Spring 2007

Bactericidal Effects of Cold Plasma Technology on *Geobacillus stearothermophilus* and *Bacillus cereus* Microorganisms

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BACTERICIDAL EFFECTS OF COLD PLASMA TECHNOLOGY ON *GEOBACILLUS STEAROTHERMOPHILUS* AND *BACILLUS CEREUS* MICROORGANISMS

by

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B.S.D.H. May 2006, Old Dominion University

A Thesis Submitted to the Faculty of
Old Dominion University in Partial Fulfillment of the
Requirement for the Degree of

MASTER OF SCIENCE

DENTAL HYGIENE

OLD DOMINION UNIVERSITY
May 2007

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ABSTRACT

BACTERICIDAL EFFECTS OF COLD PLASMA TECHNOLOGY ON *GEOBACILLUS STEAROTHERMOPHILUS* AND *BACILLUS CEREUS* MICROORGANISMS

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Non-equilibrium atmospheric pressure plasma, also known as cold plasma, is a state of matter that consists of a mix of neutral and charged particles. Plasma generates chemically reactive species and ultraviolet radiation making them useful in decontamination applications (Kong & Laroussi, 2003). Research regarding the inactivation of gram-positive bacteria, such as *Bacillus atrophaeus*, by cold plasma has been studied by Laroussi et al. (2003); however, there is limited research regarding the germicidal effectiveness of cold plasma on the microorganisms *Geobacillus stearothermophilus* and *Bacillus cereus*. The purpose of this study was to determine if cold plasma technology inactivates heat resistant microorganisms, specifically, *G. stearothermophilus* and *B. cereus* vegetative cells and spores. ***Methods:*** The study consisted of 762 *G. stearothermophilus* and *B. cereus* samples exposed to cold plasma at various times and 219 control samples (N=981). Bacteria were inoculated and exposed to either indirect or direct cold plasma, incubated for 12 to 16 hours and number of colony forming units (CFU) determined. The percentage kill and log concentration reductions were computed utilizing the CFU and data was analyzed using one-way ANOVA, Kruskal Wallis and Tukey's tests at the .05 level. ***Results:*** There was a statistically significant difference in the inactivation of *G. stearothermophilus* vegetative cells receiving indirect exposure ($p=.0001$) and direct exposure ($p=.0013$) and *B. cereus*

vegetative cells and spores exposed to indirect and direct cold plasma (p=.0001 for both). Cold plasma exposure to *G. stearothermophilus* spores demonstrated no statistically significant difference in inactivation of microorganisms receiving indirect (p=.7208) and direct (p=.0835) exposure. ***Conclusion:*** Results indicate that indirect and direct cold plasma exposure significantly inactivated *G. stearothermophilus* vegetative cells and *B. cereus* vegetative cells and spores; however, *G. stearothermophilus* spores were not significantly inactivated; therefore, sterility was not achieved.

ACKNOWLEDGMENTS

The successful completion of this thesis would not have happened without the strength from God. I want to thank Him for being there to listen to my countless prayers and for providing me with Bible verses to feel comforted in my times of joy and need.

I want to thank my committee members for their guidance and direction in conducting the research and with the development of this thesis. Gayle McCombs for providing direction and tremendous support, Wayne Hynes for the amount of wisdom and information you have taught me, Mounir Laroussi for your expertise and knowledge regarding cold plasma and Lynn Tolle for your experience in dental hygiene. Without your help, understanding and patience, this manuscript would not have happened.

To Dayanand Naik, I greatly appreciate your help with the statistical analysis and for answering all of my questions. You are a wonderful statistician!

To the American Dental Hygienists' Association Institute for Oral Health, I want to express sincere gratitude and thanks for funding this research.

My wonderful husband, Matt, you have been so supportive and helpful throughout the undergraduate and graduate Dental Hygiene programs. All of the cooked meals, cleaning, back massages and foot rubs relieved the stress that continuously accumulated. I love you with all of my heart and look forward to the next chapter in our lives!

To my brother Josh, you have been the best brother anyone could have and a shoulder I could lean and cry on. There has never been any form of jealousy between us and I am blessed to have a brother that wants the best for my life. Thank you for always being there!

To my parents, Wayne and Mary, and the rest of the Morris family, your direction and support have been a blessing and a motivation in my life. Leaving our beautiful home of Grottoes was one of the most difficult things I have ever done, but this country girl received an eye-opening experience when she left for the city! Thank you for always being there for me, traveling to be patients when I had clinic, for the countless prayers and for instilling in me the perseverance to never quit. You've always taught me to reach for the moon and if I land on a star to be happy. I'm still reaching for that moon!

I want to thank Dr. Tamer Akan for all of your wisdom, experiences, knowledge and smiles that you have shared with me. You were always willing to help and our story time together was very meaningful to me and led to the development of a wonderful friendship. Thank you for all of your help and for the wonderful diagram of the direct cold plasma chamber!

Thank you to Shannon Hensley for your help in conducting the research. You are an excellent microbiologist, teacher and friend. The best of luck in all of your future endeavors!

To Sudhakar Alla and Asma Begum, thank you so very much for the flexibility, kindness and unselfishness that you have shown me. It was such a pleasure to have met each of you. I treasure the countless hours we spent treating bacteria to cold plasma and I will never forget you.

Ann Poindexter, what a friend and inspiration you have been to me! Thank you for always being there experiencing the dental hygiene program with me. I could come to you with anything and I strongly believe that you are like a sister I never had.

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CHAPTER I

INTRODUCTION

Low temperature, non-equilibrium air plasma at atmospheric pressure, also known as cold plasma, is a state of matter that consists of a mix of neutral and charged particles. Moreover, plasmas generate chemically reactive species and ultraviolet radiation that makes this new emerging technology useful in decontamination applications (Kong & Laroussi, 2003). Research has been conducted regarding the effectiveness of cold plasmas on strains of bacteria, such as *Bacillus atrophaeus* (previously called *Bacillus subtilis*), *Escherichia coli*, and *Staphylococcus aureus* (Laroussi, 2002; Laroussi, Mendis & Rosenberg, 2003; Laroussi, Tendero, Lu, Alla & Hynes, 2006b; Lee, Paek, Ju & Lee, 2006). Results suggest that cold plasma has the capability to inactivate 90% of bacteria, such as *E. coli*, in a time frame of 15 seconds to five minutes depending on the type of medium the microorganisms are cultured on (Laroussi, 2002). However, research regarding the inactivation of *Geobacillus stearothermophilus,* formerly called *Bacillus stearothermophilus,* and *Bacillus cereus* is limited. *G. stearothermophilus* is commonly found on biological indicator test strips, utilized in dentistry, to verify sterility of resistant microorganisms (Schneider, Reich, Kirckof & Foltz, 2005). Dual species biological indicator strips contain two microorganisms, *G. stearothermophilus* and *B. atrophaeus. G. stearothermophilus* spores are used in steam or chemical vapor sterilization, and *B. atrophaeus* spores are used to monitor dry heat or ethylene oxide sterilization methods (Acosta-Gio, Mata-Portuguez, Herrero-Farias & Perez, 2002). In this study, only one heat-stable microorganism contained on a dual-species biological indicator strip, *G.*

stearothermophilus (American Type Culture Collection (ATCC) 12980), was selected because additional information regarding the bactericidal efficacy of cold plasma on another *Bacillus* strain was desired. Therefore, *B. cereus* (ATCC 14579) was chosen instead of *B. atrophaeus* since it is responsible for causing food poisoning, and at the spore stage, is extremely resistant to heat (ESR Ltd, 2006).

This study demonstrated cold plasma's effectiveness in destroying two heat resistant microorganisms, *G. stearothermophilus* and *B. cereus*. *G. stearothermophilus*, which is commonly found on dual species biological indicator sterilization strips, was selected for this study because of its use in dentistry and its extreme heat resistance. Additionally, *B. cereus* was utilized as foundational research to determine if cold plasma would inactivate *Bacillus anthracis*. *B. cereus* was assessed in addition to *G. stearothermophilus* since this microorganism is more likely to be found in a dental office than *G. stearothermophilus*. Moreover, the study sought to determine which type of cold plasma treatment (indirect and direct) had the greatest germicidal effect on *G. stearothermophilus* and *B. cereus* (vegetative cells and spores) after various exposure times. Results from cold plasma exposure times were assessed to estimate the minimum time needed to achieve inactivation.

Colony forming units (CFU) were determined after incubation for 12 to 16 hours at 55°C for *G. stearothermophilus* and 30°C for *B. cereus*, as recommended by the ATCC (American Type Culture Collection, 2006). If CFU were too numerous to accurately count (greater than 300), the results were recorded as "too numerous to count" (TNTC). The concentration of cells in the overnight culture was determined by plating out serial tenfold dilutions on to the agar. After overnight incubation the number of colonies were

counted. To determine the CFU in the original sample, the number of colonies at each dilution was multiplied by the inverse of the dilution factor (e.g. a 10^{-2} dilution would be 10^2 dilution factor) and by the inverse of the volume of liquid plated (e.g. 100uL plated equaled a volume factor of 10). This calculation determines the number of CFU/mL in the starting culture.

Statement of the Problem

G. stearothermophilus, contained on biological indicator test strips, is an effective monitor used in dentistry to determine sterility of instruments after autoclaving, and *B. cereus* is an opportunistic pathogen that may be found in dental offices. Traditional methods used for sterilization, such as ethylene oxide, steam, or dry heat, are time consuming, require adequate ventilation and may damage certain materials, such as plastics (Cuny, Bednarsh & Ecklund, 2003; Laroussi & Leipold, 2003). An improved sterilization method is desired to decrease the required sterilization cycle time, improve convenience, and provide a mechanism to sterilize materials which cannot be treated by traditional methods. According to Laroussi et al. (2006b), the "low temperature feature of non-equilibrium plasmas makes them the technology of choice in applications requiring medium preservation..." such as that with plastic dental instruments or other armamentarium. Although still experimental, the application of cold plasma as a sterilization method has the potential to achieve such improvements. Therefore, this study sought to answer the following research questions:

- What is the relationship between the length of time *G. stearothermophilus* or *B. cereus* are exposed to cold plasma and the number of CFU surviving?

- Does cold plasma have the greatest inactivation effect on vegetative cells or on spore stages for *G. stearothermophilus* and *B. cereus* following various exposure times to cold plasma?
- Does the greatest inactivation of *G. stearothermophilus* and *B. cereus* occur from indirect or direct cold plasma exposure?
- Is there a correlation between the type of bacteria and the exposure time of cold plasma in the number of CFU?

Significance of the Problem

This study explored the effectiveness of indirect and direct cold plasma technology as a potential new method of sterilization. Indirect, or "remote" cold plasma exposure occurs when microorganisms are placed away from the discharge of plasma particles, thus, the sample is located in a second or adjacent chamber (Laroussi, 2002; Laroussi, 2006). According to Laroussi, Minayeve, Dobbs & Woods (2006a), indirect exposure has a reduced amount of heat and "the charged particles do not play a role because they recombine before reaching the sample." In contrast, microorganisms receiving direct exposure come into direct contact with the plasma discharge thus, "all plasma-generated agents, including charged particles, come in contact with the sample" (Laroussi, 2002; Laroussi et al., 2006a).

This study identified the length of time necessary for cold plasma to achieve reductions in vegetative cells and spores of *G. stearothermophilus* and *B. cereus*. Sterilization of instruments is considered to occur when all forms of microorganisms have been killed, and *G. stearothermophilus* is a commonly used organism on biological indicator test strips that are used to verify sterility after exposure to steam or chemical

vapor sterilization methods (Acosta-Gio et al., 2002; Madigan & Martinko, 2006; Miller & Palenik, 2005; Samaranayake, 2002). Furthermore, *B. cereus* is an opportunistic pathogen that commonly causes food poisoning, specifically two distinct forms known as emetic and diarrheal (Helgason, Caugant, Olsen & Kolsto, 2000). *B. cereus* has been associated with infections of the eyes and possibly with periodontitis yielding benefits beyond instrument sterilization (Helgason et al., 2000). Destruction of *B. cereus* may indicate the potential cold plasma has in destroying periodontal pathogens and preventing certain diseases, such as food poisoning and eye and wound infections (Helgason et al., 2000).

The results from this study will be compared to the research conducted by Laroussi, Mendis, and Rosenberg (2003) to determine if cold plasma has the ability to effectively destroy gram-positive microorganisms, such as *G. stearothermophilus* and *B. cereus*. Research regarding the inactivation of certain gram-positive bacteria, such as *B. atrophaeus*, by cold plasma has been studied by Laroussi et al. (2003); however, there is limited literature on the germicidal effectiveness of cold plasma on *G. stearothermophilus* and *B. cereus* (Birmingham, 2004; Birmingham, 2006; Boudam, Moisan, Saoudi, Popovici, Gherardi & Massines, 2006).

Effective sterilization of instruments is required to ensure that infection control procedures are preventing cross-contamination of diseases among patients and health care personnel. A more efficient, cost effective sterilization method may benefit medical and dental offices by reducing the risk of improper sterilization. According to Schultz (2002a), experimental cold plasma units are less than $1,000; whereas, the cost of moist heat (autoclave) sterilization units may be from $1,623 to $11,040, dry heat sterilizers

cost approximately $3,795, and ethylene oxide units range from $15,000 to $20,000 (Tuttnauer Equipment, 2003-2006; Conviser, 2005).

The method of measurement utilized in this study was counting CFU and, from this measurement, the percentage kill and log concentration of the microorganisms were calculated. The CFU indicated the presence and quantity of microorganisms and was used to derive the concentration (CFU/mL) and percentage kill, a percentage of the bacteria that were killed after receiving cold plasma treatment (Laroussi, 2002; Todar, 2002).

Terms Used

Bacteria—a microorganism that is unicellular and prokaryotic and contains both DNA and RNA, is capable of growth and contains a rigid cell wall (Samaranayake, 2002).

Biological Indicator Test Strip—a filter paper inoculated with bacterial spores, such as *G. stearothermophilus* and *B. atrophaeus* or the combination of *G. stearothermophilus* and *B. atrophaeus*, which are used to determine sterility of instruments after processing through moist heat, dry heat, or ethylene-oxide sterilization methods (Miller & Palenik, 2005; Schultz, 2002b).

Cold Plasma—a non-thermal, atmospheric pressure, non-equilibrium fourth state of matter that involves weakly ionized gases consisting of positively and negatively charged ions, electrons and neutral species (Bogaerts, Neyts, Gijbels & van der Mullen, 2001; Kong & Laroussi, 2003; Laroussi, 2002; Laroussi, 2005).

Colony Forming Units (CFU)—the quantity of viable microorganism colonies that are countable following inoculation onto a suitable medium, such as agar plates, and incubated for a desired time (Todar, 2005).

D-value—amount of time required to kill 90% of microorganisms; therefore, 1 D means that 90% of the microorganisms from the original concentration were killed and 10% remain viable (Laroussi, 2002; Mazzola, Vessoni Penna & da S Martins, 2003).

Direct Cold Plasma—microorganisms come into direct contact with all plasma-generated particles (Laroussi, 2002; Laroussi, 2006).

Inactivation—microorganisms rendered nonviable or incapable of successfully living (Seton Resource Center, n.d.).

Indirect Cold Plasma—also referred to as "remote" exposure. Microorganisms are at a distance from the discharge of plasma particles, thus, the sample is placed in an adjacent or second chamber; therefore, there is a reduced amount of heat reaching the microorganisms and the charged particles and some neutral reactive species may not even reach the sample (Laroussi, 2002; Laroussi, 2006).

Percentage Kill—a percentage of microorganisms that were destroyed due to a specific type of treatment. The experimental CFU is subtracted from the control CFU, divided by the control CFU and multiplied by 100, and this percentage is a reflection of the effectiveness of a sterilization method.

Spores—bacteria that are non-growing and highly heat resistant with a structure containing layers, such as the exosporium, spore coat and spore cortex, which are absent in the vegetative cell (Madigan & Martinko, 2006)

Sterilization—occurs when all forms of microorganisms are killed (Madigan & Martinko, 2006; Miller & Palenik, 2005).

Vegetative Cells—a state of bacteria that grows and thrives under favorable environmental conditions; this state is not as resistant as spores (Executive Summary, 2005; Todar, 2005).

Assumptions

For the purpose of this study, the following assumptions regarding the use of cold plasma as a future sterilization method were:

- Cold plasma has the potential to be an effective method of sterilization as determined by the CFU and the calculations of the percentage kill and log concentrations (CFU/mL).
- *G. stearothermophilus* and *B. cereus* microorganisms exposed to cold plasma did not undergo cross-contamination from the researchers after exposure, as outlined by infection control procedures to prevent cross-contamination of the control and the experimental groups. Each of the bacteria were exposed on separate days, stored at separate laboratory benches and incubated in separate incubators. Prior to microbiology procedures, the laboratory bench was disinfected with Clorox® Bleach to further reduce the risk of cross-contamination.
- Direct cold plasma exposure would have a faster inactivation time because the sample would have direct contact with the plasma particles whereas with indirect exposure the plasma particles are in a second or adjacent chamber.
- The concept of sterilization is perceived as the killing of all forms of microorganisms (Madigan & Martinko, 2006; Miller & Palenik, 2005).

- The researchers followed the same microbiology laboratory procedures for *G. stearothermophilus* and *B. cereus* microorganisms; however, cold plasma tests determined the appropriate exposure times.
- Cross-contamination of the control group did not occur because, prior to cold plasma exposure, the control plates were taped to prevent accidental opening and contamination of the exposed plates. Also, control plates were not stored on top of or below treated plates.
- The CFU, which is used to calculate the percentage kill, is believed to be an accurate instrument to measure the effectiveness of cold plasma on the inactivation of *G. stearothermophilus*, found on dual species biological indicator strips, and *B. cereus*, a common cause of food poisoning and a microorganism discovered to be associated with periodontal disease.

Limitations

Cold plasma was assessed in this study as having the potential to become a new method of sterilization. However, factors that threatened the validity of the results were:

- Cold plasma exposed *G. stearothermophilus* and *B. cereus* in culture and not on dental instruments; therefore, the results cannot be generalized to conclude that cold plasma is an effective sterilization method until research is conducted using dental instruments.
- *B. cereus* was exposed to cold plasma instead of *B. atrophaeus*, an additional key indicator of sterility utilized on dual-species biological indicator test strips. *B. cereus* was used as foundational research to determine cold plasma's ability to inactivate *B. anthracis*, and there is a greater chance that *B. cereus* will be found

in a dental office than *G. stearothermophilus*. However, *B. cereus* was not as resistant to cold plasma exposure as *G. stearothermophilus*.

- Contamination of inoculated Petri dishes and the glass slides may have occurred, thus, threatening the validity and reliability of the results. However, this threat was minimized by using proper precautions to prevent cross-contamination between *G. stearothermophilus* and *B. cereus*. The two bacteria cultures were stored in different locations, on separate laboratory benches, and the inoculated Petri dishes were placed into separate incubators. *G. stearothermophilus* required the 55°C incubator whereas *B. cereus* needed the 30°C incubator. Researchers were not be allowed to access the two microorganisms simultaneously, thus, exposures of *G. stearothermophilus* did not occur on the same day as *B. cereus*, and vice-versa. Laboratory benches and equipment were disinfected with Clorox® Bleach prior to accessing the bacteria as an effort to decrease the risk of cross-contamination.
- Accurate readings from the CFU were necessary for the results to be valid. The CFU represent the number of microorganisms left viable following cold plasma exposure and the 12 to 16 hour incubation time. The concentration of cells in the overnight culture was determined by plating out serial tenfold dilutions on to agar. After overnight incubation, colonies were counted. To determine the CFU in the original sample, the number of colonies at each dilution was multiplied by the inverse of the dilution factor (e.g. a 10^{-2} dilution would be a 10^{2} dilution factor) and by the inverse of the volume of liquid plated (e.g. 100uL plated would be 10 volume factor). This calculation determines the number of CFU/mL in the starting

culture. Therefore, if a discrepancy in the CFU count and/or the calculation of the concentration of bacteria had occurred, validity of the results would be threatened. Having an experienced biology researcher assist with the counting of CFU minimized this threat. In addition, prior to study initiation, researchers participated in various pilot tests that required counting CFU, thus, establishing inter- and intra-rater reliability and decreasing the threat to validity.

- *G. stearothermophilus* is a resistant, thermophilic microorganism, but the effectiveness of cold plasma at inactivating all types of thermophilic microorganisms cannot be concluded from this study.

Hypotheses

The following hypotheses were tested at a .05 level:

HO_1: There will be a statistically significant difference in the germicidal effect of cold plasma treatment on vegetative cells of *Geobacillus stearothermophilus* for 15, 20, 25 and 30 minutes via the indirect chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_2: There will be a statistically significant difference in the germicidal effect of cold plasma treatment on spores of *Geobacillus stearothermophilus* for 15, 20, 25 and 30 minutes via the indirect chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_3: There will be a statistically significant difference in the germicidal effect of cold plasma treatment on vegetative cells of *Geobacillus stearothermophilus* for 1, 2, 4, 5, 6, 8, 10, 15, 20 and 30 minutes via the direct chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_4: There will be a statistically significant difference in the germicidal effect of cold plasma treatment on spores of *Geobacillus stearothermophilus* for 10, 20 and 30 minutes via the direct chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_5: There will be a statistically significant difference in the germicidal effect of cold plasma treatment on vegetative cells of *Bacillus cereus* for 1, 2, 3, 4, 5, 10, 15, 20, 25, and 30 minutes via the indirect chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_6: There will be a statistically significant difference in the germicidal effect of cold plasma treatment on spores of *Bacillus cereus* for 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 minutes via the indirect chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_7: There will be a statistically significant difference in the germicidal effect of cold plasma treatment on vegetative cells of *Bacillus cereus* for 10, 20, 30, 40, 50 seconds, and 1, 2, 4, 6, 8 and 10 minutes via the direct chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_8: There will be a statistically significant difference in the germicidal effect of cold plasma treatment on spores of *Bacillus cereus* for 30 seconds and 1, 2, 3, 4 and 5 minutes via the direct chamber, compared to those not exposed to cold plasma, as measured by CFU.

