COMPARISON OF SPORICIDAL ACTIVITIES OF COMMERCIAL DISINFECTANT WIPES FOR SURFACE DECONTAMINATION

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ABSTRACT

Li Chen: Comparison of Sporicidal Activities of Commercial Disinfectant Wipes for Surface Decontamination
(Under the direction of Sally Mauriello)

Digital intraoral receptors present an infection control challenge since they cannot be sterilized using traditional methods. This study evaluated the sporicidal effectiveness of CaviWipe®, Volo™, and Dispatch® wipes by directly treating spore strips impregnated with *Geobacillus stearothermophilus* spores and after incubation, observing for growth. Treated spore strips were progressively washed in trypticase soy broth (TSB) to determine both spore release and residual disinfectant removal. Ethylene oxide was effective in killing spores within the sealed barrier bag. All three disinfectants totally inhibited growth when disinfectant-treated strips were placed directly in TSB. Bacterial growth was recovered after Dispatch® treatment by washing the treated strip suggesting inhibition was not sporicidal. In contrast, ten second exposures of either spore strips or contaminated PSP barrier bags with CaviWipe® or Volo™ resulted in sporicidal activity comparable to overnight sterilization with ethylene oxide. These wipes are a practical approach to decontaminate PSP barriers prior to receptor processing.

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LIST OF ABBREVIATIONS

ADA	American Dental Association
C	Celsius Temperature Scale
CCD	Charge-coupled Device
CDC	Centers for Disease Control and Prevention
EtO	Ethylene Oxide
PBS	Phosphate-buffered Saline
PSP	Photostimulable Phosphor
ROS	Reactive Oxygen Species
TSB	Trypticase Soy Broth

CHAPTER 1: INTRODUCTION

History of Cross Contamination

The mode of transmission for many diseases is via saliva, blood, or aerosols. Since bacteria and viruses are present in high numbers in saliva, the oral cavity provides a fertile environment for cross-contamination among patients. Thus, the prevention of cross-contamination of microbes among patients is paramount in dentistry. Most often, the transmission of microbes has been prevented by the sterilization of instruments or through the use of disposable armamentarium. Radiographic film packets were most often treated with disinfectants or barrier bags to reduce the microbial load prior to processing and mounting. Unlike film receptors, the reuse of digital receptors in the oral cavity, both charge-coupled devices (CCD) and Photostimulable Phosphor (PSP) receptors makes it increasingly difficult to prevent cross-contamination.

The receptor technology of intraoral digital imaging has created challenging obstacles to preventing cross-contamination among patients. Currently, the Center for Disease Control (CDC) recommends heat sterilization or a high level of disinfection for the digital PSP receptors. Since the phosphor layer of the receptors can be easily damaged by high heat or steam sterilization, the CDC states that minimally the PSP receptors must be protected by FDA approved barriers. The CDC cautions practitioners that the barriers only decrease gross bio burden and that the receptors can still become contaminated upon removal from the barrier bag.

One method of sterilization that doesn't appear to harm the phosphor layer on the PSP receptor is ethylene oxide. Unfortunately, ethylene oxide is not environmentally friendly and not all dental offices have access to ethylene oxide sterilization. Moreover, radiographic equipment

such as scanners, black boxes, viewboxes, and computers used to view the images cannot be sterilize

with ethylene oxide. Contaminated surfaces can increase transfer of diseases from provider to patient, patient to provider and patient to patient. For instance, after removal of contaminated PSP receptors enclosed in barriers from the patient's mouth, the contaminated receptor is removed from the barrier bag and dropped into a black box to transfer it to the scanning room. However, it is difficult to remove the receptor from the contaminated barrier bag without touching the edges of the receptor. As a result, it is possible that receptor was contaminated after it was removed from the barrier, and then contaminated the scanner and other radiographic equipment. Furthermore, as more dental offices convert to digital imaging, it is important to investigate alternative methods for minimizing cross-contamination with the use of PSP digital receptors. Possible alternative methods may be chlorine-based and/or phenol-based disinfectants. The primary aim of this study was to break the chain of cross contamination before opening the contaminated barrier bag by wiping down the contaminated barrier bags with high level disinfectant. The specific aims of the study are:

- To evaluate sporicidal ability of ethylene oxide gas sterilization on *Geobacillus stearothermophilus* spores.
- To determine if ethylene oxide gas sterilization penetrates the barrier bag.
- To determine sporicidal ability of three types of disinfectant wipes; a sodium hypochlorite-based disinfectant (Dispatch®) and two quaternary ammonium-based disinfectants (CaviWipe® and Volo™).

CHAPTER 2: LITERATURE REVIEW

Infection control issues have emerged in dentistry with the standard of care requiring to treatment all patients using universal precautions. This change created special concerns in radiology due to the sterilization limitations of intraoral radiographic receptors (both film and digital). A temporal review of the literature illustrated the evolution of infection control in dental radiology.

Cross-Contamination with Film in Dental Radiology

The dental profession has shown concern about cross-contamination in intraoral dental radiology as early as 1978. The first reported finding was published by Stuart and Glaze, who described the potential for cross-contamination in the dental operatory.[1] Autio *et al.* demonstrated that pathogens were transferred from the oral cavity of patients to the clinical areas.[2] Rahmuatulla *et al.* found that in the radiology area, high touch areas were the headrest adjusting lock, the x-ray cone, the exposure control knob, the timer switch, the film placement area in the darkroom, the feeding area in the automatic processor, and the revolving door to the darkroom.[3] Other studies showed that the potential for cross-contamination in dental radiography was high due to the multi-step process of exposing film receptors, processing, and displaying images.[3-5] For instance, cross-contamination occurs when the operator's hands are contaminated by patient's saliva during film placement. Then, the contaminated hands touch the x-ray tube for positioning, the x-ray machine control panel for time adjustments, and conclude with activation of the exposure switch. White and Glaze reported that pathogens left on radiographic equipment were able to survive up to 48 hours.[1]

For 100 years, film has been the primary means for obtaining radiographic images.[5] Through the years, dental professionals have been able to minimize the impact of microbial cross-contamination primarily because dental film can only be exposed once and then the film was processed and mounted for viewing. Film Barriers were introduced in the early 1990's to help minimize bacterial load on the film packet. Wolfgang reported that 20% of the packets used with barrier envelopes were contaminated when removed from the envelope.[6] Those film packets that were contaminated had a low numbers of microbes.[6] Hubar et al. stated that contamination rates with barriers remained too high to ensure an acceptable safety level.[5] Due to the issue of contaminated packets, Tullner et al. described a technique for using barrier envelopes by dropping film carefully from the envelope.[7] Advances in technology, such as automatic processors, exacerbated the cross-contamination problem.

In an attempt to expedite film processing procedures, automatic film processors were introduced in 1958.[8,9] Efficiency improvements were achieved, although the processing chemicals needed for developing the film were still environmentally unfriendly and provided a nidus for bacterial growth.[11,12] Stanczyk et al. reported that microbial contamination of the processor and daylight loader occurred during film processing.[10] In addition, Bachman et al. reported that bacteria survived the processing procedure.[11] Bacterial counts on the film surface were decreased through processing, but the potential for contamination and cross-contamination remained.

