack of microbial contamination of the NOLA immediately upon removal from the packaging. However, due to the limited scope of testing, we would still recommend following the manufacturer's recommendations for processing prior to use.

More research studies with larger sample sizes would help to confirm or refute our findings and provide more legitimacy for the sterilization procedures recommended by Great Lakes Orthodontics. Also, even though the *Geobacillus stearothermophilus* used in our study is currently the standard for determining sterility, future studies using inoculum more representative of patient saliva would be valuable.

VII. REFERENCES

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