CHAPTER II

REVIEW OF THE LITERATURE

The literature contains studies on the effectiveness of cold plasma on the inactivation of bacteria, such as *Bacillus subtilis* (now known as *Bacillus atrophaeus*), *Escherichia coli*, *Staphylococcus aureaus* and *Saccharomyces cerevisiae* (Laroussi et al., 2003; Lee et al., 2006). Exposing bacteria that are on different types of media (Petri dish, liquid suspension, glass slab) has an effect on the amount of cold plasma exposure time needed for inactivation to occur (Laroussi, 2002). Furthermore, during direct cold plasma exposure, the microorganisms come into direct contact with the discharge of the plasma particles. In contrast, samples are located in a second or adjacent chamber for indirect exposure, thus, plasma particles do not directly contact the microorganisms (Laroussi, 2002; Laroussi, 2006). The effectiveness of cold plasma as a potential sterilization method in the inactivation of the microorganisms *G. stearothermophilus* and *B. cereus* have received limited research focus (Birmingham, 2004, 2006; Boudam et al., 2006). Literature on the applications of cold plasma, effects of cold plasma on microorganisms and comparing direct and indirect cold plasma exposure provided the theoretical basis for this study.

Applications of Cold Plasma

Sterilization, a daily, repetitive process, is an essential part of infection control procedures, which takes place within a dental or medical office. Sterilization destroys all forms of microorganisms and is a term that should not be used synonymously with inactivation because sterilization occurs only when all microorganisms are inactivated (Akitsu, Ohkawa, Tsuji, Kimura & Kogoma, 2005; Boudam et al., 2006; Moisan,

Barbeau, Crevier, Pelletier, Philip & Saoudi, 2002). Infection control is a major topic in health professional programs, thus learning the proper way to sterilize instruments and equipment, as well as monitoring the sterilization process, is incorporated into the didactic and clinical curriculum. Examining cold plasma as a potential sterilization method is important because this type of sterilization may become more cost effective, less timely, and produce less toxic waste as compared to the traditional types of sterilization (steam, dry heat, ethylene oxide, or chemical vapor) (Akitsu et al., 2005; Laroussi, 2002; Moisan et al., 2002). According to Laroussi (2005), cold plasma has a simple design, is practical because it does not require a bulky machine, has a low cost to operate, and does not cause thermal damage to the items it comes in contact with.

Findings from Laroussi et al. (2003) suggest that cold plasma inactivates certain microorganisms such as *E. coli* and *B. atrophaeus*. However, additional studies are needed involving various microorganisms, such as *G. stearothermophilus* and *B. cereus,* since the results from Laroussi et al. (2003) cannot be generalized to the inactivation of all microorganisms. In addition, selection-treatment interaction may have threatened external validity within the study conducted by Laroussi et al. (2003) due to the types of microorganisms used. Limited research has been conducted regarding the effects of cold plasma in the inactivation of *G. stearothermophilus* and *B. cereus* spores; therefore, research is needed to substantiate theories.

Effect of Cold Plasma on Different Types of Microorganisms

Cold plasma produces a greater structural effect on gram-negative bacteria, such as *E. coli*, than on gram-positive bacteria, such as *B. atrophaeus* (Laroussi et al., 2003). Gram-negative bacteria experience structural damage to the outer membrane following

exposure to cold plasma, whereas more resistant gram-positive bacteria do not show the degree of morphological effects seen with gram-negative bacteria (Laroussi, Richardson & Dobbs, 2002). According to Laroussi et al. (2003), even though structural damage was not observed in gram-positive bacteria following exposure, the bacteria were left nonviable suggesting that cold plasma inactivates the microorganisms without necessarily changing the structure. The microorganisms that did not experience structural changes from exposure to cold plasma, yet were still rendered nonviable, suggests that inactivation of bacteria may occur without having evident morphological damage (Laroussi et al., 2003). However, instrumentation effects may have threatened the internal validity of this study because the amount of structural damage that resulted may not have accurately been assessed by the instrument, and inconsistency among the researchers interpreting the results of the morphological changes may have occurred. Before results could be interpreted, the bacteria had to be fixed in gluteraldehyde overnight and inconsistency in this procedure among researchers may have resulted (Laroussi et al., 2003). In addition, scanning electron microscopy was used to provide images of the damage caused by exposure to cold plasma and the results interpreted from this visual image may have affected internal validity due to inconsistencies regarding inter-rater and/or intra-rater reliability.

According to Laroussi (2002), the microorganisms that survive exposure to cold plasma depend on the microorganism used. For example, gram-positive bacteria, such as *B. atrophaeus,* do not have an outer lipopolysaccharide layer found in gram-negative bacteria, but gram-positive microorganisms have a thicker, more rigid, and stronger cell wall peptidoglycan layer than gram-negative bacteria. Gram-positive bacteria experience

less structural damage from exposure to cold plasma, but are still nonviable following exposure, whereas gram-negative bacteria experience an apparent structural change as well as being inactivated (Laroussi et al., 2003). Cold plasma effects on the inactivation of microorganisms may require increasing the exposure time to account for the differences in structures of gram-positive and gram-negative bacteria to ensure that sterilization is achieved regardless of the type of bacteria exposed. The literature revealed that microorganisms which have been tested, such as *E. coli, B. atrophaeus, S. aureus, P. aeruginosa,* etc., were rendered nonviable following exposure to cold plasma (Laroussi, 2002; Laroussi et al., 2003). However, more research should be conducted regarding the effects of cold plasma on other resistant microorganisms, such as viruses and fungi; therefore, results from previous studies cannot be generalized to include cold plasma's inactivation of all forms of microorganisms.

G. stearothermophilus and *B. cereus* Characteristics

G. stearothermophilus is an extremely heat resistant, gram-positive, spore-forming, aerobic microorganism (Watanabe, Furukawa, Hirata, Koyama, Ogihara and Yamasaki, 2003). This microorganism is associated with spoilage of canned liquid foods in vending machines, such as coffee. *G. stearothermophilus* is commonly incorporated on biological indicator strips, used to monitor sterilization methods, because of its resistance (Watanabe et al., 2003). According to Watanabe et al. (2003), spores of *G. stearothermophilus* are not inactivated by room temperature treatment. Atmospheric pressure cold plasma operates at or near room temperature; however, *G. stearothermophilus* requires high temperatures for effective inactivation (Watanabe et al.,

2003). In addition, *G. stearothermophilus* has the ability to form spores, thus, making the microorganism more resistant.

B. cereus is an aerobic, spore-forming, gram-positive, opportunistic pathogen that commonly causes food poisoning and has been associated with periodontal disease and bacteremias (Beuchat, Rocelle, Clavero & Jaquette, 1997; Helgason, Okstad, Caugant, Johansen, Fouet, Mock, Hegna & Kolsto, 2000; Leonard, Zekri & Mahillon, 1998). There are stains of *B. cereus* that produce enterotoxins and emetic toxins, resulting in diarrhea and vomiting, the classic characteristics of food poisoning (Leonard et al., 1998). According to Marsili, Espie, Anderson and MacGregor (2002), *B. cereus* microorganisms demonstrate susceptibility to plasma treatment by being inactivated within 10 to 30 seconds of exposure. Additionally, when air was used as the gas for the plasma, a greater inactivation of *B. cereus* occurred after 50 seconds of treatment in comparison to using nitrogen or carbon dioxide gas mixtures. This may be due to the ozone and free radicals that are produced in the breakdown of the air gas, causing inactivation of the *B. cereus* microorganisms (Marsili et al., 2002). Since *B. cereus* was effectively inactivated by an air gas plasma treatment, it was expected that the low-voltage atmospheric pressure air plasma system utilized for this study would efficaciously inactivate this microorganism. However, *B. cereus'* ability to form spores would require a longer cold plasma exposure time than that for the vegetative cells.

Cold Plasma Effects on Spores

According to Birmingham (2006), the spore structure increases resistance to physical and chemical treatments. The spore protects the nucleic acids causing difficulties in their extraction. Laroussi et al. (2006a) reveal that gram-positive spores are

able to withstand harsh conditions that vegetative cells are unable to endure because the cell's genetics is located between a compact core protected by a wall and a protein coat. The spore structure enables the spore to remain dormant during unfavorable conditions and, when conditions improve, the spore can return to its vegetative state (Laroussi et al., 2006; Madigan & Martinko, 2006). Furthermore, the outer and inner coats contain a region consisting of peptidoglycan and its thickness varies for gram-positive and gram-negative bacteria. Gram-positive bacteria have a peptidoglycan wall of 10 to 20 layers in comparison to only 1 to 3 layers for gram-negative bacteria (Scheffers & Pinho, 2005). Peptidoglycan thickness may contribute to gram-positive microorganisms' resistance to cold plasma treatment.

Laroussi et al. (2006) reported that plasma produces cellular metabolic changes that may cause the cell to die when inactivation by cold plasma particles is not the direct cause for its death. Moisan et al. (2002) demonstrate that the "number, thickness and chemical composition of the spore-protecting layers and on the location of its DNA material" provides a limitation to spore death from plasma treatment. Additionally, Moisan et al. (2002) reveal that spreading spores on larger areas results in an initial higher death because there are fewer spores per area being exposed to cold plasma, making penetration of plasma particles more effective. The same concept occurs with higher dilution rates. The more diluted the bacteria, the shorter amount of time it requires for inactivation (Moisen et al., 2002). Overall, a factor influencing the efficacy of plasma sterilization is related to the bacterial state (vegetative cell or spore) that is being exposed (Moisen et al., 2002). Since vegetative cells do not have a protective spore structure,

inactivation of vegetative cells should occur within a shorter exposure time to cold plasma.

Effect of Cold Plasma on Microorganisms on Different Media

Exposing bacteria that are on different types of media (liquid suspension, glass slab, polypropylene) effects inactivation by cold plasma (Laroussi, 2002). Laroussi (2002) demonstrated that the survivor curves of the microorganisms are related to the medium that they were cultured on. For example, the D-value, or the time that was needed to destroy 90% of the original concentration, of *B. atrophaeus* on a glass slab was much shorter than the time required for inactivation of the same bacteria in a liquid suspension (Laroussi, 2002). Results from Laroussi (2002) suggest that different types of dental instruments and armamentarium may require varying times for effective sterilization. For example, there are different types of material used in dental instruments; therefore an instrument made of plastic may require a shorter sterilization time than a metal one, or vice-versa.

Factors that Affect Sterilization

Key factors increase the chances of a proper sterilization cycle for processing dental instruments. According to Schultz (2002b), properly loading instruments increases the effectiveness of traditional sterilization methods. Moreover, the strategic placement of instruments in an autoclave improves the chances that steam will be able to penetrate each product and that proper drying will take place. The penetration of cold plasma is affected by the type of medium receiving exposure. Laroussi (2002) suggests that cold plasma is able to inactivate microorganisms on glass at a faster rate than microorganisms in liquid suspensions. Results from the Laroussi (2002) study reveal that the D-value for

E. coli microorganisms on glass was 15 seconds whereas it took 5 minutes for *E. coli* cultured in a liquid suspension. The penetration of cold plasma as a method of sterilization may not necessarily be influenced by the placement of the dental instruments, but rather by the type of material used. Cold plasma does not require a special drying cycle following sterilization because it does not produce moisture during sterilization, unlike an autoclave.

Traditional sterilization methods require the use of a chemical indicator to determine if the appropriate temperature was reached, whereas cold plasma treatment occurs at or near room temperature (Shultz, 2002b). This present study attempted to determine if cold plasma reached the level of penetration necessary for sterility by inactivating all *G. stearothermophilus* and *B. cereus*, not whether a specific temperature was reached. Since cold plasma does not produce an increased thermal change, a chemical indicator would have been inappropriate to use since cold plasma inactivates microorganisms at or near room temperature (Kong & Laroussi, 2003).

In summary, cold plasma has the potential to become a method of sterilization in the future. Its inactivation of resistant gram-positive bacteria, such as *B. atrophaeus*, without causing significant structural damage to the microorganism suggests that cold plasma has the ability to kill without producing evident morphological changes in all microorganisms. Vegetative cells may require a shorter exposure time than spores because vegetative cells do not contain a protective spore coat. In addition, the material in which cold plasma is exposed to may have an effect on the amount of exposure time needed to adequately destroy resistant microorganisms such as *G. stearothermophilus* and *B. cereus*.

CHAPTER III

METHODS AND MATERIALS

Research Design

A multi-factorial research design was utilized during this study. Petri dishes and glass slides containing *G. stearothermophilus* or *B. cereus* were exposed to indirect or direct cold plasma at various treatment times. The experimental group consisted of *G. stearothermophilus* and *B. cereus* vegetative cells and spores that were inoculated onto trypticase soy agar (TSA) or Luria Bertani (LB) agar plates, respectively, and exposed to cold plasma at various exposure times (Table 1). The control group contained *G. stearothermophilus* and *B. cereus* vegetative cells and spores inoculated onto agar plates, not exposed to cold plasma, also referred to as "0 seconds" exposure.

Sample Description

The sample consisted of vegetative cells and spores of *G. stearothermophilus* (ATCC 12980) and *B. cereus* (ATCC 14579). The total sample size for this study was N= 981 exposed (n= 762) and unexposed samples (n= 219) (Table 2). The indirect chamber exposed 344 samples and the direct chamber exposed 418 samples (n= 762) (Tables 2 and 3).

Description of Agency Setting

The Department of Biological Sciences at Old Dominion University (ODU) was utilized to conduct all microbiology procedures. Cold plasma exposure occurred at the ODU Physical Electronics Research Institute (PERI) Lab and the Frank Reidy Research Center for Bioelectrics.

Table 1. Cold Plasma Exposure Times

Dependent and Independent Variables and Cold Plasma Exposure Times			
Dependent Variables		**Independent Variables**	
Bacteria and State	**Control (No Exposure)**	**Indirect Cold Plasma Exposure Times**	**Direct Cold Plasma Exposure Times**
G. stearothermophilus **Vegetative**	0 seconds	15 minutes	1 minute
		20 minutes	2 minutes
		25 minutes	4 minutes
		30 minutes	5 minutes
		----------	6 minutes
		----------	8 minutes
		----------	10 minutes
		----------	15 minutes
		----------	20 minutes
		----------	30 minutes
G. stearothermophilus **Spores**	0 seconds	15 minutes	10 minutes
		20 minutes	20 minutes
		25 minutes	30 minutes
		30 minutes	----------
B. cereus **Vegetative**	0 seconds	1 minute	10 seconds
		2 minutes	20 seconds
		3 minutes	30 seconds
		4 minutes	40 seconds
		5 minutes	50 seconds
		10 minutes	1 minute
		15 minutes	2 minutes
		20 minutes	4 minutes
		25 minutes	6 minutes
		30 minutes	8 minutes
		----------	10 minutes
B. cereus **Spores**	0 seconds	1 minute	30 seconds
		2 minutes	1 minute
		3 minutes	2 minutes
		4 minutes	3 minutes
		5 minutes	4 minutes
		10 minutes	5 minutes
		15 minutes	----------
		20 minutes	----------
		25 minutes	----------
		30 minutes	----------

Table 2: Total Sample Sizes per Exposure Type

Total Sample Size for Cold Plasma Exposures			
Exposure Type	**Experimental**	**Control**	**Total**
Indirect	344	96	**440**
Direct	418	123	**541**
Total Sample	**762**	**219**	**981**

Table 3. Total and Distribution of Sample Size

Sample Size and Distribution							
	Control Group Veg.	**Control Group Spores**	***G. stearo.* Veg.**	***G. stearo.* Spores**	***B. cereus* Veg.**	***B. cereus* Spores**	**Total**
Indirect	46	50	48	80	116	100	**440**
Direct	75	48	82	36	192	108	**541**
Total	**121**	**98**	**130**	**116**	**308**	**208**	**981**

Procedures and Materials

G. stearothermophilus and *B. cereus* from ATCC were obtained.

Indirect Cold Plasma Exposure

Prior to indirect cold plasma exposure, microorganisms were cultured in trypticase soy (TS) broth, diluted and plated onto TSA media for *G. stearothermophilus* and LB media for *B. cereus*. Following cold plasma treatment, experimental and control plates were incubated for 12 to 16 hours at 55°C for *G. stearothermophilus* and 30°C for *B. cereus*. The dilutions, as determined from preliminary pilot testing, were 10^{-1} and 10^{-2} for the vegetative cells and spores of *G. stearothermophilus*. Dilutions for *B. cereus* were 10^{-4} and 10^{-5} for vegetative cells and 10^{-5} and 10^{-6} for spores. An extra dilution of 10^{-6} and 10^{-7} were plated for the vegetative and spore control plates, respectively, as an effort

to reduce the risk of the *B. cereus* colonies growing too large and, thus, producing control plates that were TNTC. In addition, *B. cereus* was plated onto LB media because pilot testing determined that TSA media resulted in the formation of extremely large colonies that were unable to be accurately counted, whereas LB media provided a more suitable environment for this type of microorganism. In an effort to reduce colonies TNTC, dilution rates were determined prior to cold plasma exposure to ensure that the growth rate was between 30 and 300 colony forming units. Provided below are specific details regarding the microbiology and cold plasma lab procedures and equipment.

Indirect Exposure Microbiology Procedures

- *G. stearothermophilus* and *B. cereus* were inoculated into 5.0 mL of TS Broth by flaming a bacterial loop, swiping the loop across a T-streak of the microorganisms and dispensing the inoculated loop into the broth.
- Inoculated culture was incubated for 24 hours and for 7 days. Both cultures contained vegetative cells and spores.
- Culture was vortexed for 10 to 15 seconds and 500 µL of the inoculated culture was pipetted into 4.5 mL of 0.9% sterile saline.
- Appropriate dilution rates were made by pipetting 500 µL of previous dilution into test tubes containing 4.5 mL of 0.9% sterile saline.
- 100 µL was placed onto TSA agar media for *G. stearothermophilus* and LB media for *B. cereus* and spread-plated using a manual turntable.
- Experimental group was transported to cold plasma lab and exposed.
- Control plates were placed in refrigerator during exposure.

- Spores of the bacteria were placed on ice when transporting to the cold plasma lab and before and after receiving cold plasma exposure to prevent vegetative cells from actively growing.
- Following exposure, control and experimental plates were inverted and placed into appropriate incubators.
- After 12 to 16 hours of incubation, colonies were counted and plates were disposed in the biohazard waste container.

Indirect Exposure Cold Plasma Procedures

- Plates were placed into chamber (refer to Figure 1 and Appendices A, B and C for placement).
- Chamber door was secured.
- Cold plasma exposure was started and timed via a stop-watch.
- At end of exposure time, cold plasma was turned-off and chamber evacuated for 60 seconds.

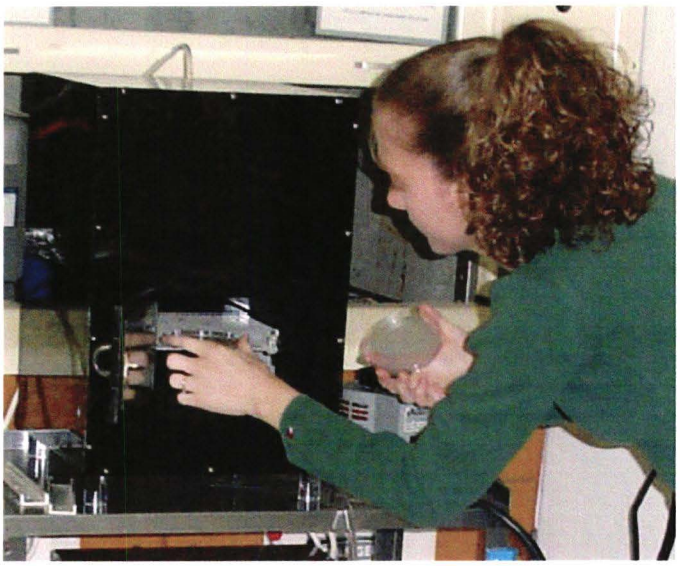


Figure 1. Placing Petri Dishes into Indirect Cold Plasma Chamber

Direct Cold Plasma Exposure

Procedures for the direct cold plasma chamber differed slightly from the indirect chamber. Additionally, slightly different pre-exposure procedures were followed for *G. stearothermophilus* and *B. cereus*. *B. cereus* followed the same inoculation and incubation procedures as *G. stearothermophilus*, however, after vortexing the culture for 10 to 15 seconds, a dilution of 10^{-2} was made and this diluted culture was exposed to cold plasma. At the cold plasma lab, the same procedures were followed as those for *G. stearothermophilus*, as well as those for dilution and plating at the microbiology lab; however, *G. stearothermophilus* was incubated at 55°C whereas *B. cereus* was incubated at 30°C (ATCC, 2006; Todar, 2005).

Direct Exposure Microbiology Procedures

- *G. stearothermophilus* and *B. cereus* were inoculated into 5.0 mL of TS Broth by flaming a bacterial loop, swiping the loop across a T-streak of the microorganisms and dispensing the inoculated loop into the broth.
- Inoculated culture was incubated for 24 hours and for 7 days. Both cultures contained vegetative cells and spores.
- Culture was vortexed for 10 to 15 seconds.
    - For *G. stearothermophilus,* culture was placed onto ice and transported to the cold plasma lab.
    - For *B. cereus*, 500 µL of the inoculated culture was pipetted into 4.5 mL of 0.9% sterile saline, appropriate dilutions determined via pilot testing were made, diluted culture of 10^{-2} was placed on ice and transported to the cold plasma lab.
- Following direct cold plasma exposure, tubes were vortexed for 10 to 15 seconds, appropriate dilutions were made and 100 µL from each dilution was spread-plated onto appropriate agar plates.
- Plates were inverted and stored in appropriate incubators for 12 to 16 hours.
- CFU were quantified and plates were discarded into the biohazard waste container.