Cross-contamination of Digital Receptors in Dental Radiology

Mid 1990's, dental professionals began to incorporate intraoral digital radiography into their dental practices. Today, approximately 58% of dental offices use an intraoral digital x-ray system and 21% plan to use it in the future. [12] The three primary digital intraoral devices used today are the Charge-Coupled Device (CCD), Complementary Metal Oxide Semiconductor (CMOS) devices and the photostimulable phosphor receptor (PSP). The CCD was first

introduced in 1987 and was the first digital image device used intraorally.[13,14] The CCD is composed of microcircuits that are silicon encased in a bulky and ridged rectangular frame. A wire cord extends from the CCD to the computer. This design makes it difficult to keep the CCD sensor and cord microbial free. Obviously, the sensor cannot be heated, steamed, or gas sterilized without damaging the electrical circuits. CMOS sensors also known as active pixel sensors are an alternative to CCD sensors but it is hard to tell them apart from CCD sensors. Another receptor that has become popular is the PSP receptor. The PSP popularity is due to its similarity to film. The major active component of the PSP receptor is the phosphor layer, which can be damaged by steam and heat sterilization. The phosphor layer is designed to absorb and store energy from the x-ray exposure (latent image) and then release a visible image when stimulated by a laser light of an appropriate wavelength (~600nm).[11] Due to the ability of light to erase the image, the PSP receptor must be inserted into a plastic envelope, similar to an infection control barrier bag, prior to exposure. Thus, the purpose of the barrier is twofold. Primarily, the barrier is used to protect the erasure of the latent image after exposure. Secondly, the barrier helps to reduce the bacterial load on the receptor. Many studies have shown that PSP receptors are not efficient in preventing cross-contamination.[13,15-18] According to a study conducted by Kalathingal *et al.*, over half of the PSP receptors at a dental school clinic were contaminated with microorganisms and some of the organisms were pathogenic.[17]

Due to the severe consequences of contaminating a digital receptor, it is paramount that strict infection control procedures are employed. Current FDA regulations allow the use of ethylene oxide to sterilize PSP receptors. Due to the harmful effects of ethylene oxide properties to people and the environment, discussions have occurred to discontinue its use. If this comes to fruition, then it will be virtually impossible to prevent cross-contamination with the PSP receptor. Thus, the introduction of PSP digital receptors in intraoral radiography has created new infection control challenges that were not experienced previously with film-based imaging.

Clinical Implications of Cross-contamination in Dental Radiography

As a result of cross-contamination, clinicians and patients are at a greater risk for being exposed to the tuberculum bacteria, herpes virus, various hepatitis strains, and other infectious diseases transmitted in blood or saliva.[14] Therefore, it is critical to treat each patient as if they have human immunodeficiency virus and hepatitis B. Moreover, not only will cross-contamination harm healthy patients, but it could also cause major health problems for immunocompromised and/or older patients.

Sterilization Methods Used with PSP Receptors

The Center for Disease Control and Prevention (CDC) states that digital radiography receptors should ideally be steam or heat-sterilized or achieve a high level of disinfection at the tuberculocidal level between patients because the receptors come into contact with mucous membranes and also possibly blood.[19] Therefore, one of the most effective sterilization methods used today for PSP receptors is ethylene oxide gas sterilization. However, ethylene oxide gas is not environmentally friendly nor do most dental offices have access to this sterilization method. According to OSHA, ethylene oxide gas is highly flammable and reactive. It is harmful to humans and it could result in respiratory irritation and lung injuries, headaches, nausea, vomiting, etc. Studies have shown that chronic exposure to ethylene oxide could also lead to cancer, reproductive effects, mutagenic change, and neurotoxicity.[20] As a consequence; there is the potential for banning the use of ethylene oxide in the future.

Since ethylene oxide gas is not human or environmentally friendly, the need to find alternative methods of sterilization is critical. Some alternative methods have been reported in the literature.[22] According to a study conducted by Negron *et al.*, a dry paper towel was more effective in removing bacteria than a paper towel soaked with disinfectant. However, the authors proposed that the finding may be due to the fact the participants did not leave the prophene solution on the surface of the barrier bag long enough for it to act as a high level disinfectant.[22]

Several studies have used alcohol wipes to disinfect PSP receptors and these findings showed that the alcohol wipes were effective in removing most bacteria, but not spores nor *Mycobacterium tuberculosis*.[13,15,17] Furthermore, alcohol is useful in killing most of the oral bacterial that have lipids in their cell membranes. 70% alcohol is the most effective concentration for killing oral microbials. The major problem with alcohol wipes was that it damaged the phosphor layer over time.[15] The cross-contamination issue has become the topic of many research projects; however, no study has reported an effective method for preventing cross contamination.

Spore and Mycobacteria Cell Structure

Since the government is strictly monitoring the use of spore-forming pathogens and *Mycobacterium tuberculosis* in research laboratories; hence, it is not practical to contaminate PSP receptors with *Mycobacteria* or spore-forming pathogens to test the effectiveness of various disinfectant wipes. However strips impregnated with non-pathogenic spores can be used to test the effectiveness of infection control methods. Spore strips are biological indicators used to test the effectiveness of sterilization (heat, EtO, etc). The CDC recommends and the state of North Carolina requires the use of spore strips weekly to test the effectiveness of sterilizers to kill spores. Any repeated bacteria growth from the spore strip after sterilization indicates machine failure and should be repaired before use.[23]

Spore forming bacteria can be in two forms, the spore form and the vegetative cell form. The vegetative form causes many diseases in humans. Table 1 shows some of the common disease causing spores. Extreme environments stress bacteria to form spores. Spores are resistant to UV, high temperature, strong acid, reactive oxygen species, and disinfectants. They can also survive harsh environments for long periods of time. Spores are harder to kill than most bacteria because spores have outer and inner coats that serve as permeability barriers and can detoxify harmful chemicals. Moreover, the outer coat acts as a barrier against host defense

proteins such as lysozyme. The coats also protect the underlying spore cortex, which keeps the spore core dehydrated. The spore core contains the DNA and other important organelles. Exosporium is a collagen-like protein layer that encapsulates the outer coat. This layer protects the spore from chemical and enzymatic treatments, and also provides a hydrophobic surface that aids in adhesive properties.[24] Spores cannot replicate until they germinate and become vegetative cells. Therefore, it is easier to kill spore forming bacteria when they are in their vegetative phase because DNA can easily be targeted.

Table 1: Common spore forming bacteria that causes diseases

Types of Spore Forming Bacteria	Modes of Transmission	Diseases
Bacillus antracis	Infected animals and animal products	Anthrax
Clostridium botulinum	Foodborne	Botulism (botulinum toxin in can food and honey)
Clostridium difficile	Healthcare facilities and through the fecal-oral route	*Clostridium difficile*-induced colitis
Clostridium perfringens	Germinate in wounds	Gas gangrene
Clostridium tetani	Spores in soil and germinate in wounds	Tetanus (lockjaw)
Baccillus cereus	Soil, vegetables, raw, processed foods, and gastrointestinal tract of humans and animal.	Food poisoning

Quaternary Ammonium Compounds and Sodium Hypochlorite

Both the CaviWipe® and the Volo™ wipes contain quaternary ammonium compounds which are diisobutylphenoxyethoxyethyl dimethyl benzyl ammonium chloride for CaviWipe®

and Alkyl dimethyl benzyl ammonium chloride for Volo™ wipe. Quaternary ammonium compounds are known to be effective as surface disinfectants for hospital settings. In health care, quaternary ammonium solutions or wipes are widely used to disinfect patient-supplies and health care equipment. Quaternary ammonium has antimicrobial characteristics depending on the substituent radicals that are attached to nitrogen atoms (i.e. alky or heterocyclic, halide, sulfate etc.). To achieve bactericidal effects, quaternary ammonium inactivates energy-producing enzymes, denature important cell proteins, and disrupt cell membrane of bacteria. According to the CDC, scientific literatures reported that these products were effective at fungicidal, bactericidal, and virucidal (lipophilic viruses), but most of them are not sporicidal, tuberculocidal, nor virucidal against hydrophilic viruses.[25] According to scientific literature, EPA-registered quaternary ammonium compounds such as Caviwipes® were used to disinfect equipment surfaces that have been contaminated by intact skin.