Direct Exposure Cold Plasma Procedures

- Pipetted 10 µL of culture onto a sterile glass slide about ½ inch from the edge
    - Experimental group's glass slide with culture was placed onto platform of direct cold plasma chamber (Figure 2).

- Control group was not placed onto direct cold plasma chamber platform.

- Direct chamber was turned on by one researcher and a timer was started simultaneously by a second researcher (Appendix D).
- After the appropriate exposure time was complete, bacteria was rinsed from glass slide into a new sterile test tube by pipetting 1 mL of 0.9% sterile saline over the 10 µL drop of bacteria.
- Test tube was placed on ice and transported back to microbiology lab.

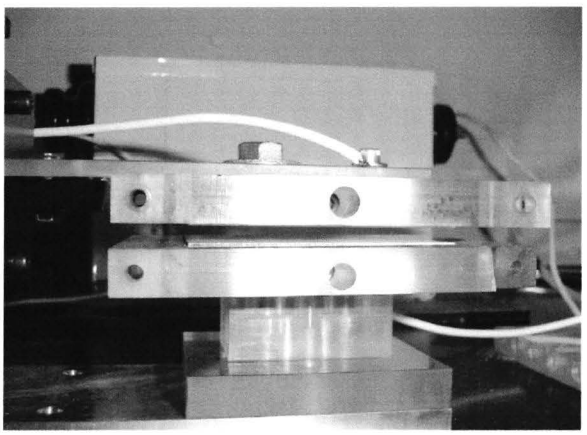


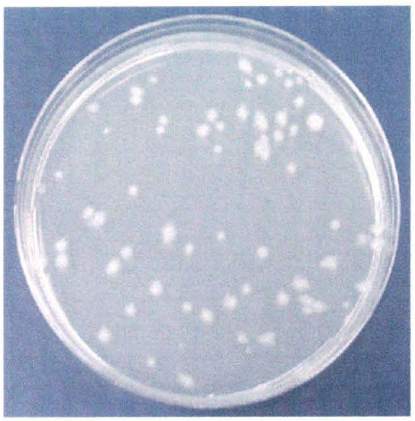
Figure 2. Platform of Direct Chamber

The experimental group of *G. stearothermophilus* vegetative cells and spores receiving indirect cold plasma exposure were treated for 15, 20, 25 and 30 minutes whereas exposure times of 10, 20 and 30 minutes were utilized for this bacteria in the direct chamber. *B. cereus* vegetative and spores experimental plates were exposed for 1, 2, 3, 4, 5, 15, 20, 25 and 30 minutes in the indirect chamber. *B. cereus* vegetative cells were exposed for 10, 20, 30, 40, 50 seconds, 1 minute, 2, 3, 4 and 5 minutes in the direct chamber, whereas the spores received direct treatment for 30 seconds, 1, 2, 3, 4, and 5

minutes (Table 1). Colonies were counted to assess the number of microorganisms that were left viable after exposure to cold plasma. The percentage kill and log concentration reduction were calculated from the CFU measurement. The concentration of cells in the overnight culture was determined by plating out serial tenfold dilutions of the culture on to the agar. After overnight incubation, the number of colonies on the plates were counted. To determine the number of colony forming units (CFU) in the original sample, the number of colonies at each dilution was multiplied by the inverse of the dilution factor (e.g. if it was a 10^{-2} dilution then the dilution factor would be 10^{2}) and by the inverse of the volume of liquid plated (e.g if 100ul was plated then the volume factor would be 10). This calculation determines the number of CFU/mL in the starting culture. Additionally, cold plasma is an innovative technology and modifications were made throughout the study to determine treatment that resulted in statistically significant kill, resulting in varying sample sizes.

Data Collection Instruments

G. stearothermophilus and *B. cereus* inoculated onto Petri dishes allowed for the quantification of CFU, which were utilized in determining the concentration of the bacteria or the CFU/mL (Figure 3). CFU were also used to calculate the percentage kill, which is the proportion of colonies that were killed via cold plasma exposure (experimental group) compared to the number of colonies in the control group. The percentage kill directly revealed the effectiveness of cold plasma in the inactivation of *G. stearothermophilus* and *B. cereus* by providing a ratio that resulted in a percentage of the bio-burden of microorganisms that were effectively inactivated by cold plasma.



*Each colony represents one CFU

Figure 3. Petri Dish Showing Bacterial Colonies

The comparison of the quantity and presence of *G. stearothermophilus* and *B. cereus* between the treatment and control groups allowed researchers to analyze the effectiveness of cold plasma's kill capabilities. In a previous study by Laroussi (2002), the inactivation factor was a measurement used to assess the effectiveness of cold plasma's potential in the inactivation of microorganisms, such as *B. atrophaeus*, and determined the number of microorganisms that remained viable following exposure. Therefore, samples of *G. stearothermophilus* and *B. cereus* were a reliable and valid indicator of the potential sterilization effectiveness of cold plasma since *G. stearothermophilus*, which are found on biological indicator test strips used to monitor sterility, are consistent in measuring the efficacy of sterilization methods (Schneider et al., 2005).

To protect the researchers throughout the conduction of this study and to reduce the risk of cross-contamination between the experimental and control groups, proper infection control procedures were utilized. Prior to beginning any microbiology

procedures, the laboratory bench was disinfected with Clorox® Bleach. *G. stearothermophilus* and *B. cereus* T-streaked Petri dishes were stored at separate laboratory benches and *G. stearothermophilus* was placed in the 55°C incubator whereas *B. cereus* was incubated in the 30°C incubator. The bacteria were exposed on separate days to reduce the risk of cross-contamination and to prevent handling the two types of microorganisms in the same time period. Thorough hand-washing was conducted prior to and following any microbiology or cold plasma procedures.

Physicist and engineering research assistants ensured proper equipment set-up and maintenance of the cold plasma chambers. In addition, a biology research assistant was utilized to assist with microbiology procedures, such as dilution, inoculation and counting procedures. Experienced research assistants contributed to the validity and reliability of the research procedures.

Protection of Human Subjects

Not applicable. Human subjects were not utilized in this study.

Statistical Analysis

Data analysis was conducted utilizing SAS® software program, version 9.1 (SAS® Products, 2007). The parametric test of one-way Analysis of Variance (ANOVA) was conducted to determine the means and standard deviations for data that was roughly normally distributed. In addition, one-way ANOVA tested the hypotheses to demonstrate if there were any statistically significant results. Secondly, the nonparametric Kruskal Wallis test was conducted for data that was not roughly normally distributed.

Tukey's Studentized Range (HSD) test was utilized to determine statistical significance between each time exposure. One-way ANOVA and the Kruskal Wallis test

determined overall statistical significance; however, Tukey's test was necessary in determining which cold plasma treatment times resulted in statistically significant inactivation of *G. stearothermophilus* and *B. cereus*.

CHAPTER IV

RESULTS

The present study explored the bactericidal effects of cold plasma technology on vegetative cells and spores of *G. stearothermophilus* and *B. cereus* microorganisms (Table 8). Furthermore, the study sought to determine the time necessary to inactivate the heat resistant microorganisms, *G. stearothermophilus* and *B. cereus,* when exposed to cold plasma for varying times and at different bacterial dilutions.

HO_1: There will be a statistically significant difference, when tested at the 0.5 level, in the germicidal effect of cold plasma treatment on vegetative cells of *Geobacillus stearothermophilus* for 15, 20, 25 and 30 minutes via the indirect chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_2: There will be a statistically significant difference, when tested at the 0.5 level, in the germicidal effect of cold plasma treatment on spores of *Geobacillus stearothermophilus* for 15, 20, 25 and 30 minutes via the indirect chamber, compared to those not exposed to cold plasma, as measured by CFU.

Results demonstrate that there is a statistically significant kill of *G. stearothermophilus* vegetative cells exposed to cold plasma in the indirect chamber (p-value of .0001); however, there is not a statistically significant kill in *G. stearothermophilus* spores receiving indirect cold plasma exposure (p-value of .7208). Mean CFU values are displayed in Table 4 and in Figure 4. Analysis demonstrates that there is a significant CFU reduction for *G. stearothermophilus* vegetative cells for all exposure times (15, 20, 25 and 30 minutes).

Percentage kill for *G. stearothermophilus* vegetative cells is 90.5% for 15 minutes, 79.21% for 20 minutes, 69.77% for 25 minutes and 72.5% for 30 minutes. However, indirect exposure on *G. stearothermophilus* spores demonstrated a kill of 56.29% at 15 minutes, 54.35% at 20 minutes, 54.6% at 25 minutes and 48.9% at 30 minutes (Table 4, Figure 8 and Figure 9). The mean log concentrations (CFU/mL) of *G. stearothermophilus* are found in Table 4.

Table 4. Comparison of Indirect Exposure of *G. stearothermophilus*

Exposure Time	*G. stearo.*	CFU Sum	Mean CFU	Standard Deviation	Standard Error	Percentage Kill (%)	Mean Log Concentration (CFU/mL)
0 seconds	Veg.	1611	134.25	113.68	32.82	0	10.1627
15 minutes	Veg.	153	12.75*	14.81	4.28	90.5	7.0173
20 minutes	Veg.	335	27.92*	28.20	8.14	79.21	8.2417
25 minutes	Veg.	487	40.58*	61.18	17.66	68.77	8.2038
30 minutes	Veg.	443	36.92*	70.37	20.31	72.5	8.2026
0 seconds	Spore	771	48.19	92.25	23.06	0	5.0346
15 minutes	Spore	337	21.06	41.73	10.43	56.29	3.2897
20 minutes	Spore	352	22.00	51.21	12.80	54.35	3.6817
25 minutes	Spore	350	21.88	42.05	50.11	54.6	4.3414
30 minutes	Spore	394	24.63	51.12	12.78	48.9	3.9782
*Indicates statistically significant CFU reduction compared to the control (0 seconds)							

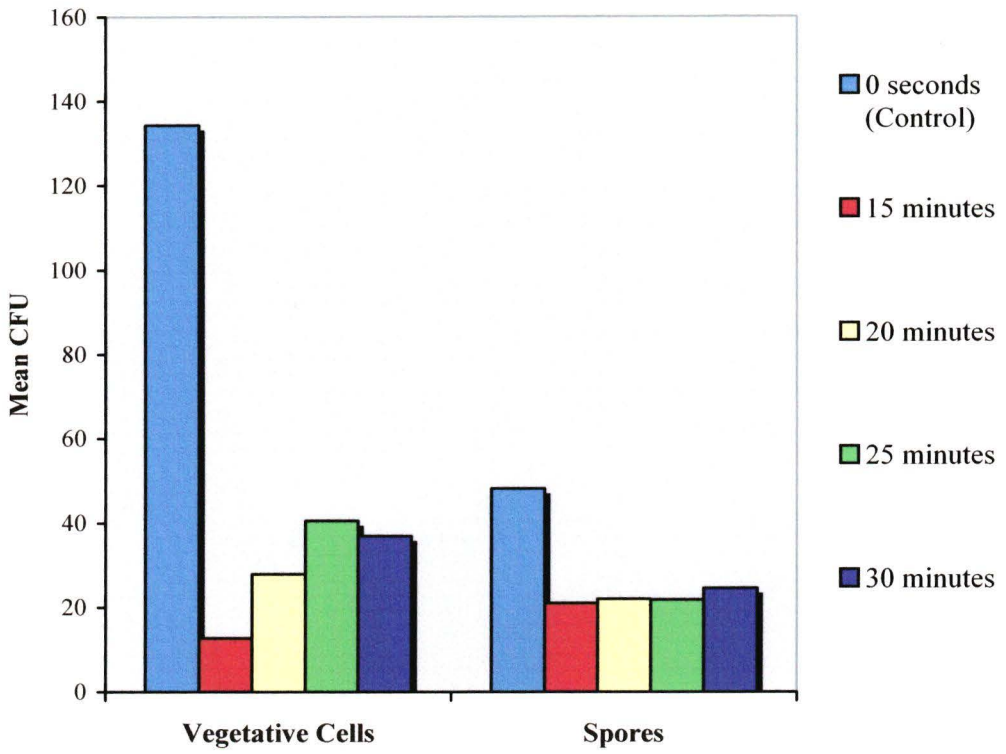


Figure 4. Mean CFU of *G. stearothermophilus* Receiving Indirect Exposure

HO_3: There will be a statistically significant difference, when tested at the 0.5 level, in the germicidal effect of cold plasma treatment on vegetative cells of *Geobacillus stearothermophilus* for 1, 2, 4, 5, 6, 8, 10, 15, 20 and 30 minutes via the direct chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_4: There will be a statistically significant difference, when tested at the 0.5 level, in the germicidal effect of cold plasma treatment on spores of *Geobacillus stearothermophilus* for 10, 20 and 30 minutes via the direct chamber, compared to those not exposed to cold plasma, as measured by CFU.

Results demonstrate that there is a statistically significant kill of *G. stearothermophilus* vegetative cells receiving cold plasma exposure via the direct chamber (p-value of .0013) with a significant reduction in CFU occurring at 10 minutes. *G. stearothermophilus* spores receiving direct exposure revealed no statistically significant reduction in CFU (p-value of .0835). Data are displayed in Table 5 and Figure 5. Percentage kill is also displayed in Table 5 and Figures 8 and 9.

Table 5. Comparison of Direct Exposure of *G. stearothermophilus*

Exposure Time	*G. stearo.*	CFU Sum	Mean CFU	Standard Deviation	Standard Error	Percentage Kill (%)	Mean Log Concentration (CFU/mL)
0 seconds	Veg.	2023	80.92	110.41	22.08	0	13.2702
1 minute	Veg.	603	150.75	113.81	56.90	70.19	13.9936
2 minutes	Veg.	620	155.00	168.64	84.32	69.35	13.0702
4 minutes	Veg.	908	181.60	123.84	55.38	55.12	13.5032
5 minutes	Veg.	123	30.75	26.17	13.09	93.92	13.3432
6 minutes	Veg.	785	130.83	135.82	55.45	61.20	13.3266
8 minutes	Veg.	807	134.50	132.40	54.05	60.11	13.5454
10 minutes	Veg.	1074	56.53*	78.07	17.91	46.91	10.0377
15 minutes	Veg.	59	14.75	12.61	6.30	97.08	12.4845
20 minutes	Veg.	216	15.43	22.81	6.10	89.32	10.0872
30 minutes	Veg.	211	21.43	30.27	9.57	89.57	11.6766
0 seconds	Spore	511	36.50	37.80	10.10	0	10.4567
10 minutes	Spore	89	7.42	8.68	2.51	82.58	7.9017
20 minutes	Spore	121	10.08	10.67	3.08	76.32	9.9735
30 minutes	Spore	376	31.33	85.37	24.64	26.42	9.7261
*Indicates statistically significant CFU reduction compared to the control (0 seconds)							

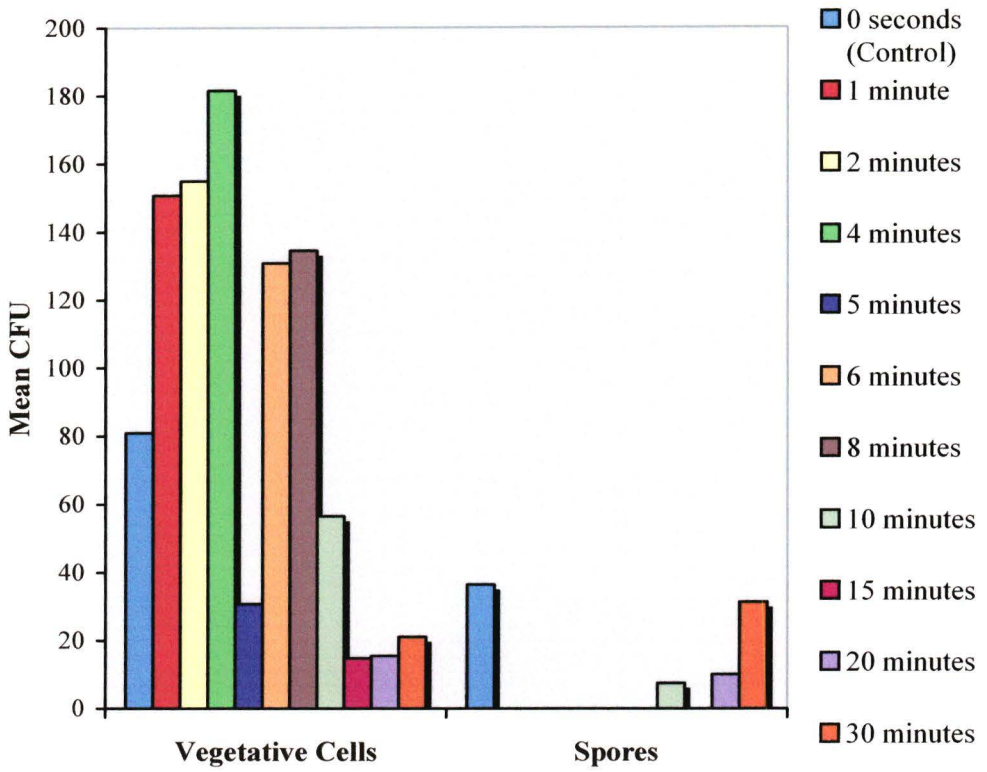


Figure 5. Mean CFU of *G. stearothermophilus* Receiving Direct Exposure

HO_5: There will be a statistically significant difference, when tested at the 0.5 level, in the germicidal effect of cold plasma treatment on vegetative cells of *Bacillus cereus* for 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 minutes via the indirect chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_6: There will be a statistically significant difference, when tested at the 0.5 level, in the germicidal effect of cold plasma treatment on spores of *Bacillus cereus* for 1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 minutes via the indirect chamber, compared to those not exposed to cold plasma, as measured by CFU.

Results reveal that there is a statistically significant kill of *B. cereus* vegetative cells and spores exposed to cold plasma in the indirect chamber, as compared to those not

exposed (p-values of .0001 for both states). All time points (1, 2, 3, 4, 5, 10, 15, 20, 25 and 30 minutes) demonstrate statistically significant reductions in CFU (Table 6 and Figure 6). Calculation of percentage kill indicates that a 100% kill starts at 4 minutes for vegetative cells and at 5 minutes for spores; however, one vegetative colony grew at 30 minutes causing a drop from 100% to 99.98% kill (Table 6, Figure 8 and Figure 9). Additionally, the mean log concentrations (CFU/mL) are provided in Table 6.

Table 6. Comparison of Indirect Exposure of *B. cereus*

Exposure Time	*B. cereus*	CFU Sum	Mean CFU	Standard Deviation	Standard Error	Percentage Kill (%)	Mean Log Concentration (CFU/mL)
0 seconds	Veg.	4639	136.44	175.78	30.15	0	12.1419
1 minute	Veg.	37	2.08*	7.66	2.21	99.20	4.2923
2 minutes	Veg.	50	4.17*	10.17	2.94	98.92	3.4007
3 minutes	Veg.	23	1.92*	6.64	1.92	99.5	1.2207
4 minutes	Veg.	0	0*	0	0	100	0.0000
5 minutes	Veg.	0	0*	0	0	100	0.0000
10 minutes	Veg.	0	0*	0	0	100	0.0000
15 minutes	Veg.	0	0*	0	0	100	0.0000
20 minutes	Veg.	0	0*	0	0	100	0.0000
25 minutes	Veg.	0	0*	0	0	100	0.0000
30 minutes	Veg.	1	0.08*	0.29	0.08	99.98	1.1513
0 seconds	Spore	539	17.97	27.22	4.97	0	11.9209
1 minutes	Spore	11	0.92*	3.18	0.92	97.96	1.3511
2 minutes	Spore	1	0.08*	0.29	0.08	99.81	1.1513
3 minutes	Spore	2	0.17*	0.39	0.11	99.63	2.4945
4 minutes	Spore	2	0.17*	0.58	0.17	99.63	1.2091
5 minutes	Spore	0	0*	0	0	100	0.0000
10 minutes	Spore	0	0*	0	0	100	0.0000
15 minutes	Spore	0	0*	0	0	100	0.0000
20 minutes	Spore	0	0*	0	0	100	0.0000
25 minutes	Spore	0	0*	0	0	100	0.0000
30 minutes	Spore	0	0*	0	0	100	0.0000
*Indicates statistically significant CFU reduction compared to the control (0 seconds)							

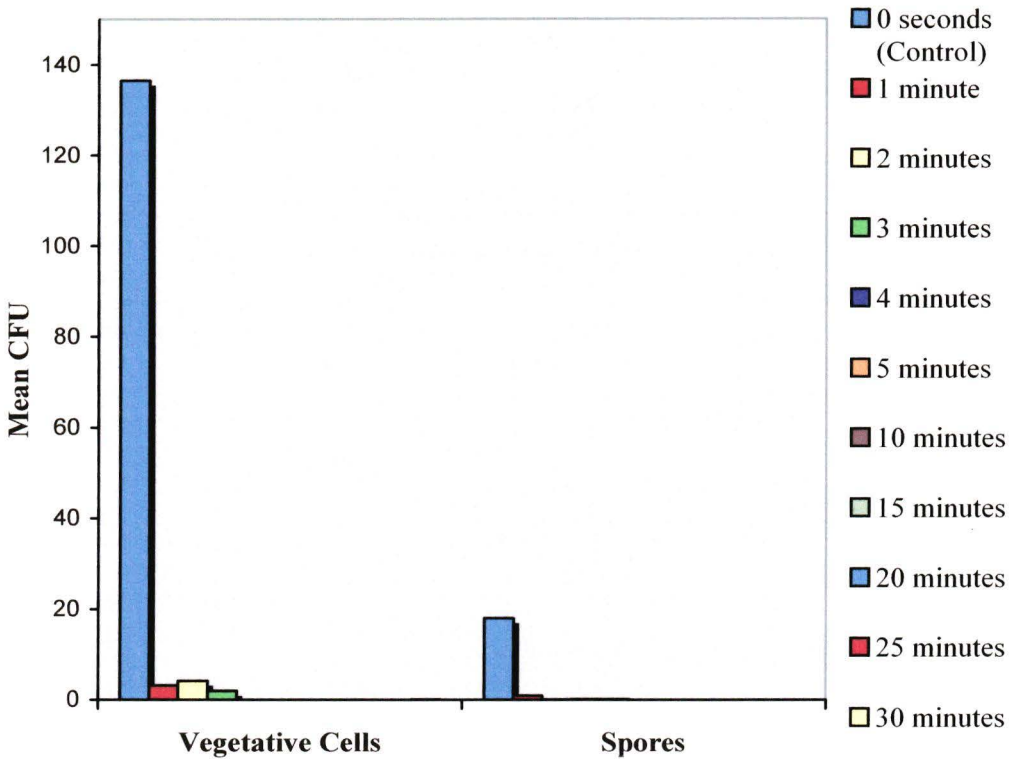


Figure 6. Mean CFU of *B. cereus* Receiving Indirect Exposure

HO_7: There will be a statistically significant difference, when tested at the 0.5 level, in the germicidal effect of cold plasma treatment on vegetative cells of *Bacillus cereus* for 10, 20, 30, 40, 50 seconds, and 1, 2, 4, 6, 8 and 10 minutes via the direct chamber, compared to those not exposed to cold plasma, as measured by CFU.

HO_8: There will be a statistically significant difference, when tested at the 0.5 level, in the germicidal effect of cold plasma treatment on spores of *Bacillus cereus* for 30 seconds, and 1, 2, 3, 4 and 5 minutes via the direct chamber, compared to those not exposed to cold plasma, as measured by CFU.

Results demonstrate that there is a statistically significant reduction in CFU for *B. cereus* vegetative cells and spores that are exposed to cold plasma via the direct chamber

(p-values of .0001 for both). *B. cereus* vegetative cells demonstrate a significant reduction in CFU starting at 50 seconds; whereas *B. cereus* spores reveal a significant kill starting at 3 minutes (Table 7 and Figure 7). Percentage kill is presented in Table 7, Figure 8 and Figure 9.

Table 7. Comparison of Direct Exposure of *B. Cereus*

Exposure Time	*B. cereus*	CFU Sum	Mean CFU	Standard Deviation	Standard Error	Percentage Kill (%)	Mean Log Concentration (CFU/mL)
0 seconds	Veg.	10067	201.34	144.88	20.49	0	17.2038
10 seconds	Veg.	3385	225.67	123.64	31.92	66.38	17.7222
20 seconds	Veg.	3105	194.06	110.50	27.62	69.16	17.6631
30 seconds	Veg.	1897	118.56	125.41	31.35	81.16	15.4614
40 seconds	Veg.	1419	94.6	121.85	31.46	85.9	14.1107
50 seconds	Veg.	133	8.87*	12.65	3.27	98.68	9.4600
1 minute	Veg.	1673	53.97*	110.74	19.89	83.38	7.2806
2 minutes	Veg.	709	47.27*	90.96	23.49	92.96	6.2018
4 minutes	Veg.	23	1.44*	3.61	0.90	99.77	1.7751
6 minutes	Veg.	18	1.13*	2.90	0.72	99.82	1.8503
8 minutes	Veg.	48	3*	10.02	2.50	99.52	1.7913
10 minutes	Spore	1	.06*	0.25	0.06	99.99	0.4318
0 seconds	Spore	6180	181.76	133.43	22.88	0	16.0282
30 seconds	Spore	1812	151	136.65	39.45	70.68	12.8474
1 minute	Spore	4697	195.71	143.83	29.36	24	14.5282
2 minutes	Spore	2748	114.5	129.22	26.38	55.53	13.5770
3 minutes	Spore	603	50.25*	117.13	33.81	90.24	4.0213
4 minutes	Spore	2	0.17*	0.39	0.11	99.97	1.9188
5 minutes	Spore	17	0.71*	2.12	0.43	99.72	1.7028
*Indicates statistically significant CFU reduction compared to the control (0 seconds)							

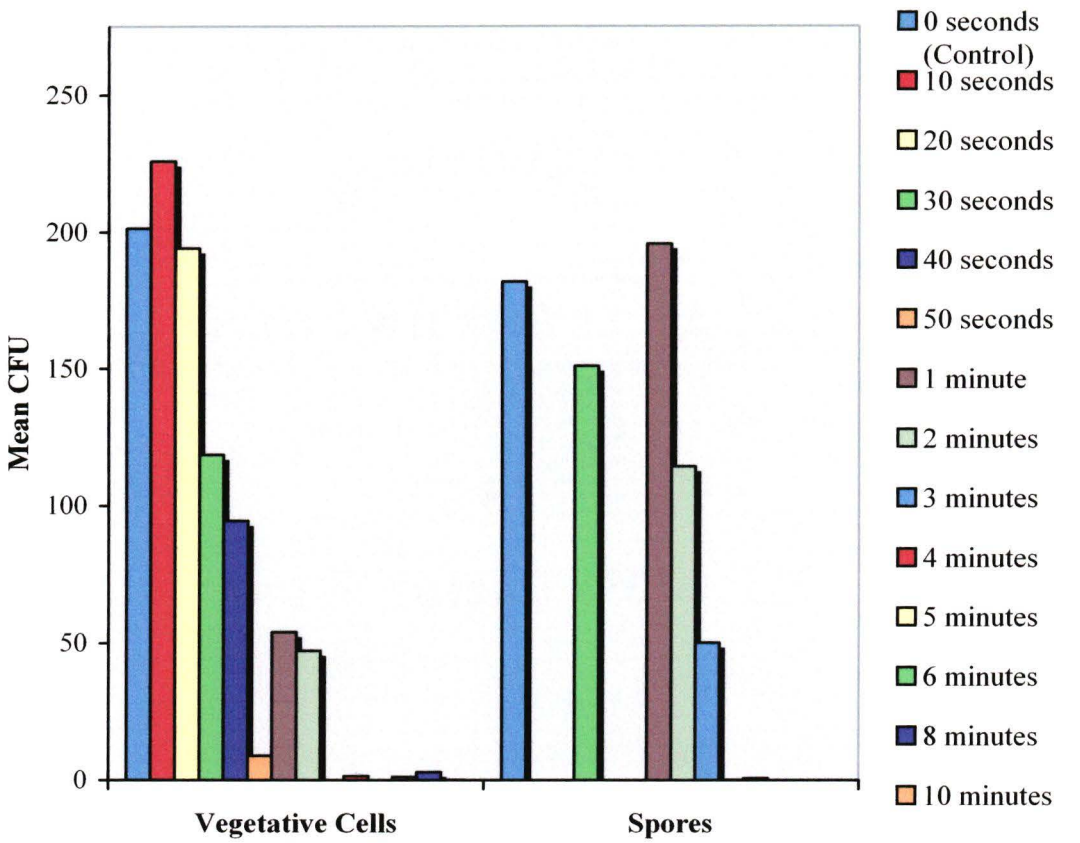


Figure 7. Mean CFU of *B. cereus* Receiving Direct Exposure

Table 8. Significance Values for Cold Plasma Exposure

Bacteria	State	Chamber	Significance
Geobacillus stearothermophilus	Vegetative	Direct	.0013*
		Indirect	.0001*
	Spore	Direct	.0835
		Indirect	.7208
Bacillus cereus	Vegetative	Direct	.0001*
		Indirect	.0001*
	Spore	Direct	.0001*
		Indirect	.0001*
* Denotes statistical significance less than or equal to .05			

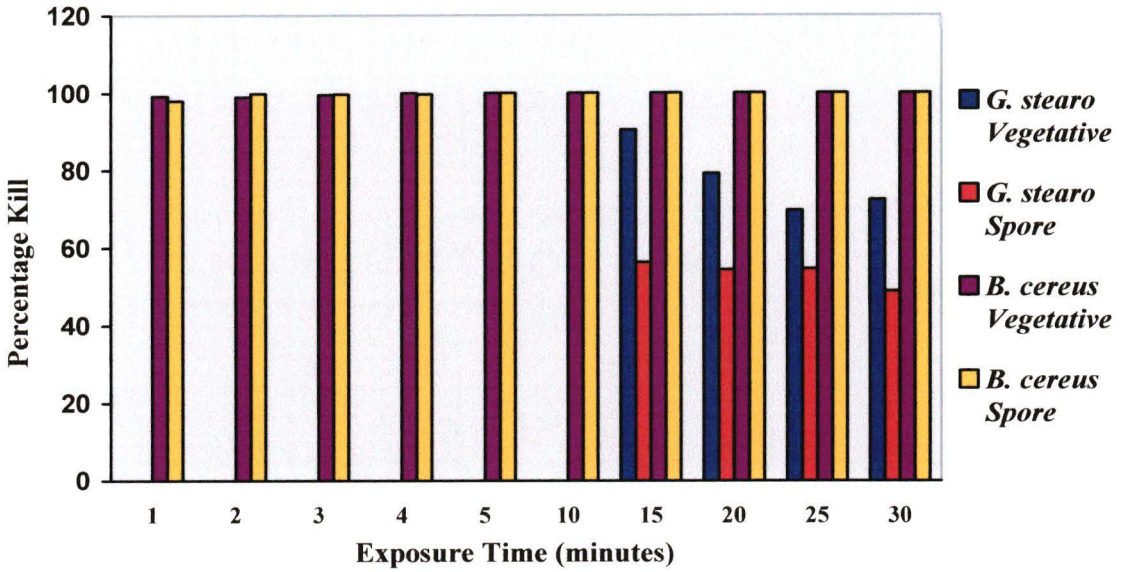


Figure 8. Percentage Kill for Indirect Cold Plasma Exposure

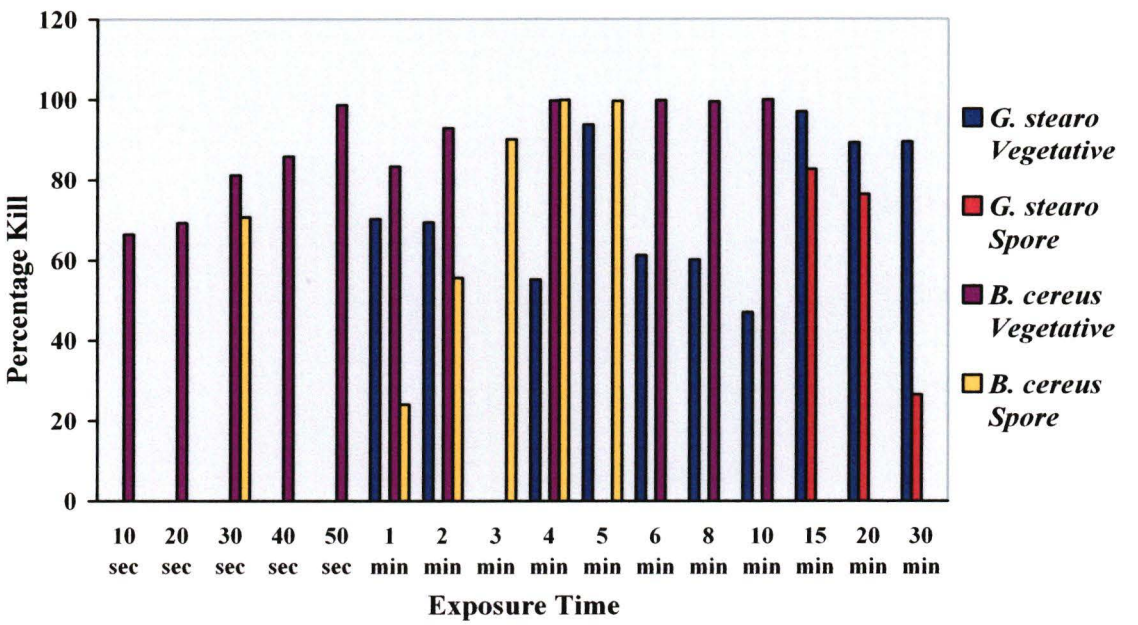


Figure 9. Percentage Kill for Direct Cold Plasma Exposure

CHAPTER V

DISCUSSION

The present study explored the germicidal effectiveness of cold plasma technology as a potential method of sterilization. This study was designed to test the bactericidal effect of cold plasma on *G. stearothermophilus* and *B. cereus*. Vegetative cells and spores were tested to assess any differences in the inactivation rate of the two forms. Since spores develop an encapsulated coating as a protective feature and vegetative cells are actively dividing and growing, it was expected by the researchers that vegetative cells would be inactivated at a faster rate than spores (Birmingham, 2006; Laroussi et al., 2006).

For the indirect chamber, the bacteria were inoculated on TSA or LB agar at various dilutions, experimental samples were exposed to cold plasma, incubated for 12 to 16 hours and CFU were counted. However, for the direct chamber, bacteria were pipetted onto a sterile glass slide and experimental samples were exposed to cold plasma before spread plating procedures occurred. Results were recorded if there was an average of 30 to 300 colonies on a plate; however, some plates revealed no growth, whereas others grew colonies TNTC. One explanation for the plates that rendered no growth was "bad" agar since the remaining plates, with fresher agar, rendered countable CFU. An explanation for TNTC was inappropriate dilution rates that yielded a bacterial culture that was too concentrated. In addition, *B. cereus* forms extremely large colonies if incubated for too long or at high temperatures; therefore, many of the TNTC for this bacteria were due to incubation times and/or temperatures.

Data was grouped for statistical analysis according to bacteria (*G. stearothermophilus* or *B. cereus*), bacteria state (vegetative or spores), cold plasma exposure (direct or indirect) and cold plasma exposure times (varies) (Appendices E & F). After data analysis, percentage kill was calculated by taking the total CFU for each bacteria, state, exposure type and time and subtracting this number from each control group's CFU, dividing this total by the control CFU and then multiplying by 100 for the percentage value. The log concentration (CFU/mL) was also calculated for each bacteria and state, exposure type and time.

Results from this study demonstrate that there is a statistically significant reduction in CFU of *G. stearothermophilus* vegetative cells, *B. cereus* vegetative cells and *B. cereus* spores exposed to indirect and direct cold plasma as compared to those not receiving exposure. However, there is not a statistically significant reduction in CFU for *G. stearothermophilus* spores exposed to either indirect or direct cold plasma.

The researchers postulate that *G. stearothermophilus* may clump together and that cold plasma may disrupt these clumps, thus, producing more CFU. An additional explanation is that cold plasma operates at or near room temperature; however, *G. stearothermophilus* spores are stable and require high temperatures for inactivation (Watanabe et al., 2003). In addition, results from this study can only be generalized to ATCC 12980 *G. stearothermophilus* and ATCC 14579 *B. cereus*.

Limitations

Due to utilizing an innovative new technology, the time required to achieve inactivation had to be determined as the study progressed, resulting in various exposure times for each bacteria, bacterial state and exposure type. In addition, trials that rendered

plates with TNTC or no growth were repeated to enhance statistical significance. However, this produced an unequal sample size and some time points contained a larger sample size than others.

Another factor that may have threatened validity and reliability was not utilizing a standardized timer when plating procedures occurred, thus, some samples may have received longer plating times than others, causing greater distribution of bacteria on the media. It is postulated that a greater distribution would produce a more uniform layer of bacteria on the media, making it more effective for cold plasma penetration. However, the researchers counted for at least 25 seconds for each plate, trying to reduce this limitation.

This study only examined CFU and additional assessment techniques may have produced greater information as to the structural changes that occur to *G. stearothermophilus* and *B. cereus* following cold plasma exposure. A limitation of this study is that scanning electron microscopy was not utilized and researchers were unable to assess structural damage that cold plasma was or was not causing.

The procedures required at the cold plasma lab for the direct chamber increased the risk for cross-contamination to occur due to the additional microbiology procedures performed prior to and after exposure. For example, glass slides were removed from the platform and rinsed with 1 mL of 0.9% sterile saline after receiving cold plasma treatment. This increased the risk of contamination from bacteria other than that tested. Additionally, a flame was not utilized at the cold plasma lab to reduce the risk of bacterial contamination on the pipette tips, test tube openings, etc.

Recommendations for Future Studies

Cold plasma has the potential to be a cost-effective, convenient and efficient method of instrument sterilization (Akitsu et al., 2005; Laroussi, 2002; Moisan et al., 2002). However, additional research is needed to assess the morphological effects caused by cold plasma on resistant bacterial strains. In this study, structural changes caused by cold plasma exposure were not assessed, but research conducted by Laroussi (2002) and Laroussi et al. (2003) utilized scanning electron microscopy to determine structural changes of bacteria. Therefore, a recommendation for future research is to use scanning electron microscopy or additional instruments of measurement that will enable the researchers to assess the structural changes that occur to microorganisms receiving cold plasma exposure.

Since the same procedures were followed for *G. stearothermophilus* and *B. cereus* for the indirect or direct chambers, it is suggested that the resistance of *G. stearothermophilus* spores may be related to bacterial clumping and cold plasma disrupting the clumps, thus, producing more CFU. Also, *G. stearothermophilus* spores demonstrate extreme stability and require high heat and pressure for inactivation (Watanabe et al., 2003). These factors contribute to the difficulty experienced in killing *G. stearothermophilus* spores using atmospheric pressure, nonthermal cold plasma and provide suggestions as to why there were no statistically significant reductions in CFU as compared to the control group. Future research assessing the structural changes that occur throughout varying cold plasma exposure times may support the bacterial clumping theory proposed by the researchers. Additionally, since *G. stearothermophilus* spores were more resistant to cold plasma than *B. cereus*, future studies are required to

determine if modifications to the cold plasma device would increase its efficacy in killing *G. stearothermophilus* spores.

Exposing bacteria on various types of media, other than agar or glass slides, is recommended. Comparisons could be made regarding the type of media and amount of time required for inactivation of *G. stearothermophilus* and *B. cereus*. It has been suggested by Laroussi (2002) that the type of media does affect cold plasma exposure times; however, this study did not assess this.

The indirect chamber exposed four Petri dishes at one time. Future studies should evaluate variability of sample placement within the chamber. The researchers kept track of the plate location within the chamber (front left, front right, back left or back right); however, the results were not analyzed differentiating between the locations (Appendices B and C). Additionally, a distance of 0.25 inch from the direct plasma output to the glass side was utilized for each exposure. A recommendation for future research would be to assess the variability of direct exposure by using different distances between the cold plasma output and the glass slide.

Lastly, research should be conducted on *G. stearothermophilus* determining the time at which total inactivation occurs. This study stopped at 30 minutes because there was variability in CFU the longer *G. stearothermophilus* was exposed. However, incorporating additional instruments of measurement would provide information as to what occurs following exposure and a time of complete inactivation of *G. stearothermophilus* may be evaluated and determined.

CHAPTER VI

SUMMARY AND CONCLUSIONS

The present study examined the bactericidal effects of cold plasma on *G. stearothermophilus* and *B. cereus* vegetative cells and spores. Results demonstrate that there is no statistically significant reduction of *G. stearothermophilus* spores exposed to indirect or direct cold plasma as compared to those not exposed. However, there is a statistically significant reduction of *G. stearothermophilus* vegetative cells and *B. cereus* receiving cold plasma exposure compared to those that did not. Based on the data analysis, six of the acting hypotheses were retained. Therefore, the following conclusions can be made:

1. Indirect and direct cold plasma exposure is statistically significant at reducing numbers of *Geobacillus stearothermophilus* vegetative cells, *Bacillus cereus* vegetative cells or spores, as evidenced by CFU (HO_1, HO_3, HO_5, HO_6, HO_7 and HO_8).
2. Indirect and direct cold plasma exposure is not statistically significant at reducing *Geobacillus stearothermophilus* spore numbers on TSA, as evidenced by CFU (HO_2 and HO_4).

As technology advances, a more efficient, cost-effective, portable and convenient sterilization method would benefit the dental and medical professions. Cold plasma has the potential to impact the health care profession beyond sterilization purposes, in particular, inactivating oral microorganisms associated with dental caries and periodontal disease. With additional research, knowledge regarding cold plasma and its bactericidal efficacy would be expanded and new applications for cold plasma would be utilized.