Sodium hypochlorite (bleach)-based disinfectants are the most widely used chlorine disinfectants in the US. They can kill a broad spectrum of bacteria without leaving toxic residues. Bleach can destroy bacteria DNA of mycobacteria. According to Lawley *et al.* sodium hypochlorite is an oxidation-based disinfectant and it is effective in inactivating vegetative and spore forms of *C. difficile*. [26] Bacteria can be inactivated by hypochlorite in several ways. For instance, it can decrease uptake of nutrients, inhibit protein synthesis, and interrupt DNA synthesis.[25] Hence, spore strips were used to test the effectiveness of hypochlorite and quaternary ammonium based wipes. It was hypothesized that they will effectively kill spores.

CHAPTER 3: METHOD AND MATERIALS

Disinfectant Wipes

This research project was a laboratory-designed study to test three different commercial disinfectant wipes (Dispatch®, Volo™, and CaviWipe®). All three disinfectant wipes claim to be tuberculocidal, bactericidal, virucidal, and fungicidal. Only Dispatch® claims to kill *C. difficile* spores in five minutes (Refer to table II).

Table II: Product details regarding the three commercial disinfection wipes, PBS control, and ethylene oxide sterilization.

Disinfectant/ Sterilization Product	Concentration	Level of Disinfection/ Sterilization	Length of Time
Dispatch®	0.65% Sodium hypochlorite Other ingredients include: Sodium Lauryl Sulfate, Sodium Metasilicate, and Sodium Hydroxide	*C. difficile* spores	5 min
		Mycobacterium tuberculosis var: bovis (BCG), HBV, HCV, HIV-1, MRSA	1 min
Volo™	N-Alkyl Dimethyl Benzyl Ammonium Chloride 0.12% N-Alkyl Dimethyl Ethyl Benzyl Ammonium Chloride 0.12% Isopropyl Alcohol 58.18%	*Mycobacterium tuberculosis* var: bovis (BCG), HBV, HCV, and MRSA	2 min
		HIV-1	1 min
CaviWipe®	Diisobutylphenoxyethoxyethyl Dimethyl Benzyl Ammonium Chloride 0.28% Isopropanol 17.20%	*Mycobacterium tuberculosis* var: bovis (BCG)	3 min
		HBV, HCV, HIV-1, and MRSA	2 min

Mesa Spore Strips

This study used Mesa Spore Strips. Each spore strip was impregnated with two species of bacterial spores, *Geobacillus stearothermophilus* and *Bacillus atrophaeus*. Mesa Spore Strips were packaged in Schleicher and Schuell filter paper (6.4mm x 38.1mm) which was enclosed in a peal open glassine paper pouch. Each spore strip contained a population of 10^5 *G. stearothermophilus* spores and 10^6 of *B. atrophaeus* spores. *G. stearothermophilus* can only grow in 60˚C and are used to determine the adequacy of steam or chemical vapour sterilization; while *B. atrophaeus* can only grow in 37˚C and are used to determine the adequacy of ethylene oxide or dry heat sterilization. Therefore, *G. stearothermophilus* spores are harder to kill then the *B. atrophaeus* spores. Hence, sporicidal ability of the disinfectant wipes was tested on *G. stearothermophilus* because they are harder to kill than *M. tuberculosis* and *Bacillus atrophaeus*.

Trypticase Soy Broth (TSB)

Trypticase Soy Broth (TSB) is a liquid nutrition-enriched culture medium that supports a wide variety of bacterial growth such as, aerobic, anaerobic, fastidious and non-fastidious bacteria, fungi, etc. The active reagents in TSB includes: Casein digest peptone, bacto soytone (peptic digest of soybean meal), dextrose, sodium chloride, and dipotassium hydrogen phosphate. Casein digest peptone and peptic digest of soybean meal provide amino acids and other nitrogenous substances; dextrose is a glucose that provides energy for the bacteria; sodium chloride maintains the osmotic equilibrium; and dibasic potassium phosphate serves as a buffer for the broth. Therefore, this medium supported the growth of both *G. stearothermophilus* and *B. atrophaeus*.

1X Phosphate Buffered Saline (PBS)

Phosphate buffered saline solution is a salt-based solution that contains sodium phosphate, sodium chloride, potassium phosphate. The osmolality and ion concentration are very similar to those of the human body. The pH of the solution is 7.4. It is often used to

maintain the osmolarity of the cells. This solution is very similar to TSB broth except that it doesn't have any of the nutrition in it; therefore, this solution was used to prepare wet wipes which served as the control for the experiments.

Ethylene Oxide Sterilization

Ethylene oxide is the standard procedure currently used to handle and sterilize PSP digital receptors at the UNC School of Dentistry. According to CDC, the efficacy of the system was determined by its ability to kill 6 $\log_{10}$ *B. atrophaeus* spores.[27] Sterilization process required 100% ethylene oxide (EtO). EtO is a colorless gas that is flammable. The four essential parameters are: EtO gas concentration range from 450 to 1200mg/l; temperature range from 37-63 degree C.: relative humidity 40-80%; and exposure time 1 to 6 hours. This sterilization procedure consists of three stages. Stage one is the pre-conditioning stage which allows the PSP receptors to be preheated and humidified. Secondly, the sterilization stage releases adequate amount of ethylene oxide gas to sterilize the receptors. Lastly, EtO is absorbed by materials and for that reason, following sterilization the PSP receptors must go through the Aeration stage. This stage allows the PSP receptors to go through a degassing phase to remove ethylene oxide gas residue. Ethylene oxide sterilization process requires a total of 24 hours.

Laboratory Preparation for Different Experiments

TSB and 1xPBS Soaked Gauzes Preparations

TSB was prepared by mixing 30.0g of TSB powder in 800mL of deionized water and was evenly mixed by placing it in a magnetic stirrer. The solution was then transferred into a 2L Erlenmeyer flask and autoclave at 121.0°C for 15 minutes. Depending on the experiment design, some of the solution was transferred to glass test tubes and some was transferred to 50ml centrifuge tubes. Each glass test tube contained 3.7ml of TSB while each centrifuge tube contained 35ml of TSB. PBS solution comes in 10x concentration. For the purpose of this

research it was diluted to 1x solution using deionized water and autoclaved. Sterilized gauzes were soaked in 1x concentration PBS to serve as control wet wipes.

Spore Strip Treatment Procedure

At room temperature *G. stearothermophilus* spores remain in spore form. Once the temperature reaches 60˚C, spores will germinate and become vegetative cells. For all the experiments, spores were treated with disinfectant wipes in their spore form. Spore strips were treated with CaviWipe®, Dispatch® Wipes, and Volo™ Wipes. To ensure a sterile environment, a sterile gauze was placed on top of a plastic weighing tray and then a treatment wipe was placed on top of a sterile gauze. Five spore strips were laid side by side on top of a treatment wipe. Another treatment wipe was placed on top of the spore strips. To ensure the spore strips were properly soaked with chemical, a plastic weighing tray was placed on top of the treatment wipe with a small laboratory glass bottle seated on top of the tray to exert pressure (Figure 1).

The same procedure was used for the positive control group as the treatment group, except instead of using the treatment wipes, sterile gauzes were soaked in a 1x Dulbecco's Phosphate-Buffered Saline solution to treat the five spore strips.