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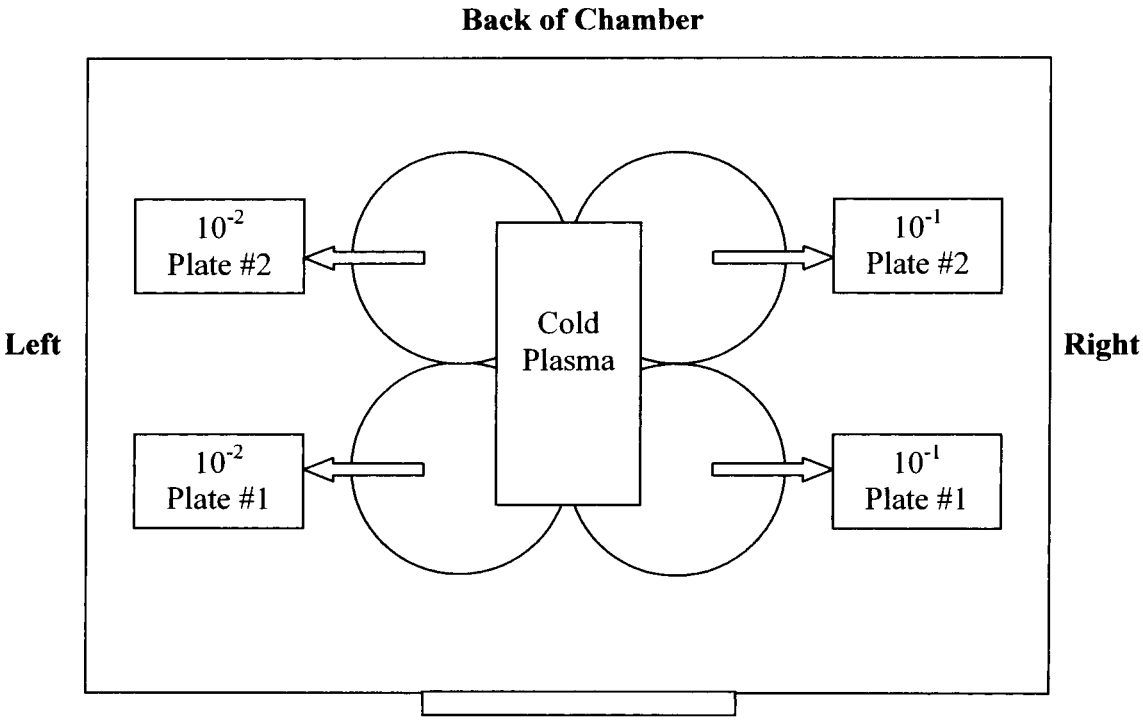
Appendix A

Indirect Cold Plasma Chamber

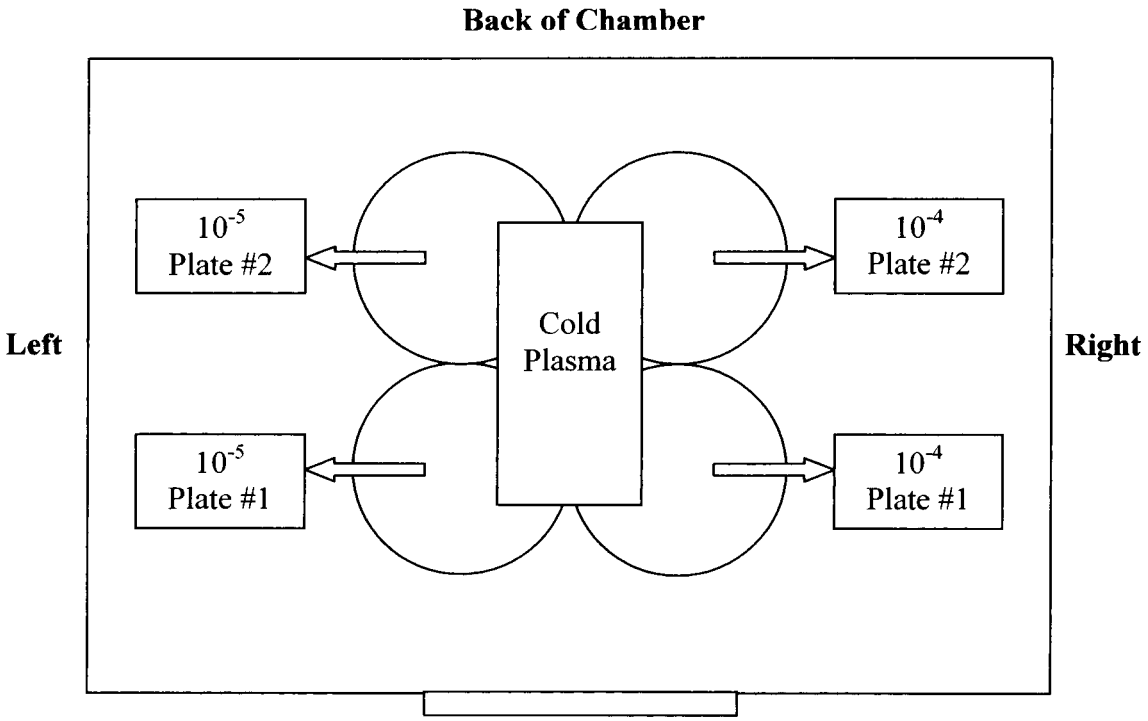
Appendix B

Cold Plasma Indirect Chamber
Placement of Plates for *G. stearothermophilus*



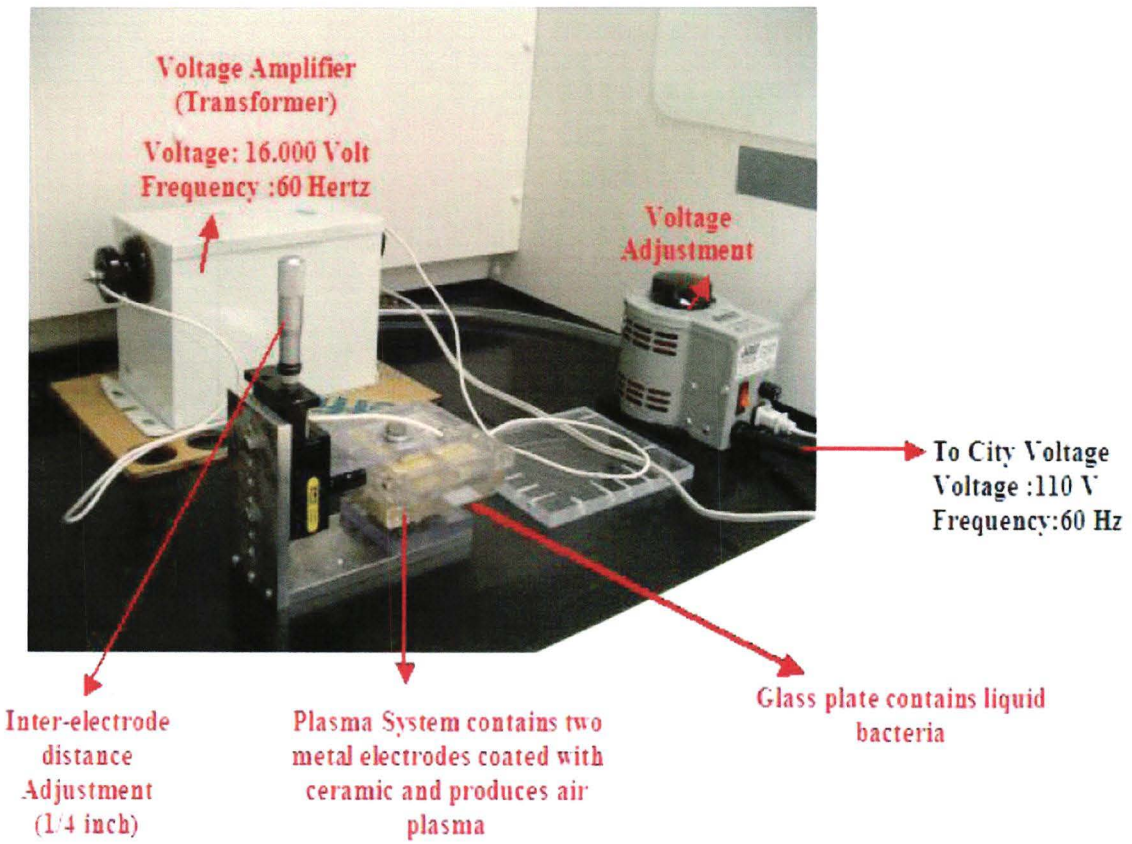
Appendix C

Cold Plasma Indirect Chamber
Placement of Plates for *B. cereus*



Appendix D

Direct Cold Plasma Chamber



(Courtesy of Dr. Tamar Akan)

Appendix E

Raw Data:
Indirect Cold Plasma Chamber

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
1	*Geobacillus stearothermophilus*	V	0	-1	1	**290**	**$2.89x10^4$**
1	*Geobacillus stearothermophilus*	V	0	-1	2	**287**	
1	*Geobacillus stearothermophilus*	V	0	-2	1	**71**	**$5.45x10^4$**
1	*Geobacillus stearothermophilus*	V	0	-2	2	**38**	
1	*Geobacillus stearothermophilus*	V	15	-1	1	**37**	**$3.05x10^3$**
1	*Geobacillus stearothermophilus*	V	15	-1	2	**24**	
1	*Geobacillus stearothermophilus*	V	15	-2	1	**6**	**$5.5x10^3$**
1	*Geobacillus stearothermophilus*	V	15	-2	2	**5**	
1	*Geobacillus stearothermophilus*	V	20	-1	1	**64**	**$6.7x10^3$**
1	*Geobacillus stearothermophilus*	V	20	-1	2	**70**	
1	*Geobacillus stearothermophilus*	V	20	-2	1	**9**	**$1.85x10^4$**
1	*Geobacillus stearothermophilus*	V	20	-2	2	**28**	
1	*Geobacillus stearothermophilus*	V	25	-1	1	**130**	**$7.1x10^3$**
1	*Geobacillus stearothermophilus*	V	25	-1	2	**12**	
1	*Geobacillus stearothermophilus*	V	25	-2	1	**8**	**$6.5x10^3$**
1	*Geobacillus stearothermophilus*	V	25	-2	2	**5**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
1	*Geobacillus stearothermophilus*	V	30	-1	1	**250**	**$1.59x10^4$**
1	*Geobacillus stearothermophilus*	V	30	-1	2	**67**	
1	*Geobacillus stearothermophilus*	V	30	-2	1	**5**	**$7.0x10^3$**
1	*Geobacillus stearothermophilus*	V	30	-2	2	**9**	
2	*Geobacillus stearothermophilus*	V	0	-1	1	**170**	**$2.23x10^4$**
2	*Geobacillus stearothermophilus*	V	0	-1	2	**275**	
2	*Geobacillus stearothermophilus*	V	0	-2	1	**18**	**$2.2x10^4$**
2	*Geobacillus stearothermophilus*	V	0	-2	2	**26**	
2	*Geobacillus stearothermophilus*	V	15	-1	1	**3**	**$3.0x10^2$**
2	*Geobacillus stearothermophilus*	V	15	-1	2	**3**	
2	*Geobacillus stearothermophilus*	V	15	-2	1	**3**	**$1.5x10^3$**
2	*Geobacillus stearothermophilus*	V	15	-2	2	**0**	
2	*Geobacillus stearothermophilus*	V	20	-1	1	**8**	**$2.25x10^3$**
2	*Geobacillus stearothermophilus*	V	20	-1	2	**37**	
2	*Geobacillus stearothermophilus*	V	20	-2	1	**2**	**$1.5x10^3$**
2	*Geobacillus stearothermophilus*	V	20	-2	2	**1**	
2	*Geobacillus stearothermophilus*	V	25	-1	1	**4**	**$7.0x10^2$**
2	*Geobacillus stearothermophilus*	V	25	-1	2	**10**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
2	*Geobacillus stearothermophilus*	V	25	-2	1	**1**	**$1.0x10^3$**
2	*Geobacillus stearothermophilus*	V	25	-2	2	**1**	
2	*Geobacillus stearothermophilus*	V	30	-1	1	**1**	**$1.45x10^3$**
2	*Geobacillus stearothermophilus*	V	30	-1	2	**28**	
2	*Geobacillus stearothermophilus*	V	30	-2	1	**4**	**$3.5x10^3$**
2	*Geobacillus stearothermophilus*	V	30	-2	2	**3**	
3	*Geobacillus stearothermophilus*	V	0	-1	1	**142**	**$1.92x10^4$**
3	*Geobacillus stearothermophilus*	V	0	-1	2	**241**	
3	*Geobacillus stearothermophilus*	V	0	-2	1	**39**	**$2.65x10^4$**
3	*Geobacillus stearothermophilus*	V	0	-2	2	**14**	
3	*Geobacillus stearothermophilus*	V	15	-1	1	**25**	**$3.3x10^3$**
3	*Geobacillus stearothermophilus*	V	15	-1	2	**41**	
3	*Geobacillus stearothermophilus*	V	15	-2	1	**1**	**$3.0x10^3$**
3	*Geobacillus stearothermophilus*	V	15	-2	2	**5**	
3	*Geobacillus stearothermophilus*	V	20	-1	1	**74**	**$5.5x10^3$**
3	*Geobacillus stearothermophilus*	V	20	-1	2	**36**	
3	*Geobacillus stearothermophilus*	V	20	-2	1	**5**	**$3.0x10^3$**
3	*Geobacillus stearothermophilus*	V	20	-2	2	**1**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
3	*Geobacillus stearothermophilus*	V	25	-1	1	**157**	**$1.47x10^4$**
3	*Geobacillus stearothermophilus*	V	25	-1	2	**137**	
3	*Geobacillus stearothermophilus*	V	25	-2	1	**7**	**$1.1x10^4$**
3	*Geobacillus stearothermophilus*	V	25	-2	2	**15**	
3	*Geobacillus stearothermophilus*	V	30	-1	1	**12**	**$3.2x10^3$**
3	*Geobacillus stearothermophilus*	V	30	-1	2	**52**	
3	*Geobacillus stearothermophilus*	V	30	-2	1	**5**	**$6.0x10^3$**
3	*Geobacillus stearothermophilus*	V	30	-2	2	**7**	
1	*Geobacillus stearothermophilus*	S	0	-1	1	**12**	**$1.5x10^3$**
1	*Geobacillus stearothermophilus*	S	0	-1	2	**18**	
1	*Geobacillus stearothermophilus*	S	0	-2	1	**0**	**0**
1	*Geobacillus stearothermophilus*	S	0	-2	2	**0**	
1	*Geobacillus stearothermophilus*	S	15	-1	1	**0**	**0**
1	*Geobacillus stearothermophilus*	S	15	-1	2	**0**	
1	*Geobacillus stearothermophilus*	S	15	-2	1	**0**	**0**
1	*Geobacillus stearothermophilus*	S	15	-2	2	**0**	
1	*Geobacillus stearothermophilus*	S	20	-1	1	**0**	**5.0x10**
1	*Geobacillus stearothermophilus*	S	20	-1	2	**1**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
1	*Geobacillus stearothermophilus*	S	20	-2	1	**0**	**0**
1	*Geobacillus stearothermophilus*	S	20	-2	2	**0**	
1	*Geobacillus stearothermophilus*	S	25	-1	1	**1**	**5.0x10**
1	*Geobacillus stearothermophilus*	S	25	-1	2	**0**	
1	*Geobacillus stearothermophilus*	S	25	-2	1	**0**	**0**
1	*Geobacillus stearothermophilus*	S	25	-2	2	**0**	
1	*Geobacillus stearothermophilus*	S	30	-1	1	**0**	**5.0x10**
1	*Geobacillus stearothermophilus*	S	30	-1	2	**1**	
1	*Geobacillus stearothermophilus*	S	30	-2	1	**1**	**$5.0x10^2$**
1	*Geobacillus stearothermophilus*	S	30	-2	2	**0**	
2	*Geobacillus stearothermophilus*	S	0	-1	1	**2**	**$1.0x10^2$**
2	*Geobacillus stearothermophilus*	S	0	-1	2	**0**	
2	*Geobacillus stearothermophilus*	S	0	-2	1	**0**	**0**
2	*Geobacillus stearothermophilus*	S	0	-2	2	**0**	
2	*Geobacillus stearothermophilus*	S	15	-1	1	**0**	**0**
2	*Geobacillus stearothermophilus*	S	15	-1	2	**0**	
2	*Geobacillus stearothermophilus*	S	15	-2	1	**0**	**0**
2	*Geobacillus stearothermophilus*	S	15	-2	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
2	*Geobacillus stearothermophilus*	S	20	-1	1	**0**	**0**
2	*Geobacillus stearothermophilus*	S	20	-1	2	**0**	
2	*Geobacillus stearothermophilus*	S	20	-2	1	**0**	**0**
2	*Geobacillus stearothermophilus*	S	20	-2	2	**0**	
2	*Geobacillus stearothermophilus*	S	25	-1	1	**0**	**0**
2	*Geobacillus stearothermophilus*	S	25	-1	2	**0**	
2	*Geobacillus stearothermophilus*	S	25	-2	1	**0**	**0**
2	*Geobacillus stearothermophilus*	S	25	-2	2	**0**	
2	*Geobacillus stearothermophilus*	S	30	-1	1	**0**	**5.0x10**
2	*Geobacillus stearothermophilus*	S	30	-1	2	**1**	
2	*Geobacillus stearothermophilus*	S	30	-2	1	**0**	**0**
2	*Geobacillus stearothermophilus*	S	30	-2	2	**0**	
3	*Geobacillus stearothermophilus*	S	0	-1	1	**0**	**$4.35x10^3$**
3	*Geobacillus stearothermophilus*	S	0	-1	2	**87**	
3	*Geobacillus stearothermophilus*	S	0	-2	1	**23**	**$1.15x10^4$**
3	*Geobacillus stearothermophilus*	S	0	-2	2	**0**	
3	*Geobacillus stearothermophilus*	S	15	-1	1	**3**	**$3.35x10^3$**
3	*Geobacillus stearothermophilus*	S	15	-1	2	**64**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
3	*Geobacillus stearothermophilus*	S	15	-2	1	**0**	**0**
3	*Geobacillus stearothermophilus*	S	15	-2	2	**0**	
3	*Geobacillus stearothermophilus*	S	20	-1	1	**0**	**$1.5x10^3$**
3	*Geobacillus stearothermophilus*	S	20	-1	2	**30**	
3	*Geobacillus stearothermophilus*	S	20	-2	1	**8**	**$4.0x10^3$**
3	*Geobacillus stearothermophilus*	S	20	-2	2	**0**	
3	*Geobacillus stearothermophilus*	S	25	-1	1	**0**	**$3.1x10^3$**
3	*Geobacillus stearothermophilus*	S	25	-1	2	**62**	
3	*Geobacillus stearothermophilus*	S	25	-2	1	**7**	**$9.5x10^3$**
3	*Geobacillus stearothermophilus*	S	25	-2	2	**12**	
3	*Geobacillus stearothermophilus*	S	30	-1	1	**0**	**$5.25x10^3$**
3	*Geobacillus stearothermophilus*	S	30	-1	2	**105**	
3	*Geobacillus stearothermophilus*	S	30	-2	1	**0**	**0**
3	*Geobacillus stearothermophilus*	S	30	-2	2	**0**	
4	*Geobacillus stearothermophilus*	S	0	-1	1	**NA**	**PLATES**
4	*Geobacillus stearothermophilus*	S	0	-1	2	**NA**	
4	*Geobacillus stearothermophilus*	S	0	-2	1	**NA**	**WERE**
4	*Geobacillus stearothermophilus*	S	0	-2	2	**NA**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
4	*Geobacillus stearothermophilus*	S	15	-1	1	**NA**	**EXTREMELY**
4	*Geobacillus stearothermophilus*	S	15	-1	2	**NA**	
4	*Geobacillus stearothermophilus*	S	15	-2	1	**NA**	**WET**
4	*Geobacillus stearothermophilus*	S	15	-2	2	**NA**	
4	*Geobacillus stearothermophilus*	S	20	-1	1	**NA**	**AND**
4	*Geobacillus stearothermophilus*	S	20	-1	2	**NA**	
4	*Geobacillus stearothermophilus*	S	20	-2	1	**NA**	**NO**
4	*Geobacillus stearothermophilus*	S	20	-2	2	**NA**	
4	*Geobacillus stearothermophilus*	S	25	-1	1	**NA**	**COLONIES**
4	*Geobacillus stearothermophilus*	S	25	-1	2	**NA**	
4	*Geobacillus stearothermophilus*	S	25	-2	1	**NA**	**HAD**
4	*Geobacillus stearothermophilus*	S	25	-2	2	**NA**	
4	*Geobacillus stearothermophilus*	S	30	-1	1	**NA**	**GROWN**
4	*Geobacillus stearothermophilus*	S	30	-1	2	**NA**	
4	*Geobacillus stearothermophilus*	S	30	-2	1	**NA**	**ON PLATES!**
4	*Geobacillus stearothermophilus*	S	30	-2	2	**NA**	
5	*Geobacillus stearothermophilus*	S	0	-1	1	**255**	**$2.76x10^4$**
5	*Geobacillus stearothermophilus*	S	0	-1	2	**297**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
5	*Geobacillus stearothermophilus*	S	0	-2	1	**43**	**$3.85x10^4$**
5	*Geobacillus stearothermophilus*	S	0	-2	2	**34**	
5	*Geobacillus stearothermophilus*	S	15	-1	1	**134**	**$1.19x10^4$**
5	*Geobacillus stearothermophilus*	S	15	-1	2	**103**	
5	*Geobacillus stearothermophilus*	S	15	-2	1	**16**	**$1.65x10^4$**
5	*Geobacillus stearothermophilus*	S	15	-2	2	**17**	
5	*Geobacillus stearothermophilus*	S	20	-1	1	**186**	**$1.46x10^4$**
5	*Geobacillus stearothermophilus*	S	20	-1	2	**106**	
5	*Geobacillus stearothermophilus*	S	20	-2	1	**16**	**$1.05x10^4$**
5	*Geobacillus stearothermophilus*	S	20	-2	2	**5**	
5	*Geobacillus stearothermophilus*	S	25	-1	1	**91**	**$1.19x10^4$**
5	*Geobacillus stearothermophilus*	S	25	-1	2	**146**	
5	*Geobacillus stearothermophilus*	S	25	-2	1	**10**	**$1.55x10^4$**
5	*Geobacillus stearothermophilus*	S	25	-2	2	**21**	
5	*Geobacillus stearothermophilus*	S	30	-1	1	**180**	**$1.25x10^4$**
5	*Geobacillus stearothermophilus*	S	30	-1	2	**69**	
5	*Geobacillus stearothermophilus*	S	30	-2	1	**15**	**$1.85x10^4$**
5	*Geobacillus stearothermophilus*	S	30	-2	2	**22**	
1	*Bacillus cereus*	V	0	-4	1	**TNTC**	**TNTC**
1	*Bacillus cereus*	V	0	-4	2	**TNTC**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
1	*Bacillus cereus*	V	0	-5	1	**TNTC**	**TNTC**
1	*Bacillus cereus*	V	0	-5	2	**TNTC**	
1	*Bacillus cereus*	V	15	-4	1	**0**	**0**
1	*Bacillus cereus*	V	15	-4	2	**0**	
1	*Bacillus cereus*	V	15	-5	1	**0**	**0**
1	*Bacillus cereus*	V	15	-5	2	**0**	
1	*Bacillus cereus*	V	20	-4	1	**0**	**0**
1	*Bacillus cereus*	V	20	-4	2	**0**	
1	*Bacillus cereus*	V	20	-5	1	**0**	**0**
1	*Bacillus cereus*	V	20	-5	2	**0**	
1	*Bacillus cereus*	V	25	-4	1	**0**	**0**
1	*Bacillus cereus*	V	25	-4	2	**0**	
1	*Bacillus cereus*	V	25	-5	1	**0**	**0**
1	*Bacillus cereus*	V	25	-5	2	**0**	
1	*Bacillus cereus*	V	30	-4	1	**0**	**0**
1	*Bacillus cereus*	V	30	-4	2	**0**	
1	*Bacillus cereus*	V	30	-5	1	**1**	**$5.0x10^5$**
1	*Bacillus cereus*	V	30	-5	2	**0**	
2	*Bacillus cereus*	V	0	-4	1	**228**	**$2.09x10^7$**
2	*Bacillus cereus*	V	0	-4	2	**189**	
2	*Bacillus cereus*	V	0	-5	1	**20**	**$2.05x10^7$**
2	*Bacillus cereus*	V	0	-5	2	**21**	
2	*Bacillus cereus*	V	0	-6	1	**0**	**0**
2	*Bacillus cereus*	V	0	-6	2	**0**	
2	*Bacillus cereus*	V	15	-4	1	**0**	**0**
2	*Bacillus cereus*	V	15	-4	2	**0**	
2	*Bacillus cereus*	V	15	-5	1	**0**	**0**
2	*Bacillus cereus*	V	15	-5	2	**0**	
2	*Bacillus cereus*	V	20	-4	1	**0**	**0**
2	*Bacillus cereus*	V	20	-4	2	**0**	
2	*Bacillus cereus*	V	20	-5	1	**0**	**0**
2	*Bacillus cereus*	V	20	-5	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
2	*Bacillus cereus*	V	25	-4	1	**0**	**0**
2	*Bacillus cereus*	V	25	-4	2	**0**	
2	*Bacillus cereus*	V	25	-5	1	**0**	**0**
2	*Bacillus cereus*	V	25	-5	2	**0**	
2	*Bacillus cereus*	V	30	-4	1	**0**	**0**
2	*Bacillus cereus*	V	30	-4	2	**0**	
2	*Bacillus cereus*	V	30	-5	1	**0**	**0**
2	*Bacillus cereus*	V	30	-5	2	**0**	
3	*Bacillus cereus*	V	0	-4	1	**201**	**$1.48x10^7$**
3	*Bacillus cereus*	V	0	-4	2	**194**	
3	*Bacillus cereus*	V	0	-5	1	**4**	**$1.05x10^7$**
3	*Bacillus cereus*	V	0	-5	2	**17**	
3	*Bacillus cereus*	V	0	-6	1	**0**	**0**
3	*Bacillus cereus*	V	0	-6	2	**0**	
3	*Bacillus cereus*	V	5	-4	1	**0**	**0**
3	*Bacillus cereus*	V	5	-4	2	**0**	
3	*Bacillus cereus*	V	5	-5	1	**0**	**0**
3	*Bacillus cereus*	V	5	-5	2	**0**	
3	*Bacillus cereus*	V	10	-4	1	**0**	**0**
3	*Bacillus cereus*	V	10	-4	2	**0**	
3	*Bacillus cereus*	V	10	-5	1	**0**	**0**
3	*Bacillus cereus*	V	10	-5	2	**0**	
3	*Bacillus cereus*	V	15	-4	1	**0**	**0**
3	*Bacillus cereus*	V	15	-4	2	**0**	
3	*Bacillus cereus*	V	15	-5	1	**0**	**0**
3	*Bacillus cereus*	V	15	-5	2	**0**	
3	*Bacillus cereus*	V	20	-4	1	**0**	**0**
3	*Bacillus cereus*	V	20	-4	2	**0**	
3	*Bacillus cereus*	V	20	-5	1	**0**	**0**
3	*Bacillus cereus*	V	20	-5	2	**0**	
3	*Bacillus cereus*	V	25	-4	1	**0**	**0**
3	*Bacillus cereus*	V	25	-4	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
3	*Bacillus cereus*	V	25	-5	1	**0**	**0**
3	*Bacillus cereus*	V	25	-5	2	**0**	
3	*Bacillus cereus*	V	30	-4	1	**0**	**0**
3	*Bacillus cereus*	V	30	-4	2	**0**	
3	*Bacillus cereus*	V	30	-5	1	**0**	**0**
3	*Bacillus cereus*	V	30	-5	2	**0**	
4	*Bacillus cereus*	V	0	-4	1	**498**	**$4.8x10^7$**
4	*Bacillus cereus*	V	0	-4	2	**462**	
4	*Bacillus cereus*	V	0	-5	1	**2**	**$3.5x10^6$**
4	*Bacillus cereus*	V	0	-5	2	**5**	
4	*Bacillus cereus*	V	0	-6	1	**0**	**0**
4	*Bacillus cereus*	V	0	-6	2	**0**	
4	*Bacillus cereus*	V	1	-4	1	**1**	**$5.0x10^4$**
4	*Bacillus cereus*	V	1	-4	2	**0**	
4	*Bacillus cereus*	V	1	-5	1	**0**	**0**
4	*Bacillus cereus*	V	1	-5	2	**0**	
4	*Bacillus cereus*	V	2	-4	1	**0**	**0**
4	*Bacillus cereus*	V	2	-4	2	**0**	
4	*Bacillus cereus*	V	2	-5	1	**0**	**0**
4	*Bacillus cereus*	V	2	-5	2	**0**	
4	*Bacillus cereus*	V	3	-4	1	**0**	**0**
4	*Bacillus cereus*	V	3	-4	2	**0**	
4	*Bacillus cereus*	V	3	-5	1	**0**	**0**
4	*Bacillus cereus*	V	3	-5	2	**0**	
4	*Bacillus cereus*	V	4	-4	1	**0**	**0**
4	*Bacillus cereus*	V	4	-4	2	**0**	
4	*Bacillus cereus*	V	4	-5	1	**0**	**0**
4	*Bacillus cereus*	V	4	-5	2	**0**	
4	*Bacillus cereus*	V	5	-4	1	**0**	**0**
4	*Bacillus cereus*	V	5	-4	2	**0**	
4	*Bacillus cereus*	V	5	-5	1	**0**	**0**
4	*Bacillus cereus*	V	5	-5	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
5	*Bacillus cereus*	V	0	-4	1	**223**	**$2.14x10^7$**
5	*Bacillus cereus*	V	0	-4	2	**204**	
5	*Bacillus cereus*	V	0	-5	1	**0**	**0**
5	*Bacillus cereus*	V	0	-5	2	**0**	
5	*Bacillus cereus*	V	0	-6	1	**0**	**0**
5	*Bacillus cereus*	V	0	-6	2	**0**	
5	*Bacillus cereus*	V	1	-4	1	**9**	**$5.0x10^5$**
5	*Bacillus cereus*	V	1	-4	2	**1**	
5	*Bacillus cereus*	V	1	-5	1	**0**	**0**
5	*Bacillus cereus*	V	1	-5	2	**0**	
5	*Bacillus cereus*	V	2	-4	1	**0**	**$5.0x10^4$**
5	*Bacillus cereus*	V	2	-4	2	**1**	
5	*Bacillus cereus*	V	2	-5	1	**0**	**0**
5	*Bacillus cereus*	V	2	-5	2	**0**	
5	*Bacillus cereus*	V	3	-4	1	**0**	**0**
5	*Bacillus cereus*	V	3	-4	2	**0**	
5	*Bacillus cereus*	V	3	-5	1	**0**	**0**
5	*Bacillus cereus*	V	3	-5	2	**0**	
5	*Bacillus cereus*	V	4	-4	1	**0**	**0**
5	*Bacillus cereus*	V	4	-4	2	**0**	
5	*Bacillus cereus*	V	4	-5	1	**0**	**0**
5	*Bacillus cereus*	V	4	-5	2	**0**	
5	*Bacillus cereus*	V	5	-4	1	**0**	**0**
5	*Bacillus cereus*	V	5	-4	2	**0**	
5	*Bacillus cereus*	V	5	-5	1	**0**	**0**
5	*Bacillus cereus*	V	5	-5	2	**0**	
6	*Bacillus cereus*	V	0	-4	1	**611**	**$5.25x10^7$**
6	*Bacillus cereus*	V	0	-4	2	**438**	
6	*Bacillus cereus*	V	0	-5	1	**57**	**$5.05x10^7$**
6	*Bacillus cereus*	V	0	-5	2	**44**	
6	*Bacillus cereus*	V	0	-6	1	**7**	**$8.5x10^7$**
6	*Bacillus cereus*	V	0	-6	2	**10**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
6	*Bacillus cereus*	V	1	-4	1	**26**	**$1.3x10^6$**
6	*Bacillus cereus*	V	1	-4	2	**0**	
6	*Bacillus cereus*	V	1	-5	1	**0**	**0**
6	*Bacillus cereus*	V	1	-5	2	**0**	
6	*Bacillus cereus*	V	2	-4	1	**33**	**$2.45x10^6$**
6	*Bacillus cereus*	V	2	-4	2	**16**	
6	*Bacillus cereus*	V	2	-5	1	**0**	**0**
6	*Bacillus cereus*	V	2	-5	2	**0**	
6	*Bacillus cereus*	V	3	-4	1	**23**	**$1.15x10^6$**
6	*Bacillus cereus*	V	3	-4	2	**0**	
6	*Bacillus cereus*	V	3	-5	1	**0**	**0**
6	*Bacillus cereus*	V	3	-5	2	**0**	
6	*Bacillus cereus*	V	4	-4	1	**0**	**0**
6	*Bacillus cereus*	V	4	-4	2	**0**	
6	*Bacillus cereus*	V	4	-5	1	**0**	**0**
6	*Bacillus cereus*	V	4	-5	2	**0**	
6	*Bacillus cereus*	V	5	-4	1	**0**	**0**
6	*Bacillus cereus*	V	5	-4	2	**0**	
6	*Bacillus cereus*	V	5	-5	1	**0**	**0**
6	*Bacillus cereus*	V	5	-5	2	**0**	
1	*Bacillus cereus*	S	0	-5	1	**59**	**$4.55x10^7$**
1	*Bacillus cereus*	S	0	-5	2	**32**	
1	*Bacillus cereus*	S	0	-6	1	**10**	**$8.5x10^7$**
1	*Bacillus cereus*	S	0	-6	2	**7**	
1	*Bacillus cereus*	S	0	-7	1	**0**	**$5.0x10^7$**
1	*Bacillus cereus*	S	0	-7	2	**1**	
1	*Bacillus cereus*	S	15	-5	1	**0**	**0**
1	*Bacillus cereus*	S	15	-5	2	**0**	
1	*Bacillus cereus*	S	15	-6	1	**0**	**0**
1	*Bacillus cereus*	S	15	-6	2	**0**	
1	*Bacillus cereus*	S	20	-5	1	**0**	**0**
1	*Bacillus cereus*	S	20	-5	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
1	*Bacillus cereus*	S	20	-6	1	**0**	**0**
1	*Bacillus cereus*	S	20	-6	2	**0**	
1	*Bacillus cereus*	S	25	-5	1	**0**	**0**
1	*Bacillus cereus*	S	25	-5	2	**0**	
1	*Bacillus cereus*	S	25	-6	1	**0**	**0**
1	*Bacillus cereus*	S	25	-6	2	**0**	
1	*Bacillus cereus*	S	30	-5	1	**0**	**0**
1	*Bacillus cereus*	S	30	-5	2	**0**	
1	*Bacillus cereus*	S	30	-6	1	**0**	**0**
1	*Bacillus cereus*	S	30	-6	2	**0**	
2	*Bacillus cereus*	S	0	-5	1	**98**	**$8.2x10^7$**
2	*Bacillus cereus*	S	0	-5	2	**66**	
2	*Bacillus cereus*	S	0	-6	1	**4**	**$4.5x10^7$**
2	*Bacillus cereus*	S	0	-6	2	**5**	
2	*Bacillus cereus*	S	0	-7	1	**0**	**0**
2	*Bacillus cereus*	S	0	-7	2	**0**	
2	*Bacillus cereus*	S	5	-5	1	**0**	**0**
2	*Bacillus cereus*	S	5	-5	2	**0**	
2	*Bacillus cereus*	S	5	-6	1	**0**	**0**
2	*Bacillus cereus*	S	5	-6	2	**0**	
2	*Bacillus cereus*	S	10	-5	1	**0**	**0**
2	*Bacillus cereus*	S	10	-5	2	**0**	
2	*Bacillus cereus*	S	10	-6	1	**0**	**0**
2	*Bacillus cereus*	S	10	-6	2	**0**	
2	*Bacillus cereus*	S	15	-5	1	**0**	**0**
2	*Bacillus cereus*	S	15	-5	2	**0**	
2	*Bacillus cereus*	S	15	-6	1	**0**	**0**
2	*Bacillus cereus*	S	15	-6	2	**0**	
2	*Bacillus cereus*	S	20	-5	1	**0**	**0**
2	*Bacillus cereus*	S	20	-5	2	**0**	
2	*Bacillus cereus*	S	20	-6	1	**0**	**0**
2	*Bacillus cereus*	S	20	-6	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
2	*Bacillus cereus*	S	25	-5	1	**0**	**0**
2	*Bacillus cereus*	S	25	-5	2	**0**	
2	*Bacillus cereus*	S	25	-6	1	**0**	**0**
2	*Bacillus cereus*	S	25	-6	2	**0**	
2	*Bacillus cereus*	S	30	-5	1	**0**	**0**
2	*Bacillus cereus*	S	30	-5	2	**0**	
2	*Bacillus cereus*	S	30	-6	1	**0**	**0**
2	*Bacillus cereus*	S	30	-6	2	**0**	
3	*Bacillus cereus*	S	0	-5	1	**24**	**$2.0x10^7$**
3	*Bacillus cereus*	S	0	-5	2	**16**	
3	*Bacillus cereus*	S	0	-6	1	**3**	**$1.5x10^7$**
3	*Bacillus cereus*	S	0	-6	2	**0**	
3	*Bacillus cereus*	S	0	-7	1	**0**	**0**
3	*Bacillus cereus*	S	0	-7	2	**0**	
3	*Bacillus cereus*	S	1	-5	1	**0**	**0**
3	*Bacillus cereus*	S	1	-5	2	**0**	
3	*Bacillus cereus*	S	1	-6	1	**0**	**0**
3	*Bacillus cereus*	S	1	-6	2	**0**	
3	*Bacillus cereus*	S	2	-5	1	**0**	**0**
3	*Bacillus cereus*	S	2	-5	2	**0**	
3	*Bacillus cereus*	S	2	-6	1	**0**	**0**
3	*Bacillus cereus*	S	2	-6	2	**0**	
3	*Bacillus cereus*	S	3	-5	1	**0**	**0**
3	*Bacillus cereus*	S	3	-5	2	**0**	
3	*Bacillus cereus*	S	3	-6	1	**0**	**0**
3	*Bacillus cereus*	S	3	-6	2	**0**	
3	*Bacillus cereus*	S	4	-5	1	**0**	**0**
3	*Bacillus cereus*	S	4	-5	2	**0**	
3	*Bacillus cereus*	S	4	-6	1	**0**	**0**
3	*Bacillus cereus*	S	4	-6	2	**0**	
3	*Bacillus cereus*	S	5	-5	1	**0**	**0**
3	*Bacillus cereus*	S	5	-5	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
3	*Bacillus cereus*	S	5	-6	1	**0**	**0**
3	*Bacillus cereus*	S	5	-6	2	**0**	
4	*Bacillus cereus*	S	0	-5	1	**70**	**$7.25x10^7$**
4	*Bacillus cereus*	S	0	-5	2	**75**	
4	*Bacillus cereus*	S	0	-6	1	**16**	**$1.95x10^8$**
4	*Bacillus cereus*	S	0	-6	2	**23**	
4	*Bacillus cereus*	S	0	-7	1	**4**	**$2.5x10^8$**
4	*Bacillus cereus*	S	0	-7	2	**1**	
4	*Bacillus cereus*	S	1	-5	1	**0**	**0**
4	*Bacillus cereus*	S	1	-5	2	**0**	
4	*Bacillus cereus*	S	1	-6	1	**0**	**0**
4	*Bacillus cereus*	S	1	-6	2	**0**	
4	*Bacillus cereus*	S	2	-5	1	**0**	**0**
4	*Bacillus cereus*	S	2	-5	2	**0**	
4	*Bacillus cereus*	S	2	-6	1	**0**	**0**
4	*Bacillus cereus*	S	2	-6	2	**0**	
4	*Bacillus cereus*	S	3	-5	1	**0**	**0**
4	*Bacillus cereus*	S	3	-5	2	**0**	
4	*Bacillus cereus*	S	3	-6	1	**0**	**0**
4	*Bacillus cereus*	S	3	-6	2	**0**	
4	*Bacillus cereus*	S	4	-5	1	**2**	**$1.0x10^6$**
4	*Bacillus cereus*	S	4	-5	2	**0**	
4	*Bacillus cereus*	S	4	-6	1	**0**	**0**
4	*Bacillus cereus*	S	4	-6	2	**0**	
4	*Bacillus cereus*	S	5	-5	1	**0**	**0**
4	*Bacillus cereus*	S	5	-5	2	**0**	
4	*Bacillus cereus*	S	5	-6	1	**0**	**0**
4	*Bacillus cereus*	S	5	-6	2	**0**	
5	*Bacillus cereus*	S	0	-5	1	**17**	**$1.25x10^7$**
5	*Bacillus cereus*	S	0	-5	2	**8**	
5	*Bacillus cereus*	S	0	-6	1	**0**	**0**
5	*Bacillus cereus*	S	0	-6	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Exposure Time (minutes)	Dilution	Plate #	CFU	Concentration (CFU/mL)
5	*Bacillus cereus*	S	0	-7	1	**0**	**0**
5	*Bacillus cereus*	S	0	-7	2	**0**	
5	*Bacillus cereus*	S	1	-5	1	**0**	**$5.5x10^6$**
5	*Bacillus cereus*	S	1	-5	2	**11**	
5	*Bacillus cereus*	S	1	-6	1	**0**	**0**
5	*Bacillus cereus*	S	1	-6	2	**0**	
5	*Bacillus cereus*	S	2	-5	1	**1**	**$5.0x10^5$**
5	*Bacillus cereus*	S	2	-5	2	**0**	
5	*Bacillus cereus*	S	2	-6	1	**0**	**0**
5	*Bacillus cereus*	S	2	-6	2	**0**	
5	*Bacillus cereus*	S	3	-5	1	**1**	**$5.0x10^5$**
5	*Bacillus cereus*	S	3	-5	2	**0**	
5	*Bacillus cereus*	S	3	-6	1	**1**	**$5.0x10^6$**
5	*Bacillus cereus*	S	3	-6	2	**0**	
5	*Bacillus cereus*	S	4	-5	1	**0**	**0**
5	*Bacillus cereus*	S	4	-5	2	**0**	
5	*Bacillus cereus*	S	4	-6	1	**0**	**0**
5	*Bacillus cereus*	S	4	-6	2	**0**	
5	*Bacillus cereus*	S	5	-5	1	**0**	**0**
5	*Bacillus cereus*	S	5	-5	2	**0**	
5	*Bacillus cereus*	S	5	-6	1	**0**	**0**
5	*Bacillus cereus*	S	5	-6	2	**0**	