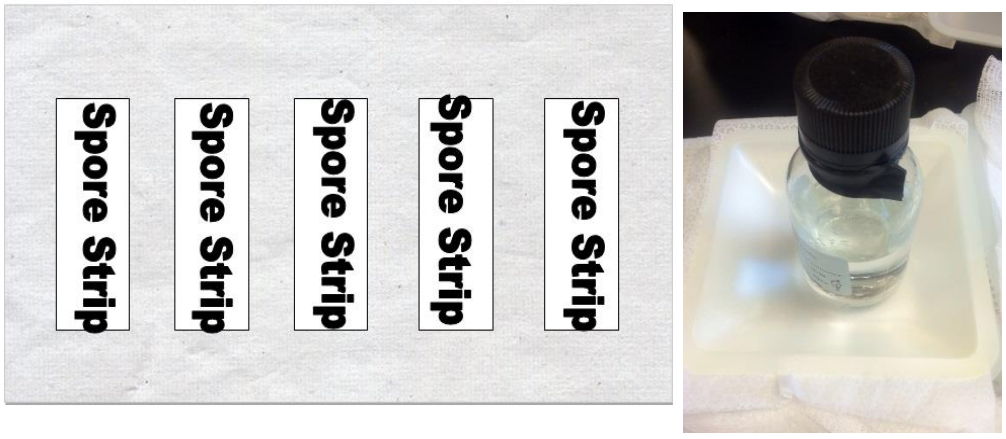


Figure 1: Spore strips placed on top of a treatment wipe with a plastic weighing tray and glass bottle placed on top of the treatment wipe for pressure.

Gold Standard Experiment: Ethylene Oxide Sterilization

Ethylene oxide sterilization of PSP receptors enclosed in barriers, 18 receptors representing Full Mouth Series (FMX) sets were packaged together in one sterilization bag (3.5 inch x 5.25 in Crosstex Dual-Process Indicators sterilization package). To determine if the ethylene oxide penetrated through the layered sets, three unopened spore strips were placed individually inside of three sealed PSP receptor barrier bags. The spore strips were strategically placed inside the FMX sets. One spore strip was placed on top, one was placed in the middle, and the other one was placed at the bottom. After EtO sterilization, the principal investigator removed the spore strips from the barrier bags. The spore strips were transferred into fresh TSB tubes for bacteria growth. This process was repeated three times.

Experiment I: Direct Treatment of Spore Strips for Variable Time Limits

Five Minutes Treatment

A total of 20 spore strips were used to test the positive control and treatment groups (CaviWipe®, Dispatch® Wipes, and Volo™ Wipes) for five minutes because the manufacturer of Dispatch® Wipes claimed to kill C. difficile spores in five minutes. Each treatment group used five spore strips. After five minutes treatment, each of the spore strips were aseptically transferred to a 3.7ml TSB-filled test tube by using flamed forceps and incubated at 60˚C for growth for 24 hours. In addition, five spore strips were used for the control group. The purpose of the control was to design an equivalent wetting procedure similar to the treatment group. This ensures that the wetting procedure doesn't reduce the spore count in the strip to the point that the spore cannot be detected.

One Minute Treatment

To test if the shorter treatment time was sufficient to inhibit spore growth, the treatment time was reduced to one minute for CaviWipe®, Dispatch® Wipes, and Volo™ Wipes. For all

experiments, the treatment and control TSB-filled tubes were observed for a maximum of seven days. Observations ceased once bacterial growth (cloudiness) was visually observed.

Fresh Spore Strip Added

After 24 hours of incubation, if there was any inhibition of bacterial growth, then it was important to determine whether this occurred due to residual effects of the disinfectant or if the spores were killed initially upon contact with the disinfectant prior to incubation. Hence, a second spore strip was added to both one and five minutes treatment groups and then incubated at 60˚C for 24 hours.

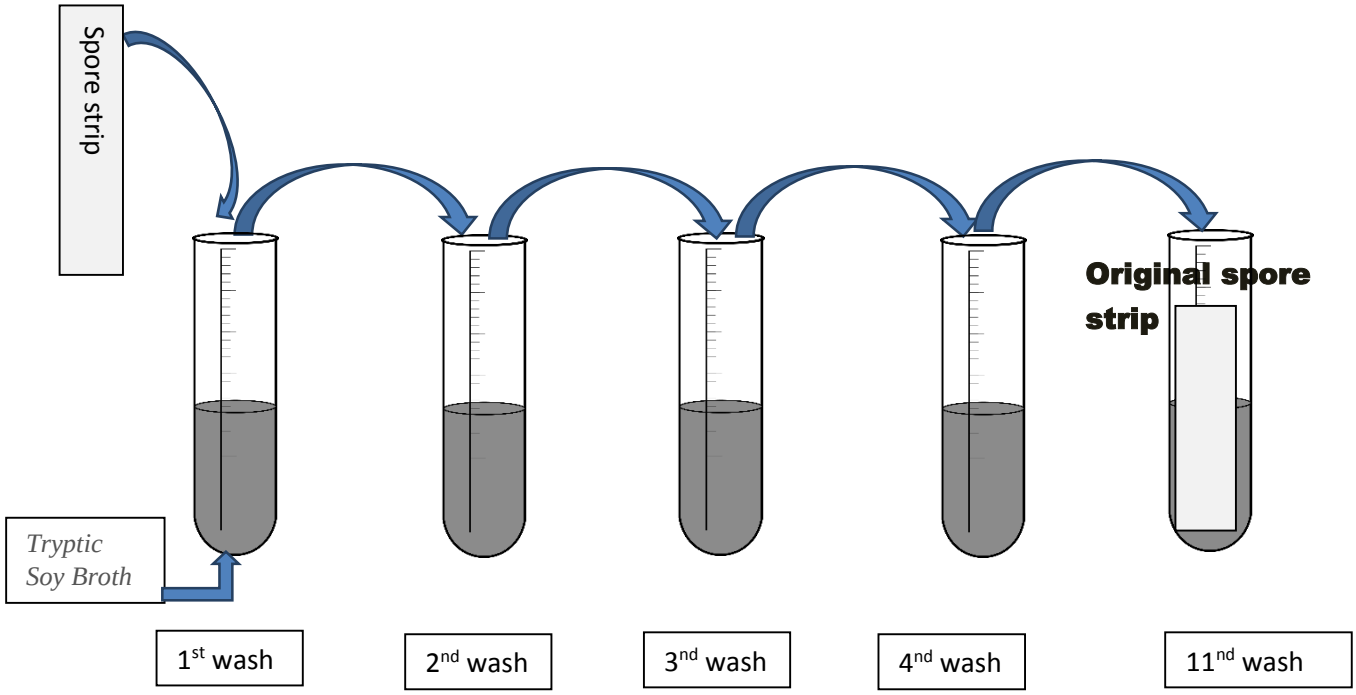
Experiment II: Progressive Washing of Reagent and Spore Strips

Treated spore strips were progressively washed in TSB to remove the residual reagent from the treated strips. Spore strips were treated with each treatment wipe (CaviWipe®, Volo™ wipe, or Dispatch® wipe) for five minutes. Then, these treated spore strips were placed in 3.7ml of TSB-filled test tube and vortexed vigorously. Then, the strips were subsequently aseptically transferred to 3.7ml TSB-filled tubes up to 11 times to remove reagent from treated spore strips. The media used to wash the treated strip and the media that contained the treated spore strip were incubated at 60˚C for 24 hours for bacterial growth (Refer to Figure 2). The same procedure was performed on the positive control group using 1x PBS wipe. This procedure was repeated with one minute treatment time.

Second Spore Strip Added

After 24 hours, a second fresh spore strip was added in the test tubes that remained negative in order to determine the end point of residue activity. The TSB-filled tubes were incubated again at 60˚C for growth for 24 hours.

Figure 2: Progressive washing of treated spore strip.