Appendix F

Raw Data:
Direct Cold Plasma Chamber

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
1	*Bacillus cereus*	V	0	0	0	1	TNTC	TNTC
1	*Bacillus cereus*	V	0	0	0	2	TNTC	
1	*Bacillus cereus*	V	0	0	-1	1	TNTC	TNTC
1	*Bacillus cereus*	V	0	0	-1	2	TNTC	
1	*Bacillus cereus*	V	0	0	-2	1	TNTC	TNTC
1	*Bacillus cereus*	V	0	0	-2	2	TNTC	
1	*Bacillus cereus*	V	0	0	-3	1	TNTC	TNTC
1	*Bacillus cereus*	V	0	0	-3	2	TNTC	
1	*Bacillus cereus*	V	0	0	-4	1	TNTC	TNTC
1	*Bacillus cereus*	V	0	0	-4	2	TNTC	
1	*Bacillus cereus*	V	0	60	0	1	45	$3.2x10^4$
1	*Bacillus cereus*	V	0	60	0	2	19	
1	*Bacillus cereus*	V	0	60	-1	1	3	$3.0x10^4$
1	*Bacillus cereus*	V	0	60	-1	2	3	
1	*Bacillus cereus*	V	0	60	-2	1	0	0
1	*Bacillus cereus*	V	0	60	-2	2	0	
1	*Bacillus cereus*	V	0	120	0	1	71	$4.6x10^4$
1	*Bacillus cereus*	V	0	120	0	2	21	
1	*Bacillus cereus*	V	0	120	-1	1	4	$4.0x10^4$
1	*Bacillus cereus*	V	0	120	-1	2	4	
1	*Bacillus cereus*	V	0	120	-2	1	0	0
1	*Bacillus cereus*	V	0	120	-2	2	0	
1	*Bacillus cereus*	V	0	240	0	1	9	$1.05x10^4$
1	*Bacillus cereus*	V	0	240	0	2	12	
1	*Bacillus cereus*	V	0	240	-1	1	0	$1.0x10^4$
1	*Bacillus cereus*	V	0	240	-1	2	2	
1	*Bacillus cereus*	V	0	240	-2	1	0	0
1	*Bacillus cereus*	V	0	240	-2	2	0	
1	*Bacillus cereus*	V	0	360	0	1	8	$8.5x10^3$
1	*Bacillus cereus*	V	0	360	0	2	9	
1	*Bacillus cereus*	V	0	360	-1	1	0	0
1	*Bacillus cereus*	V	0	360	-1	2	0	
1	*Bacillus cereus*	V	0	360	-2	1	0	$5.0x10^3$
1	*Bacillus cereus*	V	0	360	-2	2	1	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
1	*Bacillus cereus*	V	0	480	0	1	**40**	**$2.35x10^4$**
1	*Bacillus cereus*	V	0	480	0	2	**7**	
1	*Bacillus cereus*	V	0	480	-1	1	**0**	**$5.0x10^3$**
1	*Bacillus cereus*	V	0	480	-1	2	**1**	
1	*Bacillus cereus*	V	0	480	-2	1	**0**	**0**
1	*Bacillus cereus*	V	0	480	-2	2	**0**	
1	*Bacillus cereus*	V	0	600	0	1	**0**	**$5.0x10^2$**
1	*Bacillus cereus*	V	0	600	0	2	**1**	
1	*Bacillus cereus*	V	0	600	-1	1	**0**	**0**
1	*Bacillus cereus*	V	0	600	-1	2	**0**	
1	*Bacillus cereus*	V	0	600	-2	1	**0**	**0**
1	*Bacillus cereus*	V	0	600	-2	2	**0**	
1	*Bacillus cereus*	V	-2	0	0	1	**TNTC**	**TNTC**
1	*Bacillus cereus*	V	-2	0	0	2	**TNTC**	
1	*Bacillus cereus*	V	-2	0	-1	1	**TNTC**	**TNTC**
1	*Bacillus cereus*	V	-2	0	-1	2	**TNTC**	
1	*Bacillus cereus*	V	-2	0	-2	1	**342**	**$3.31x10^9$**
1	*Bacillus cereus*	V	-2	0	-2	2	**320**	
1	*Bacillus cereus*	V	-2	60	0	1	**0**	**0**
1	*Bacillus cereus*	V	-2	60	0	2	**0**	
1	*Bacillus cereus*	V	-2	60	-1	1	**0**	**0**
1	*Bacillus cereus*	V	-2	60	-1	2	**0**	
1	*Bacillus cereus*	V	-2	60	-2	1	**0**	**0**
1	*Bacillus cereus*	V	-2	60	-2	2	**0**	
1	*Bacillus cereus*	V	-2	120	0	1	**0**	**0**
1	*Bacillus cereus*	V	-2	120	0	2	**0**	
1	*Bacillus cereus*	V	-2	120	-1	1	**0**	**0**
1	*Bacillus cereus*	V	-2	120	-1	2	**0**	
1	*Bacillus cereus*	V	-2	120	-2	1	**0**	**0**
1	*Bacillus cereus*	V	-2	120	-2	2	**NA**	
1	*Bacillus cereus*	V	-2	240	0	1	**0**	**0**
1	*Bacillus cereus*	V	-2	240	0	2	**0**	
1	*Bacillus cereus*	V	-2	240	-1	1	**0**	**0**
1	*Bacillus cereus*	V	-2	240	-1	2	**0**	
1	*Bacillus cereus*	V	-2	240	-2	1	**0**	**0**
1	*Bacillus cereus*	V	-2	240	-2	2	**0**	
1	*Bacillus cereus*	V	-2	360	0	1	**0**	**0**
1	*Bacillus cereus*	V	-2	360	0	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
1	*Bacillus cereus*	V	-2	360	-1	1	**0**	**0**
1	*Bacillus cereus*	V	-2	360	-1	2	**0**	
1	*Bacillus cereus*	V	-2	360	-2	1	**0**	**0**
1	*Bacillus cereus*	V	-2	360	-2	2	**0**	
1	*Bacillus cereus*	V	-2	480	0	1	**0**	**0**
1	*Bacillus cereus*	V	-2	480	0	2	**0**	
1	*Bacillus cereus*	V	-2	480	-1	1	**0**	**0**
1	*Bacillus cereus*	V	-2	480	-1	2	**0**	
1	*Bacillus cereus*	V	-2	480	-2	1	**0**	**0**
1	*Bacillus cereus*	V	-2	480	-2	2	**0**	
1	*Bacillus cereus*	V	-2	600	0	1	**0**	**0**
1	*Bacillus cereus*	V	-2	600	0	2	**0**	
1	*Bacillus cereus*	V	-2	600	-1	1	**0**	**0**
1	*Bacillus cereus*	V	-2	600	-1	2	**0**	
1	*Bacillus cereus*	V	-2	600	-2	1	**0**	**0**
1	*Bacillus cereus*	V	-2	600	-2	2	**0**	
2	*Bacillus cereus*	V	0	0	-4	1	**TNTC**	**TNTC**
2	*Bacillus cereus*	V	0	0	-4	2	**TNTC**	
2	*Bacillus cereus*	V	0	0	-5	1	**TNTC**	**TNTC**
2	*Bacillus cereus*	V	0	0	-5	2	**TNTC**	
2	*Bacillus cereus*	V	0	0	-6	1	**25**	**$2.15x10^{10}$**
2	*Bacillus cereus*	V	0	0	-6	2	**18**	
2	*Bacillus cereus*	V	0	60	0	1	**TNTC**	**TNTC**
2	*Bacillus cereus*	V	0	60	0	2	**TNTC**	
2	*Bacillus cereus*	V	0	60	-1	1	**TNTC**	**TNTC**
2	*Bacillus cereus*	V	0	60	-1	2	**TNTC**	
2	*Bacillus cereus*	V	0	120	0	1	**221**	**$2.21x10^{5}$**
2	*Bacillus cereus*	V	0	120	0	2	**TNTC**	
2	*Bacillus cereus*	V	0	120	-1	1	**61**	**$4.35x10^{5}$**
2	*Bacillus cereus*	V	0	120	-1	2	**26**	
2	*Bacillus cereus*	V	0	240	0	1	**0**	**0**
2	*Bacillus cereus*	V	0	240	0	2	**0**	
2	*Bacillus cereus*	V	0	240	-1	1	**0**	**0**
2	*Bacillus cereus*	V	0	240	-1	2	**0**	
2	*Bacillus cereus*	V	0	360	0	1	**0**	**0**
2	*Bacillus cereus*	V	0	360	0	2	**0**	
2	*Bacillus cereus*	V	0	360	-1	1	**0**	**0**
2	*Bacillus cereus*	V	0	360	-1	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
2	*Bacillus cereus*	V	0	480	0	1	**0**	**0**
2	*Bacillus cereus*	V	0	480	0	2	**0**	
2	*Bacillus cereus*	V	0	480	-1	1	**0**	**0**
2	*Bacillus cereus*	V	0	480	-1	2	**0**	
2	*Bacillus cereus*	V	0	600	0	1	**0**	**0**
2	*Bacillus cereus*	V	0	600	0	2	**0**	
2	*Bacillus cereus*	V	0	600	-1	1	**0**	**0**
2	*Bacillus cereus*	V	0	600	-1	2	**0**	
2	*Bacillus cereus*	V	-2	0	-2	1	**TNTC**	**TNTC**
2	*Bacillus cereus*	V	-2	0	-2	2	**TNTC**	
2	*Bacillus cereus*	V	-2	0	-3	1	**138**	**$1.17x10^{10}$**
2	*Bacillus cereus*	V	-2	0	-3	2	**95**	
2	*Bacillus cereus*	V	-2	10	0	1	**359**	**$3.6x10^{7}$**
2	*Bacillus cereus*	V	-2	10	0	2	**361**	
2	*Bacillus cereus*	V	-2	10	-1	1	**63**	**$6.3x10^{7}$**
2	*Bacillus cereus*	V	-2	10	-1	2	**NA**	
2	*Bacillus cereus*	V	-2	20	0	1	**102**	**$1.34x10^{7}$**
2	*Bacillus cereus*	V	-2	20	0	2	**165**	
2	*Bacillus cereus*	V	-2	20	-1	1	**121**	**$7.4x10^{7}$**
2	*Bacillus cereus*	V	-2	20	-1	2	**27**	
2	*Bacillus cereus*	V	-2	30	0	1	**175**	**$1.75x10^{7}$**
2	*Bacillus cereus*	V	-2	30	0	2	**TNTC**	
2	*Bacillus cereus*	V	-2	30	-1	1	**17**	**$1.7x10^{7}$**
2	*Bacillus cereus*	V	-2	30	-1	2	**TNTC**	
2	*Bacillus cereus*	V	-2	40	0	1	**54**	**$5.4x10^{6}$**
2	*Bacillus cereus*	V	-2	40	0	2	**NA**	
2	*Bacillus cereus*	V	-2	40	-1	1	**7**	**$6.5x10^{6}$**
2	*Bacillus cereus*	V	-2	40	-1	2	**6**	
2	*Bacillus cereus*	V	-2	50	0	1	**NA**	**$4.5x10^{6}$**
2	*Bacillus cereus*	V	-2	50	0	2	**45**	
2	*Bacillus cereus*	V	-2	50	-1	1	**10**	**$9.5x10^{6}$**
2	*Bacillus cereus*	V	-2	50	-1	2	**9**	
2	*Bacillus cereus*	V	-2	60	0	1	**21**	**$3.0x10^{6}$**
2	*Bacillus cereus*	V	-2	60	0	2	**39**	
2	*Bacillus cereus*	V	-2	60	-1	1	**0**	**$1.0x10^{6}$**
2	*Bacillus cereus*	V	-2	60	-1	2	**2**	
3	*Bacillus cereus*	V	-2	0	0	1	**TNTC**	**TNTC**
3	*Bacillus cereus*	V	-2	0	0	2	**TNTC**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
3	*Bacillus cereus*	V	-2	0	-1	1	TNTC	$9.0x10^7$
3	*Bacillus cereus*	V	-2	0	-1	2	90	
3	*Bacillus cereus*	V	-2	0	-2	1	4	$4.5x10^7$
3	*Bacillus cereus*	V	-2	0	-2	2	5	
3	*Bacillus cereus*	V	-2	0	-3	1	3	$2.0x10^8$
3	*Bacillus cereus*	V	-2	0	-3	2	1	
3	*Bacillus cereus*	V	-2	10	0	1	165	$1.6x10^7$
3	*Bacillus cereus*	V	-2	10	0	2	167	
3	*Bacillus cereus*	V	-2	10	-1	1	14	$1.4x10^7$
3	*Bacillus cereus*	V	-2	10	-1	2	TNTC	
3	*Bacillus cereus*	V	-2	20	0	1	TNTC	$1.22x10^7$
3	*Bacillus cereus*	V	-2	20	0	2	122	
3	*Bacillus cereus*	V	-2	20	-1	1	TNTC	TNTC
3	*Bacillus cereus*	V	-2	20	-1	2	TNTC	
3	*Bacillus cereus*	V	-2	30	0	1	7	$8.5x10^5$
3	*Bacillus cereus*	V	-2	30	0	2	10	
3	*Bacillus cereus*	V	-2	30	-1	1	1	$5.0x10^5$
3	*Bacillus cereus*	V	-2	30	-1	2	0	
3	*Bacillus cereus*	V	-2	40	0	1	TNTC	TNTC
3	*Bacillus cereus*	V	-2	40	0	2	TNTC	
3	*Bacillus cereus*	V	-2	40	-1	1	16	$2.1x10^7$
3	*Bacillus cereus*	V	-2	40	-1	2	26	
3	*Bacillus cereus*	V	-2	50	0	1	10	$1.75x10^6$
3	*Bacillus cereus*	V	-2	50	0	2	25	
3	*Bacillus cereus*	V	-2	50	-1	1	0	$5.0x10^5$
3	*Bacillus cereus*	V	-2	50	-1	2	1	
3	*Bacillus cereus*	V	-2	60	0	1	NA	$3.0x10^5$
3	*Bacillus cereus*	V	-2	60	0	2	3	
3	*Bacillus cereus*	V	-2	60	-1	1	0	0
3	*Bacillus cereus*	V	-2	60	-1	2	0	
4	*Bacillus cereus*	V	-2	0	0	1	324	$3.25x10^7$
4	*Bacillus cereus*	V	-2	0	0	2	326	
4	*Bacillus cereus*	V	-2	0	-1	1	23	$2.7x10^7$
4	*Bacillus cereus*	V	-2	0	-1	2	31	
4	*Bacillus cereus*	V	-2	0	-2	1	4	$2.0x10^7$
4	*Bacillus cereus*	V	-2	0	-2	2	0	
4	*Bacillus cereus*	V	-2	0	-3	1	0	0
4	*Bacillus cereus*	V	-2	0	-3	2	0	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
4	*Bacillus cereus*	V	-2	10	0	1	**297**	**$3.15x10^7$**
4	*Bacillus cereus*	V	-2	10	0	2	**333**	
4	*Bacillus cereus*	V	-2	10	-1	1	**60**	**$6.0x10^6$**
4	*Bacillus cereus*	V	-2	10	-1	2	**TNTC**	
4	*Bacillus cereus*	V	-2	20	0	1	**83**	**$4.95x10^6$**
4	*Bacillus cereus*	V	-2	20	0	2	**16**	
4	*Bacillus cereus*	V	-2	20	-1	1	**TNTC**	**TNTC**
4	*Bacillus cereus*	V	-2	20	-1	2	**TNTC**	
4	*Bacillus cereus*	V	-2	30	0	1	**104**	**$8.85x10^6$**
4	*Bacillus cereus*	V	-2	30	0	2	**73**	
4	*Bacillus cereus*	V	-2	30	-1	1	**15**	**$1.25x10^7$**
4	*Bacillus cereus*	V	-2	30	-1	2	**10**	
4	*Bacillus cereus*	V	-2	40	0	1	**7**	**$5.5x10^5$**
4	*Bacillus cereus*	V	-2	40	0	2	**4**	
4	*Bacillus cereus*	V	-2	40	-1	1	**0**	**0**
4	*Bacillus cereus*	V	-2	40	-1	2	**0**	
4	*Bacillus cereus*	V	-2	50	0	1	**19**	**$1.5x10^6$**
4	*Bacillus cereus*	V	-2	50	0	2	**11**	
4	*Bacillus cereus*	V	-2	50	-1	1	**0**	**0**
4	*Bacillus cereus*	V	-2	50	-1	2	**0**	
4	*Bacillus cereus*	V	-2	60	0	1	**0**	**$5.0x10^4$**
4	*Bacillus cereus*	V	-2	60	0	2	**1**	
4	*Bacillus cereus*	V	-2	60	-1	1	**0**	**0**
4	*Bacillus cereus*	V	-2	60	-1	2	**0**	
5	*Bacillus cereus*	V	-2	0	0	1	**480**	**$4.8x10^7$**
5	*Bacillus cereus*	V	-2	0	0	2	**TNTC**	
5	*Bacillus cereus*	V	-2	0	-1	1	**TNTC**	**TNTC**
5	*Bacillus cereus*	V	-2	0	-1	2	**TNTC**	
5	*Bacillus cereus*	V	-2	0	-2	1	**9**	**$6.0x10^7$**
5	*Bacillus cereus*	V	-2	0	-2	2	**3**	
5	*Bacillus cereus*	V	-2	0	-3	1	**0**	**0**
5	*Bacillus cereus*	V	-2	0	-3	2	**0**	
5	*Bacillus cereus*	V	-2	10	0	1	**TNTC**	**TNTC**
5	*Bacillus cereus*	V	-2	10	0	2	**TNTC**	
5	*Bacillus cereus*	V	-2	10	-1	1	**TNTC**	**$6.1x10^7$**
5	*Bacillus cereus*	V	-2	10	-1	2	**61**	
5	*Bacillus cereus*	V	-2	20	0	1	**TNTC**	**$2.55x10^7$**
5	*Bacillus cereus*	V	-2	20	0	2	**255**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
5	*Bacillus cereus*	V	-2	20	-1	1	107	$1.07x10^8$
5	*Bacillus cereus*	V	-2	20	-1	2	TNTC	
5	*Bacillus cereus*	V	-2	30	0	1	TNTC	$2.16x10^7$
5	*Bacillus cereus*	V	-2	30	0	2	216	
5	*Bacillus cereus*	V	-2	30	-1	1	65	$6.5x10^7$
5	*Bacillus cereus*	V	-2	30	-1	2	TNTC	
5	*Bacillus cereus*	V	-2	40	0	1	170	$1.8x10^7$
5	*Bacillus cereus*	V	-2	40	0	2	190	
5	*Bacillus cereus*	V	-2	40	-1	1	36	$3.6x10^7$
5	*Bacillus cereus*	V	-2	40	-1	2	TNTC	
5	*Bacillus cereus*	V	-2	50	0	1	2	$1.5x10^5$
5	*Bacillus cereus*	V	-2	50	0	2	1	
5	*Bacillus cereus*	V	-2	50	-1	1	0	0
5	*Bacillus cereus*	V	-2	50	-1	2	0	
5	*Bacillus cereus*	V	-2	60	0	1	28	$2.8x10^6$
5	*Bacillus cereus*	V	-2	60	0	2	TNTC	
5	*Bacillus cereus*	V	-2	60	-1	1	2	$2.0x10^6$
5	*Bacillus cereus*	V	-2	60	-1	2	2	
1	*Bacillus cereus*	S	0	0	0	1	TNTC	TNTC
1	*Bacillus cereus*	S	0	0	0	2	TNTC	
1	*Bacillus cereus*	S	0	0	-1	1	TNTC	TNTC
1	*Bacillus cereus*	S	0	0	-1	2	TNTC	
1	*Bacillus cereus*	S	0	0	-2	1	183	$1.86x10^7$
1	*Bacillus cereus*	S	0	0	-2	2	188	
1	*Bacillus cereus*	S	0	0	-3	1	21	$2.1x10^7$
1	*Bacillus cereus*	S	0	0	-3	2	TNTC	
1	*Bacillus cereus*	S	0	30	0	1	TNTC	TNTC
1	*Bacillus cereus*	S	0	30	0	2	TNTC	
1	*Bacillus cereus*	S	0	30	-1	1	TNTC	TNTC
1	*Bacillus cereus*	S	0	30	-1	2	TNTC	
1	*Bacillus cereus*	S	0	30	-2	1	TNTC	$1.32x10^7$
1	*Bacillus cereus*	S	0	30	-2	2	132	
1	*Bacillus cereus*	S	0	60	0	1	TNTC	TNTC
1	*Bacillus cereus*	S	0	60	0	2	TNTC	
1	*Bacillus cereus*	S	0	60	-1	1	TNTC	TNTC
1	*Bacillus cereus*	S	0	60	-1	2	TNTC	
1	*Bacillus cereus*	S	0	60	-2	1	123	$1.23x10^7$
1	*Bacillus cereus*	S	0	60	-2	2	TNTC	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
1	*Bacillus cereus*	S	0	120	0	1	**TNTC**	**TNTC**
1	*Bacillus cereus*	S	0	120	0	2	**TNTC**	
1	*Bacillus cereus*	S	0	120	-1	1	**TNTC**	**TNTC**
1	*Bacillus cereus*	S	0	120	-1	2	**TNTC**	
1	*Bacillus cereus*	S	0	120	-2	1	**183**	**$1.83x10^7$**
1	*Bacillus cereus*	S	0	120	-2	2	**TNTC**	
1	*Bacillus cereus*	S	0	300	0	1	**7**	**$7.5x10^3$**
1	*Bacillus cereus*	S	0	300	0	2	**8**	
1	*Bacillus cereus*	S	0	300	-1	1	**0**	**$5.0x10^3$**
1	*Bacillus cereus*	S	0	300	-1	2	**1**	
1	*Bacillus cereus*	S	0	300	-2	1	**0**	**0**
1	*Bacillus cereus*	S	0	300	-2	2	**0**	
1	*Bacillus cereus*	S	-2	0	0	1	**129**	**$1.28x10^7$**
1	*Bacillus cereus*	S	-2	0	0	2	**127**	
1	*Bacillus cereus*	S	-2	0	-1	1	**13**	**$1.7x10^7$**
1	*Bacillus cereus*	S	-2	0	-1	2	**21**	
1	*Bacillus cereus*	S	-2	0	-2	1	**3**	**$1.5x10^7$**
1	*Bacillus cereus*	S	-2	0	-2	2	**0**	
1	*Bacillus cereus*	S	-2	0	-3	1	**0**	**0**
1	*Bacillus cereus*	S	-2	0	-3	2	**0**	
1	*Bacillus cereus*	S	-2	30	0	1	**65**	**$5.35x10^6$**
1	*Bacillus cereus*	S	-2	30	0	2	**42**	
1	*Bacillus cereus*	S	-2	30	-1	1	**22**	**$3.4x10^7$**
1	*Bacillus cereus*	S	-2	30	-1	2	**46**	
1	*Bacillus cereus*	S	-2	30	-2	1	**0**	**0**
1	*Bacillus cereus*	S	-2	30	-2	2	**0**	
1	*Bacillus cereus*	S	-2	60	0	1	**36**	**$5.2x10^6$**
1	*Bacillus cereus*	S	-2	60	0	2	**68**	
1	*Bacillus cereus*	S	-2	60	-1	1	**17**	**$1.25x10^7$**
1	*Bacillus cereus*	S	-2	60	-1	2	**8**	
1	*Bacillus cereus*	S	-2	60	-2	1	**0**	**$5.0x10^6$**
1	*Bacillus cereus*	S	-2	60	-2	2	**1**	
1	*Bacillus cereus*	S	-2	120	0	1	**46**	**$3.65x10^6$**
1	*Bacillus cereus*	S	-2	120	0	2	**27**	
1	*Bacillus cereus*	S	-2	120	-1	1	**1**	**$5.0x10^5$**
1	*Bacillus cereus*	S	-2	120	-1	2	**0**	
1	*Bacillus cereus*	S	-2	120	-2	1	**0**	**0**
1	*Bacillus cereus*	S	-2	120	-2	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
1	*Bacillus cereus*	S	-2	300	0	1	**0**	**0**
1	*Bacillus cereus*	S	-2	300	0	2	**0**	
1	*Bacillus cereus*	S	-2	300	-1	1	**0**	**$5.0x10^5$**
1	*Bacillus cereus*	S	-2	300	-1	2	**1**	
1	*Bacillus cereus*	S	-2	300	-2	1	**0**	**0**
1	*Bacillus cereus*	S	-2	300	-2	2	**0**	
2	*Bacillus cereus*	S	-2	0	0	1	**TNTC**	**TNTC**
2	*Bacillus cereus*	S	-2	0	0	2	**TNTC**	
2	*Bacillus cereus*	S	-2	0	-1	1	**TNTC**	**$3.83x10^8$**
2	*Bacillus cereus*	S	-2	0	-1	2	**383**	
2	*Bacillus cereus*	S	-2	0	-2	1	**4**	**$4.0x10^7$**
2	*Bacillus cereus*	S	-2	0	-2	2	**TNTC**	
2	*Bacillus cereus*	S	-2	60	0	1	**384**	**$3.84x10^7$**
2	*Bacillus cereus*	S	-2	60	0	2	**TNTC**	
2	*Bacillus cereus*	S	-2	60	-1	1	**0**	**0**
2	*Bacillus cereus*	S	-2	60	-1	2	**0**	
2	*Bacillus cereus*	S	-2	120	0	1	**TNTC**	**TNTC**
2	*Bacillus cereus*	S	-2	120	0	2	**TNTC**	
2	*Bacillus cereus*	S	-2	120	-1	1	**18**	**$1.55x10^7$**
2	*Bacillus cereus*	S	-2	120	-1	2	**13**	
2	*Bacillus cereus*	S	-2	180	0	1	**TNTC**	**TNTC**
2	*Bacillus cereus*	S	-2	180	0	2	**TNTC**	
2	*Bacillus cereus*	S	-2	180	-1	1	**1**	**$5.0x10^5$**
2	*Bacillus cereus*	S	-2	180	-1	2	**0**	
2	*Bacillus cereus*	S	-2	240	0	1	**1**	**$1.0x10^5$**
2	*Bacillus cereus*	S	-2	240	0	2	**1**	
2	*Bacillus cereus*	S	-2	240	-1	1	**0**	**0**
2	*Bacillus cereus*	S	-2	240	-1	2	**0**	
2	*Bacillus cereus*	S	-2	300	0	1	**0**	**0**
2	*Bacillus cereus*	S	-2	300	0	2	**0**	
2	*Bacillus cereus*	S	-2	300	-1	1	**0**	**0**
2	*Bacillus cereus*	S	-2	300	-1	2	**0**	
3	*Bacillus cereus*	S	-2	0	0	1	**TNTC**	**TNTC**
3	*Bacillus cereus*	S	-2	0	0	2	**TNTC**	
3	*Bacillus cereus*	S	-2	0	-1	1	**217**	**$2.17x10^8$**
3	*Bacillus cereus*	S	-2	0	-1	2	**TNTC**	
3	*Bacillus cereus*	S	-2	0	-2	1	**14**	**$1.55x10^8$**
3	*Bacillus cereus*	S	-2	0	-2	2	**17**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
3	*Bacillus cereus*	S	-2	60	0	1	**363**	**$3.63x10^7$**
3	*Bacillus cereus*	S	-2	60	0	2	**TNTC**	
3	*Bacillus cereus*	S	-2	60	-1	1	**TNTC**	**TNTC**
3	*Bacillus cereus*	S	-2	60	-1	2	**TNTC**	
3	*Bacillus cereus*	S	-2	120	0	1	**99**	**$8.9x10^6$**
3	*Bacillus cereus*	S	-2	120	0	2	**79**	
3	*Bacillus cereus*	S	-2	120	-1	1	**7**	**$9.0x10^6$**
3	*Bacillus cereus*	S	-2	120	-1	2	**11**	
3	*Bacillus cereus*	S	-2	180	0	1	**0**	**0**
3	*Bacillus cereus*	S	-2	180	0	2	**0**	
3	*Bacillus cereus*	S	-2	180	-1	1	**0**	**0**
3	*Bacillus cereus*	S	-2	180	-1	2	**0**	
3	*Bacillus cereus*	S	-2	240	0	1	**0**	**0**
3	*Bacillus cereus*	S	-2	240	0	2	**0**	
3	*Bacillus cereus*	S	-2	240	-1	1	**0**	**0**
3	*Bacillus cereus*	S	-2	240	-1	2	**0**	
3	*Bacillus cereus*	S	-2	300	0	1	**0**	**0**
3	*Bacillus cereus*	S	-2	300	0	2	**0**	
3	*Bacillus cereus*	S	-2	300	-1	1	**0**	**0**
3	*Bacillus cereus*	S	-2	300	-1	2	**0**	
3	*Bacillus cereus*	S	-2	0	0	1	**TNTC**	**TNTC**
3	*Bacillus cereus*	S	-2	0	0	2	**TNTC**	
3	*Bacillus cereus*	S	-2	0	-1	1	**155**	**$1.65x10^8$**
3	*Bacillus cereus*	S	-2	0	-1	2	**175**	
3	*Bacillus cereus*	S	-2	0	-2	1	**15**	**$1.5x10^8$**
3	*Bacillus cereus*	S	-2	0	-2	2	**TNTC**	
3	*Bacillus cereus*	S	-2	60	0	1	**TNTC**	**TNTC**
3	*Bacillus cereus*	S	-2	60	0	2	**TNTC**	
3	*Bacillus cereus*	S	-2	60	-1	1	**85**	**$8.5x10^7$**
3	*Bacillus cereus*	S	-2	60	-1	2	**TNTC**	
3	*Bacillus cereus*	S	-2	120	0	1	**72**	**$7.55x10^6$**
3	*Bacillus cereus*	S	-2	120	0	2	**79**	
3	*Bacillus cereus*	S	-2	120	-1	1	**4**	**$3.0x10^6$**
3	*Bacillus cereus*	S	-2	120	-1	2	**2**	
3	*Bacillus cereus*	S	-2	180	0	1	**0**	**0**
3	*Bacillus cereus*	S	-2	180	0	2	**0**	
3	*Bacillus cereus*	S	-2	180	-1	1	**0**	**0**
3	*Bacillus cereus*	S	-2	180	-1	2	**0**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
3	*Bacillus cereus*	S	-2	240	0	1	**0**	**0**
3	*Bacillus cereus*	S	-2	240	0	2	**0**	
3	*Bacillus cereus*	S	-2	240	-1	1	**0**	**0**
3	*Bacillus cereus*	S	-2	240	-1	2	**0**	
3	*Bacillus cereus*	S	-2	300	0	1	**0**	**0**
3	*Bacillus cereus*	S	-2	300	0	2	**0**	
3	*Bacillus cereus*	S	-2	300	-1	1	**0**	**0**
3	*Bacillus cereus*	S	-2	300	-1	2	**0**	
1	*Geobacillus stearothermophilus*	V	0	0	0	1	**TNTC**	**TNTC**
1	*Geobacillus stearothermophilus*	V	0	0	0	2	**TNTC**	
1	*Geobacillus stearothermophilus*	V	0	0	-1	1	**170**	**$1.24x10^6$**
1	*Geobacillus stearothermophilus*	V	0	0	-1	2	**77**	
1	*Geobacillus stearothermophilus*	V	0	0	-2	1	**3**	**$1.1x10^6$**
1	*Geobacillus stearothermophilus*	V	0	0	-2	2	**19**	
1	*Geobacillus stearothermophilus*	V	0	0	-3	1	**3**	**$3.0x10^6$**
1	*Geobacillus stearothermophilus*	V	0	0	-3	2	**3**	
1	*Geobacillus stearothermophilus*	V	0	60	0	1	**TNTC**	**TNTC**
1	*Geobacillus stearothermophilus*	V	0	60	0	2	**NA**	
1	*Geobacillus stearothermophilus*	V	0	60	-1	1	**176**	**$1.14x10^6$**
1	*Geobacillus stearothermophilus*	V	0	60	-1	2	**52**	
1	*Geobacillus stearothermophilus*	V	0	60	-2	1	**74**	**$7.4x10^6$**
1	*Geobacillus stearothermophilus*	V	0	60	-2	2	**NA**	
1	*Geobacillus stearothermophilus*	V	0	120	0	1	**TNTC**	**TNTC**
1	*Geobacillus stearothermophilus*	V	0	120	0	2	**TNTC**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
1	*Geobacillus stearothermophilus*	V	0	120	-1	1	**NA**	**NA**
1	*Geobacillus stearothermophilus*	V	0	120	-1	2	**NA**	
1	*Geobacillus stearothermophilus*	V	0	120	-2	1	**14**	**$9.0x10^5$**
1	*Geobacillus stearothermophilus*	V	0	120	-2	2	**4**	
1	*Geobacillus stearothermophilus*	V	0	240	0	1	**TNTC**	**TNTC**
1	*Geobacillus stearothermophilus*	V	0	240	0	2	**TNTC**	
1	*Geobacillus stearothermophilus*	V	0	240	-1	1	**121**	**$1.48x10^6$**
1	*Geobacillus stearothermophilus*	V	0	240	-1	2	**174**	
1	*Geobacillus stearothermophilus*	V	0	240	-2	1	**11**	**$1.1x10^6$**
1	*Geobacillus stearothermophilus*	V	0	240	-2	2	**NA**	
1	*Geobacillus stearothermophilus*	V	0	360	0	1	**TNTC**	**TNTC**
1	*Geobacillus stearothermophilus*	V	0	360	0	2	**TNTC**	
1	*Geobacillus stearothermophilus*	V	0	360	-1	1	**89**	**$8.2x10^5$**
1	*Geobacillus stearothermophilus*	V	0	360	-1	2	**75**	
1	*Geobacillus stearothermophilus*	V	0	360	-2	1	**11**	**$9.5x10^5$**
1	*Geobacillus stearothermophilus*	V	0	360	-2	2	**8**	
1	*Geobacillus stearothermophilus*	V	0	480	0	1	**TNTC**	**TNTC**
1	*Geobacillus stearothermophilus*	V	0	480	0	2	**TNTC**	
1	*Geobacillus stearothermophilus*	V	0	480	-1	1	**62**	**$8.1x10^5$**
1	*Geobacillus stearothermophilus*	V	0	480	-1	2	**100**	
1	*Geobacillus stearothermophilus*	V	0	480	-2	1	**32**	**$2.15x10^6$**
1	*Geobacillus stearothermophilus*	V	0	480	-2	2	**11**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
1	*Geobacillus stearothermophilus*	V	0	600	0	1	**286**	**$2.86x10^5$**
1	*Geobacillus stearothermophilus*	V	0	600	0	2	**NA**	
1	*Geobacillus stearothermophilus*	V	0	600	-1	1	**111**	**$9.6x10^5$**
1	*Geobacillus stearothermophilus*	V	0	600	-1	2	**81**	
1	*Geobacillus stearothermophilus*	V	0	600	-2	1	**7**	**$3.5x10^5$**
1	*Geobacillus stearothermophilus*	V	0	600	-2	2	**0**	
2	*Geobacillus stearothermophilus*	V	0	0	-1	1	**78**	**$6.35x10^5$**
2	*Geobacillus stearothermophilus*	V	0	0	-1	2	**49**	
2	*Geobacillus stearothermophilus*	V	0	0	-2	1	**2**	**$6.5x10^5$**
2	*Geobacillus stearothermophilus*	V	0	0	-2	2	**11**	
2	*Geobacillus stearothermophilus*	V	0	300	-1	1	**60**	**$5.25x10^5$**
2	*Geobacillus stearothermophilus*	V	0	300	-1	2	**45**	
2	*Geobacillus stearothermophilus*	V	0	300	-2	1	**14**	**$9.0x10^5$**
2	*Geobacillus stearothermophilus*	V	0	300	-2	2	**4**	
2	*Geobacillus stearothermophilus*	V	0	600	-1	1	**57**	**$4.25x10^5$**
2	*Geobacillus stearothermophilus*	V	0	600	-1	2	**28**	
2	*Geobacillus stearothermophilus*	V	0	600	-2	1	**0**	**$5.0x10^4$**
2	*Geobacillus stearothermophilus*	V	0	600	-2	2	**1**	
2	*Geobacillus stearothermophilus*	V	0	900	-1	1	**29**	**$2.5x10^5$**
2	*Geobacillus stearothermophilus*	V	0	900	-1	2	**21**	
2	*Geobacillus stearothermophilus*	V	0	900	-2	1	**1**	**$4.5x10^5$**
2	*Geobacillus stearothermophilus*	V	0	900	-2	2	**8**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
2	*Geobacillus stearothermophilus*	V	0	1200	-1	1	**19**	**$2.0x10^5$**
2	*Geobacillus stearothermophilus*	V	0	1200	-1	2	**21**	
2	*Geobacillus stearothermophilus*	V	0	1200	-2	1	**6**	**$4.5x10^5$**
2	*Geobacillus stearothermophilus*	V	0	1200	-2	2	**3**	
3	*Geobacillus stearothermophilus*	V	0	0	-1	1	**64**	**$3.6x10^5$**
3	*Geobacillus stearothermophilus*	V	0	0	-1	2	**8**	
3	*Geobacillus stearothermophilus*	V	0	0	-2	1	**5**	**$5.5x10^5$**
3	*Geobacillus stearothermophilus*	V	0	0	-2	2	**6**	
3	*Geobacillus stearothermophilus*	V	0	600	-1	1	**3**	**$3.0x10^4$**
3	*Geobacillus stearothermophilus*	V	0	600	-1	2	**3**	
3	*Geobacillus stearothermophilus*	V	0	600	-2	1	**0**	**0**
3	*Geobacillus stearothermophilus*	V	0	600	-2	2	**0**	
3	*Geobacillus stearothermophilus*	V	0	1200	-1	1	**1**	**$3.0x10^4$**
3	*Geobacillus stearothermophilus*	V	0	1200	-1	2	**5**	
3	*Geobacillus stearothermophilus*	V	0	1200	-2	1	**0**	**0**
3	*Geobacillus stearothermophilus*	V	0	1200	-2	2	**0**	
3	*Geobacillus stearothermophilus*	V	0	1800	-1	1	**4**	**$4.0x10^4$**
3	*Geobacillus stearothermophilus*	V	0	1800	-1	2	**4**	
3	*Geobacillus stearothermophilus*	V	0	1800	-2	1	**3**	**$2.0x10^5$**
3	*Geobacillus stearothermophilus*	V	0	1800	-2	2	**1**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
4	*Geobacillus stearothermophilus*	V	0	0	-1	1	**56**	**$5.05x10^5$**
4	*Geobacillus stearothermophilus*	V	0	0	-1	2	**44**	
4	*Geobacillus stearothermophilus*	V	0	0	-1	3	**1**	
4	*Geobacillus stearothermophilus*	V	0	0	-2	1	**11**	**$5.0x10^5$**
4	*Geobacillus stearothermophilus*	V	0	0	-2	2	**3**	
4	*Geobacillus stearothermophilus*	V	0	0	-2	3	**1**	
4	*Geobacillus stearothermophilus*	V	0	600	-1	1	**29**	**$2.37x10^5$**
4	*Geobacillus stearothermophilus*	V	0	600	-1	2	**31**	
4	*Geobacillus stearothermophilus*	V	0	600	-1	3	**11**	
4	*Geobacillus stearothermophilus*	V	0	1200	-1	1	**16**	**$8.67x10^5$**
4	*Geobacillus stearothermophilus*	V	0	1200	-1	2	**2**	
4	*Geobacillus stearothermophilus*	V	0	1200	-1	3	**8**	
4	*Geobacillus stearothermophilus*	V	0	1800	-1	1	**4**	**$3.67x10^4$**
4	*Geobacillus stearothermophilus*	V	0	1800	-1	2	**3**	
4	*Geobacillus stearothermophilus*	V	0	1800	-1	3	**4**	
5	*Geobacillus stearothermophilus*	V	0	0	-1	1	**213**	**$2.53x10^6$**
5	*Geobacillus stearothermophilus*	V	0	0	-1	2	**293**	
5	*Geobacillus stearothermophilus*	V	0	0	-1	3	**TNTC**	
5	*Geobacillus stearothermophilus*	V	0	600	-1	1	**100**	**$1.42x10^6$**
5	*Geobacillus stearothermophilus*	V	0	600	-1	2	**180**	
5	*Geobacillus stearothermophilus*	V	0	600	-1	3	**146**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
5	*Geobacillus stearothermophilus*	V	0	1200	-1	1	**81**	**$1.35x10^5$**
5	*Geobacillus stearothermophilus*	V	0	1200	-1	2	**48**	
5	*Geobacillus stearothermophilus*	V	0	1200	-1	3	**6**	
5	*Geobacillus stearothermophilus*	V	0	1800	-1	1	**86**	**$6.27x10^5$**
5	*Geobacillus stearothermophilus*	V	0	1800	-1	2	**54**	
5	*Geobacillus stearothermophilus*	V	0	1800	-1	3	**48**	
1	*Geobacillus stearothermophilus*	S	0	0	0	1	**100**	**$8.15x10^4$**
1	*Geobacillus stearothermophilus*	S	0	0	0	2	**63**	
1	*Geobacillus stearothermophilus*	S	0	0	-1	1	**3**	**$5.0x10^4$**
1	*Geobacillus stearothermophilus*	S	0	0	-1	2	**7**	
1	*Geobacillus stearothermophilus*	S	0	0	-2	1	**1**	**$5.0x10^4$**
1	*Geobacillus stearothermophilus*	S	0	0	-2	2	**0**	
1	*Geobacillus stearothermophilus*	S	0	600	0	1	**25**	**$1.95x10^4$**
1	*Geobacillus stearothermophilus*	S	0	600	0	2	**14**	
1	*Geobacillus stearothermophilus*	S	0	600	-1	1	**3**	**$1.5x10^4$**
1	*Geobacillus stearothermophilus*	S	0	600	-1	2	**0**	
1	*Geobacillus stearothermophilus*	S	0	1200	0	1	**40**	**$2.75x10^4$**
1	*Geobacillus stearothermophilus*	S	0	1200	0	2	**15**	
1	*Geobacillus stearothermophilus*	S	0	1200	-1	1	**2**	**$2.0x10^4$**
1	*Geobacillus stearothermophilus*	S	0	1200	-1	2	**2**	
1	*Geobacillus stearothermophilus*	S	0	1800	0	1	**26**	**$2.5x10^4$**
1	*Geobacillus stearothermophilus*	S	0	1800	0	2	**24**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
1	*Geobacillus stearothermophilus*	S	0	1800	-1	1	**3**	**$2.0x10^4$**
1	*Geobacillus stearothermophilus*	S	0	1800	-1	2	**1**	
2	*Geobacillus stearothermophilus*	S	0	0	0	1	**74**	**$8.65x10^4$**
2	*Geobacillus stearothermophilus*	S	0	0	0	2	**99**	
2	*Geobacillus stearothermophilus*	S	0	0	-1	1	**7**	**$8.5x10^4$**
2	*Geobacillus stearothermophilus*	S	0	0	-1	2	**10**	
2	*Geobacillus stearothermophilus*	S	0	600	0	1	**15**	**$1.75x10^4$**
2	*Geobacillus stearothermophilus*	S	0	600	0	2	**20**	
2	*Geobacillus stearothermophilus*	S	0	600	-1	1	**2**	**$1.5x10^4$**
2	*Geobacillus stearothermophilus*	S	0	600	-1	2	**1**	
2	*Geobacillus stearothermophilus*	S	0	1200	0	1	**4**	**$1.05x10^4$**
2	*Geobacillus stearothermophilus*	S	0	1200	0	2	**17**	
2	*Geobacillus stearothermophilus*	S	0	1200	-1	1	**4**	**$5.0x10^4$**
2	*Geobacillus stearothermophilus*	S	0	1200	-1	2	**6**	
2	*Geobacillus stearothermophilus*	S	0	1800	0	1	**TNTC**	**$2.0x10^3$**
2	*Geobacillus stearothermophilus*	S	0	1800	0	2	**2**	
2	*Geobacillus stearothermophilus*	S	0	1800	-1	1	**2**	**$1.5x10^4$**
2	*Geobacillus stearothermophilus*	S	0	1800	-1	2	**1**	
3	*Geobacillus stearothermophilus*	S	0	0	0	1	**62**	**$6.1x10^6$**
3	*Geobacillus stearothermophilus*	S	0	0	0	2	**60**	
3	*Geobacillus stearothermophilus*	S	0	0	-1	1	**15**	**$1.25x10^7$**
3	*Geobacillus stearothermophilus*	S	0	0	-1	2	**10**	