Experiment III: Progressive Washing of PBS-Treated Spore Strip

Five spore strips were treated with PBS-soaked gauze for five minutes. PBS-treated spore strips were progressively washed 14 times by vortexing in 3.7ml of TSB-filled test tube. Vortexed strips were subsequently aseptically transferred to 3.7ml TSB-filled tubes and vortexed up to 14 times to determine release of spores after each wash.

Experiment IV: Determining Number of Spores Released After First Wash

These series of experiments were conducted to determine the number of spores that were released from the spore strips during the progressive washing procedures. This process was evaluated in two parts: a spore strip saturated with PBS and a spore strip untreated (dry).

Experiment IV part I: PBS Treated Spore Strip

In order to determine how many spores were released from the spore strip after washing and vortexing, one to ten serial dilutions were performed on the washes. Initially, three PBS-

treated spore strips were vortexed vigorously in a 3.7ml TSB-filled test tube. Then, from these test tubes, a 400µL spore contaminated TSB media was aseptically transferred to a second 3.7 ml TSB-filled tube, and the second TSB-filled tube was vortexed. A 400 µL of the media in the contaminated-second test tube was transferred to a third 3.7mL TSB-filled test tube and was vortexed. This procedure was subsequently repeated up to six times for a total of six TSB-filled tubes (Refer to Figure 3).

Experiment IV part II: Untreated Spore Strips

In order to see if wetting the spore strip would cause more spores being released from the strip, one to ten serial dilutions were performed on untreated spore strip. The same procedure was followed for the untreated spore strips.

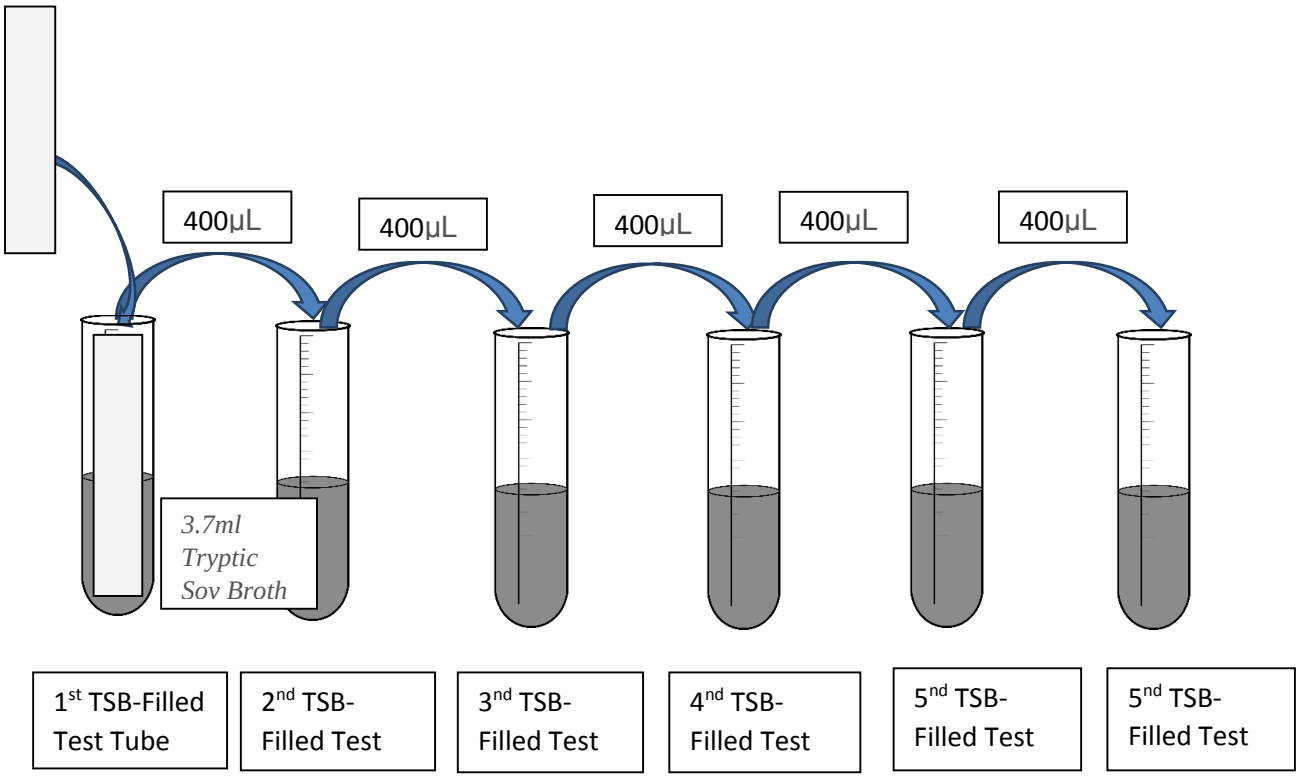


Figure 3: Tenfold serial dilution on spore contaminated TSB-filled test tube.

Experiment V: Determining Disinfectant Level of Packaged Barrier Bags

Experiment V part I: Positive Control for Contaminated PSP Barrier Bags

A total of 15 PSP barrier bags were sterilized by EtO gas sterilization prior to placing PSP receptors into the barrier bag. To ensure that there was no residual ethylene oxide gas remaining in the barrier bag after sterilization, the following experiment was conducted. Nine spore strips were placed into a 50ml centrifuge plastic tube which contained 35 ml TSB broth. The sterilized barrier bags were placed in the spore contaminated TSB broth, vortexed, and then the barrier bag was transferred to a fresh TSB-filled tube and incubated at 60˚C, and observed for growth 24 hours later.

Air Dried Contaminated Barrier Bag

Five sterilized PSP barrier bags were used. Each barrier bag was dipped in the contaminated TSB broth and transferred into a sterile Petri dish to air dry overnight. The following day, the air dried contaminated barrier bags were transferred to a fresh 3.7ml TSB-filled test tube and incubated at 60˚C for growth for 24 hours.

Mechanical Action of Wiping

In order to test if the mechanical action of wiping was removing the spores, 1x PBS soaked gauzes were used. Each spore strip was dipped in contaminated broth and then wiped with 1x PBS soaked gauze. Then, the PSP barrier bags were transferred into TSB broth and incubated at 60˚C for growth for 24 hours.

Experiment V part II: Spore Contaminated Barrier Treated with Disinfectant Wipes

After positive controls confirmed that the spores stayed on the barrier bags, the contaminated barrier bags was treated with the disinfectant wipes. Five sterile PSP barrier bags were used. Each of the PSP barrier bags were contaminated with spores by dipping them into the spore contaminated broth. Each of the contaminated barrier bags was wiped with CaviWipe®, Volo™ wipe, and Dispatch® for approximately 10 seconds to simulate clinical

radiology barrier bag wiping time and then transferred into a 35ml TSB centrifuge tube. All tubes were incubated at 60˚C and observed for bacterial growth 24 hours later.

Data Analysis

Each TSB broth culture was assessed for visual turbidity at 24hours and up to 7 days. Percentage of growth was calculated for each disinfectant. All experiments were repeated at least three times.

CHAPTER 4: RESULT

For the gold standard EtO experiment, after 12 hours of the incubation period, no growth was observed in the media that contained EtO treated spore strips. Moreover, no visual turbidity was observed in the treatment groups even after the seven days of incubation period.

In experiment I, twenty spore strips were directly treated with disinfection wipes (CaviWipe®, Volo™ wipe, and Dispatch®) and buffered saline (control group) for both a one treatment time and then a five minute treatment time. In contrast to the PBS treated spore strip (control group), all disinfectant-treated groups presented with no visual turbidity, which suggested a total inhibition.

A second spore strip was added and at 48 hours, no growth was observed in the media. An exception of one out of five CaviWipe® (one minute treatment) group was positive. Therefore, the positive test tube was Gram stained. The microscopic observation showed that the bacteria growing in the positive test tube were thin and long rods (bacillus) as compared to the control tube with shorter and bigger rods (false positive result). Fibers from the spore strip were also observed. For both experiments, no visual turbidity was observed in the treatment groups even after seven days of incubation period.

Figure 4 showed spore growth of treated spore strips that were progressively washed up to 11 times. In contrast to the control group, no growth was observed in both CaviWipe® and Volo™ groups with removal of treated strips all the way out to 11 washes. In contrast to CaviWipe® and Volo™ groups, Figure 4 showed that growth was observed in all the Dispatch® treated-spore

strip media with removal of the spore strip. However, the first six tubes that contained the treated spore strip showed no growth.

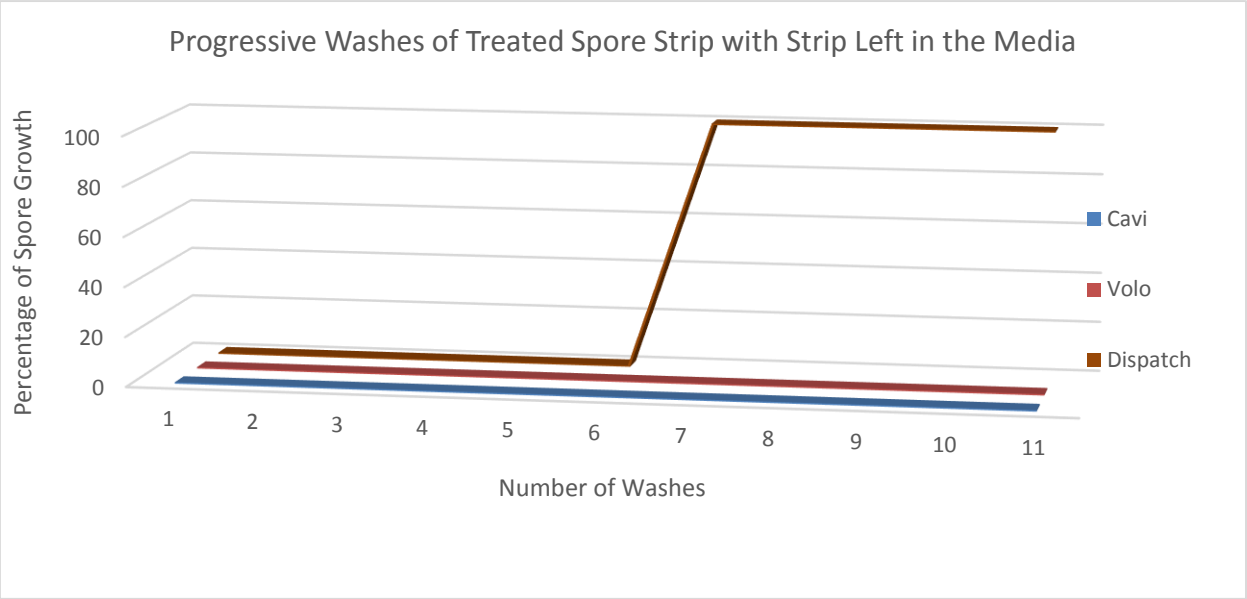


Figure 4: Spore growth when disinfectant-treated spore strips were left in the last wash of each progressive wash.

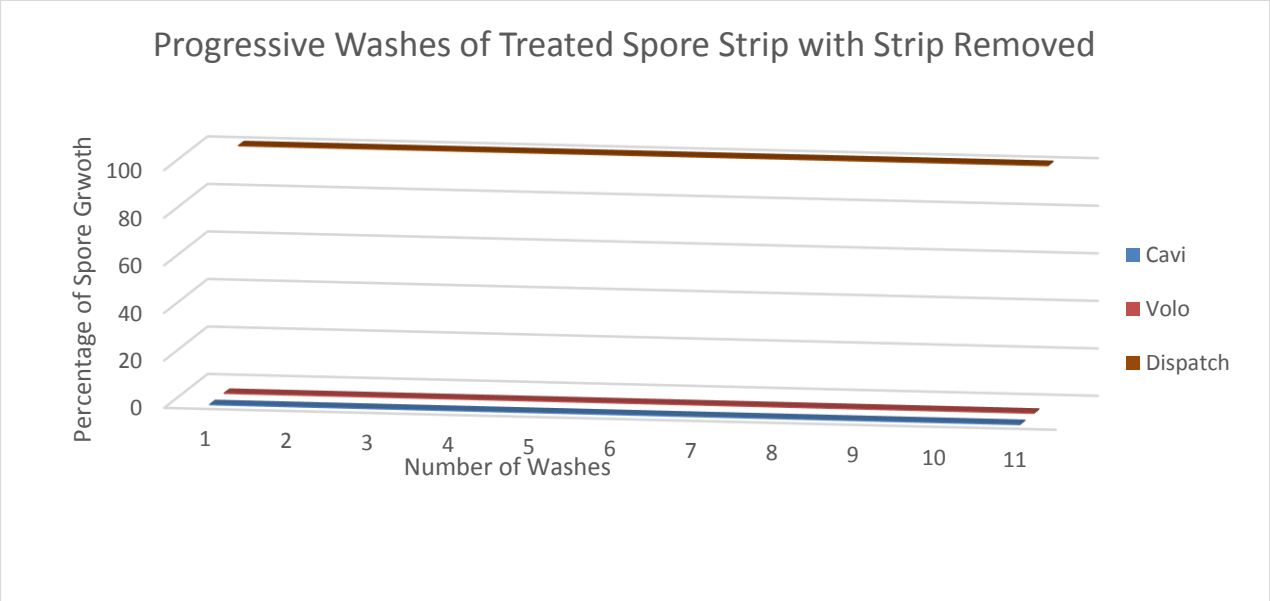


Figure 5: Spore growth when disinfectant-treated spore strips were removed from the media after each progressive wash.

Furthermore, after a second spore strip was added to both CaviWipe® and Volo ™, no growth was observed in the first three washes for both CaviWipe® and Volo ™ groups (Figure 5). Figure 6 also showed that when comparing CaviWipe® and Volo™, there was inconsistency in growth between the fourth and seventh washes. For the CaviWipe® group, 100% growth was observed after the sixth wash, while for the Volo™ group 100% growth was observed after the seventh wash.

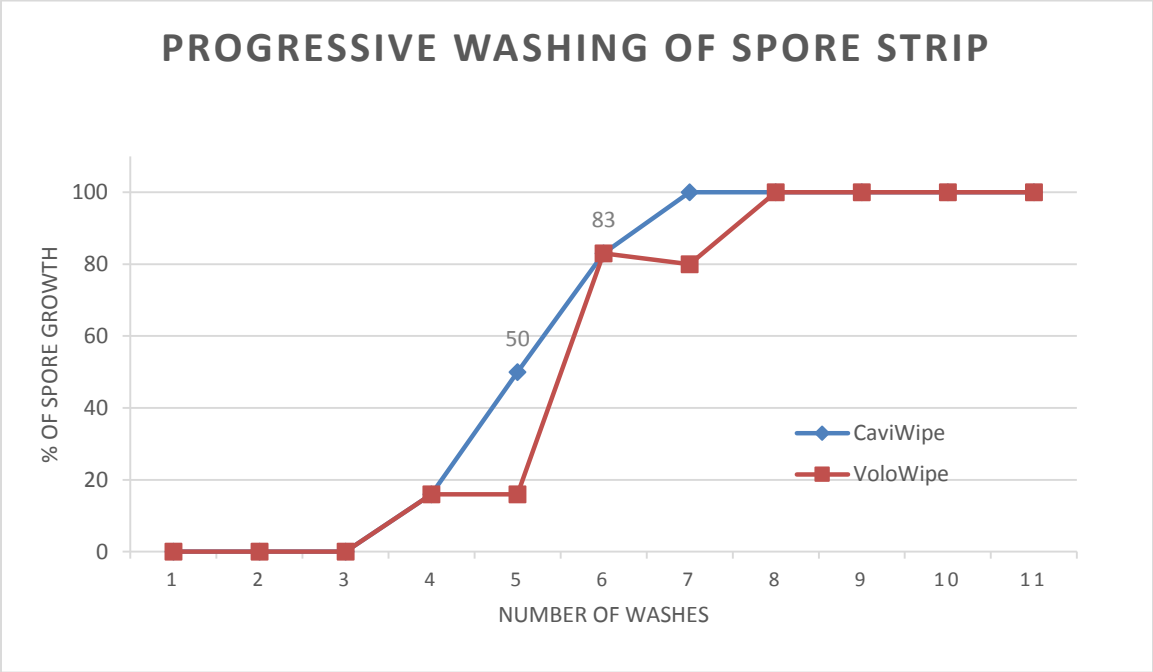


Figure 6: Number of washes required to remove reagent from treated spore strip. Outcome was determined by positive (visual turbidity) and negative (no visual turbidity)

Figure 4 showed that for the Dispatch® group, only the first six wash media that contained the original treated spore strip presented with no growth; consequently, a second strip was added to these media. After incubating for another 24 hours, the result showed that growth was recovered in third and fifth washes, while the rest of the media that was negative remained negative.

After 12 hours of incubation, the progressive washing of PBS-treated spore strips showed viable growth in all the media (total of 14 washes). Even the last tube that contained the original PBS treated strip presented with visual turbidity.

In less than 12 hours of incubation, the one to ten serial dilution of PBS-treated spore strips displayed visual turbidity on the second, third, and fourth tubes. No new growth was observed after 12 hours. After 12 hours, the tenfold dilution of five untreated spore strips showed that three spore strips have growth up to the third tubes, while two of the spore strips have growth up to the second TSB-filled tubes.

In order to make these experiments clinical applicable, spore contaminated barrier bags was treated with disinfectant wipes for 10 seconds. Growth was recovered in TSB tubes that contained the contaminated barrier bags. Growth was also recovered in contaminated barrier bags that were air dry. Moreover, after wiping contaminated barrier bags with PBS-soaked gauze, growth was presented on all five TSB tubes that contained the barrier bags. After 24 hours of incubation period, no growth was recovered in all disinfected (CaviWipe®, Volo™, and Dispatch®) treated barrier bags.

CHAPTER 5: DISCUSSION

Gold standard experiment tested the sporicidal ability of EtO sterilization by dispersing the spore strip in a package of contaminated PSP receptors and the result showed no growth. This indicated that the ethylene oxide gas was able to penetrate through the barrier bags and kill the spores inside the sealed barrier bags. It was unexpected that gas was able to penetrate inside the barrier bags because gas is not evenly distributed in the chamber and no studies have shown that gas can penetrate through the barrier and disinfect the receptor. In addition, it was unknown whether the layered barrier bags within the sterilization bag allowed gas penetration.

For experiment I, the spore strips were directly treated with disinfectant wipes for both one and five minutes and they all resulted in no growth. There are three plausible reasons for these findings. First, it was possible that spores were reduced by the wetting procedure during treatment. Secondly, it was possible that during the incubation period, the residue reagent was inhibiting the growth of the vegetated cells. Thirdly, it was possible that the reagent killed the spores during the treatment process (before incubation).

The first hypothesis was disproved by the control group which was designed as an equivalent wetting procedure similar to the treatment group. All the control groups resulted in bacterial growth. This ensured that the wetting procedure didn't reduce the spore count in the spore strips to the point where no spores could be detected. Also it showed that after incubation, the spores were able to germinate properly.

A second untreated spore strip was added to the TSB media that contained the disinfectant treated spore strip. Again no growth was observed in all the groups, with the

exception of one false positive from the CaviWipe® group. This result indicated there was residue disinfectant reagent in the TSB media because it was sufficient to inhibit the growth of a second untreated spore strip. Therefore, it was possible that during incubation, the residue reagent was killing the vegetative form of the spores instead of directly killing the spores. Therefore, the next experiment was to remove the residual reagent by progressive washing of the treated spore strip. If spore growth was observed after removing the residual reagent, then the initial treatment did not kill the spores but killed the vegetative cells.

For experiment II Part I, progressive washing of the CaviWipe® and Volo™ -treated spore strips resulted in no growth (refer to Figure 4). In order to determine the end point of residue activity, a second fresh spore strip was added to all the media. The results of the second untreated spore strip indicated that it took at least three washes to remove the residual activity for both the CaviWipe® and Volo™ groups (refer to Figure 5). This suggested that after three washes, residual reagent began to wash off because growth from the second strip was observed. The growth on the second strip indicated that there was either no reagent or not enough reagent to inhibit bacteria growth. After the seventh wash, all the residual reagent was completely washed off because there was 100% growth in all media that contained the second untreated spore strip. In contrast to the treatment groups, growth was observed in all of the control groups (11 washes). Hence, if the spores from the spore strip were not killed, then growth was expected up to 11 washes.

PBS treated spore strips were washed 14 times with the intention to remove all the spores from the spore strip. However, the results showed viable growth in all the tubes. This suggested that 14 washes of a PBS-treated spore strip were not sufficient to remove all the spores from the spore strip. This was a surprise because there was no reduction of visual turbidity even on the last washed medium that contained the original strip. Therefore, tenfold dilution was performed on a treated spore strip to see how many spores were released after the

first wash. The tenfold dilution of PBS treated and untreated spore strip showed that less than one percent of the spores were released after the first wash which explained why there was growth up to 14 washes.

These results disproved hypothesis II which stated that residual reagent was killing the vegetative form of the spores. Even after the residual reagent was washed off the spore strip, there was no growth (refer to Figures IV and V). Therefore, experiment II (progressive washing) validated the third hypothesis which stated that the reagent killed the spores during the treatment process (before incubation) for both CaviWipe® and Volo™ groups.

Surprisingly, in contrast to the CaviWipe® and Volo™ groups, growth was observed in all the Dispatch® TSB media, except the first six media that contained the original treated spore strip (refer to Figure 4). Therefore, a second untreated spore strip was added to determine if there was residue reagent sufficient to inhibit the second fresh spore strip. Unexpectedly, growth was observed on the third and fifth TSB media only. It was possible that reagent stayed with the strip because growth was observed in the media with removal of treated spore strip. Two more experiments of the progressive washing up to six washes were performed to confirm this result. Again, the result showed that the media that contained the treated spore strip presented with no growth, but the media with removal of the strip presented with growth. Furthermore, 100% growth was observed after the sixth progressive wash, which indicated that after the sixth wash the reagent was washed off because the treated spore strip began to grow with no residual reagent left in the media to inhibit growth. These results indicated that Dispatch® wipe cannot kill spores because growth was observed in all the media used to wash treated spore strips (refer to Figure 4).

There were eight possible explanations for the results of the Dispatch® treated groups (refer to Figure IV). First, growth was observed in all the wash media and this could be due to the fact that the active ingredient in Dispatch® was composed of sodium hypochlorite chlorine

(bleach) and bleach reacts with everything. Therefore, it is possible that bleach was neutralized when it was placed in the TSB media. This explanation was supported by Omidbakhsh who reported that bleach activities were reduced in the presence of organic matter.[28]

The third possible explanation is the pH of solution could have interfered with the antimicrobial activity of the reagent. Sodium hypochlorite was more active in lower pH but less stable. Therefore, detergent was often added to the formula to increase pH. Omidbakhsh's research and CDC confirmed that an increase in pH improved antimicrobial activity of quaternary ammonium compounds but decreased the activities of sodium hypochlorite.[28]

The fourth possible explanation for the reagent to stay with the spore strip may be the result of incubation temperature which triggered the treated spore trip to release the reagent to the media. Therefore, when the media was incubated at 60˚C, it triggered the spores to germinate; at the same time it triggered the disinfectant-treated spore strip to release the disinfectant reagent to the media. This may explain why in the first six progressive washings, only the media that contained the treated spore strip remained negative while rest of the media used to wash the treated spore strip exhibited growth (Refer to Figures 4 and 5).

The fifth possible explanation for inhibition of the second spore strip was because after vortexing, the second spore strip tends to stick to the first treated spore strip. Therefore, reagent from the treated spore could transfer to the second spore strip, thus inhibiting growth.

The sixth possible explanation is the result of the Dispatch® treated group could be due to the reactive oxygen species (ROS) that signals the vegetative cell to form spore. Spore forming bacteria become spore due to stress from the outside environment (i.e. chemical, heat, etc.). Vice versa, it could also trigger the spore to form bacteria due to stress from outside the environment. The active ingredient in Dispatch® is bleach and it contains oxygen that can be metabolized to form active oxygen species. Therefore, it was possible that ROS (highly reactive radical) was the stressor that triggers the vegetative cells to form spores. If the reagent stays

with the spore strip, then the media that contained the treated spore strip has the reactive oxygen species that acts as stressor which signals the vegetative cells to form spores. Therefore, this could explain why in the first six progressive washings of Dispatch® group, only the media that contained the treated spore strip remained negative because the media contained the residue reagent which was the stressor. On the other hand, if ROS was taken away, the spores germinate again and this could be the reason why growth was recovered in the media after removal of the treated spore strip. Again, Dispatch® reagent stay with the treated spore strips; therefore, when the treated spore strip was removed, the stressor (ROS) was also removed which allowed the spore to germinate when incubated at 60°C. One possible way to test if vegetative cells can be triggered by bleach to form spores is by placing an untreated spore strip in TSB media and then allow it to germinate to form vegetative cells. Then, transfer the vegetative cells to the new TSB media and add bleach to the media. If no growth was observed, then there are two possibilities: either the reagent killed the vegetative cells or it triggered the vegetative cells to form spores.

A seventh possible explanation may be that a higher concentration of sodium hypochlorite and contact time were needed to kill spores. In this research, Dispatch® contained 0.65% Sodium Hypochlorite and the maximum contact time was five minutes. According to Omidbakhsh's research, bleach at high concentration (5.25%) and ten minutes of contact time was sufficient to kill *C. difficile* spores on surfaces. However, a lower concentration of bleach or less than a five-minute treatment time was not sufficient to kill *C. difficile* spores.[28] High concentrations of sodium hypochlorite is harmful to human body. According to the CDC, high concentrations can lead to ocular irritation or oropharyngeal, and gastric burns. Also, according to Russell *et al.*, a low concentration of bleach can inhibit vegetative bacteria in seconds, but a higher concentration is required to kill *M. tuberculosis*.

Lastly, it is possible that Dispatch® killed *C. difficile* in five minutes because it was easier to kill then *G. stearothermophilus*. For instance, research conducted by Lawley *et al*. tested sodium hypochlorite on both *G. stearothermophilus* (from spore strip) and *C. difficile* spores with 1% sodium hypochlorite. Their findings suggested that strong oxidizing active ingredients such as sodium hypochlorite can inactivate *C. difficile* and *G. stearothermophilus* spores by blocking spore-mediated transmission. However, their finding suggested it takes more than 20 minutes to inactivate 10^6 *G. sterothermophilus*, but only takes five minutes to kill *C. difficile*. [26]

Experiment IV Part I and II showed the limitations of this study which included variability of the methodology (vortexing, disinfectant-soaking procedure, etc.) which resulted in inconsistency in the results. The results concluded that between 100-1000 spores were released after the first wash, which was negligible compared with one million spores in the strip. The results also suggested pre-wetting the spore strip does not make a difference. Furthermore, no additional growth was observed after the first 24 hour of incubation period. The growth rate was faster then what the CDC stated. The CDC recommended to incubate the spore strip for seven days in order to determine 99.9% kill.[23] The results of this experiment suggested that spores grew over night (less than 12 hours). Therefore it is possible that one to ten spores were sufficient for a positive overnight culture with the same density of growth as 10^6 spores. The inconsistency of spores being released after the first wash (PBS treated spore strip/untreated spore strip) explained why there was inconsistency in growth during the progressive washing (Figure 5).

For Experiment V, the result of spore contaminated barrier bags showed that a 10 second treatment of disinfectant wipes was sufficient to inhibit spore growth because no growth was observed after incubation. In this experiment, several control groups were tested to ensure that barrier bags were able to be contaminated. The result of the first control group confirmed that spores could attach to the surface of PSP barrier bags. The result of the second control group

confirmed that the wipe itself would not wipe away the spores by air drying the spore contaminated PSP barrier bags.

CHAPTER 6: CONCLUSION

This study assessed the effectiveness of sporicidal ability of three commercial disinfectant wipes commonly used in the dental office. The CDC approved spore test strips impregnated with *G. stearothermophilus* spores were used to determine the achieved level of disinfection. Treatment involved both direct saturation of spore strips with disinfectant and progressive washing of treated spore strips. Direct treatment of the spore strips with all the disinfectant wipes demonstrated total inhibition of the vegetative cells outgrowth.

If the experiment stopped at the direct treatment of spore strips, Dispatch® would have been misinterpreted as sporicidal. In progressive washing experiments, both Volo™ and CaviWipes® groups demonstrated the ability to directly kill the spore because even when the reagent was washed off the treated spore strips, growth was not recovered. In contrast, as soon as the Dispatch® treated spore strips were removed from the TSB media, the residual spores were able to grow at 60 ˚C. Surprisingly, when the treated strips were present in the TSB media, the entire test tube became an inhibitory tube; therefore, residual reagent was able to inhibit the growth of the second untreated spore strips. This indicated that Dispatch® was effective in inhibiting vegetative cells outgrowth. It is possible that once the TSB tubes were placed in the incubator at 60°C, the spores would try to germinate, but stopped growing because they sensed sodium hypochlorite as a threat. However, as soon as the treated spore strip was removed from the media, the residual spores no longer sensed the threat and then they began to grow. Tenfold serial dilution of the spore strip showed one spore was sufficient for positive overnight culture with the same density of growth as 10^6 spores. Therefore, additional studies should be conducted on Dispatch® Wipe to better understand it.

Both Volo™ and CaviWipe® are proven to be sporicidal, while Dispatch® was only effective in inhibiting vegetative cells. Therefore, Volo™ and CaviWipe® may be a practical approach for decontaminating PSP barriered bag surfaces. The chain cross contamination in radiology could be broken by using sporicidal disinfectant wipes. For instance, once the contaminated barriered bag is removed from a patient's mouth, it could be wiped down with Volo™ or CaviWipe® (or wipes with similar active ingredients) for 10 seconds, then the barrier bag can be opened to retrieve the PSP receptor. If the barrier bag is clean, then it will not contaminate the receptor and therefore will not contaminate the scanner and other radiographic equipment. Therefore, using disinfectant wipes could minimize the transfer of infectious diseases from patient to provider and vice versa.

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