Exposure Number	Bacteria	State of Bacteria	Dilution for Exposure	Exposure Time (seconds)	Dilution for plates	Plate #	CFU	Concentration (CFU/mL)
3	*Geobacillus stearothermophilus*	S	0	600	0	1	**4**	**$3.25x10^5$**
3	*Geobacillus stearothermophilus*	S	0	600	0	2	**3**	
3	*Geobacillus stearothermophilus*	S	0	600	-1	1	**2**	**$1.0x10^6$**
3	*Geobacillus stearothermophilus*	S	0	600	-1	2	**0**	
3	*Geobacillus stearothermophilus*	S	0	1200	0	1	**11**	**$1.1x10^6$**
3	*Geobacillus stearothermophilus*	S	0	1200	0	2	**11**	
3	*Geobacillus stearothermophilus*	S	0	1200	-1	1	**4**	**$4.5x10^6$**
3	*Geobacillus stearothermophilus*	S	0	1200	-1	2	**5**	
3	*Geobacillus stearothermophilus*	S	0	1800	0	1	**4**	**$3.5x10^5$**
3	*Geobacillus stearothermophilus*	S	0	1800	0	2	**3**	
3	*Geobacillus stearothermophilus*	S	0	1800	-1	1	**3**	**$4.5x10^4$**
3	*Geobacillus stearothermophilus*	S	0	1800	-1	2	**6**	

Appendix G

Curriculum Vitae

ANGELA MORRIS, RDH

EDUCATION:

Bachelor of Science in Dental Hygiene; Minor: Community Health — 2006
Old Dominion University, Norfolk, Virginia

Master of Science in Dental Hygiene; Specialization: Research — Expected 2007
Old Dominion University, Norfolk, Virginia

EXPERIENCE:

Teaching

January – May 2007	Teaching Assistant – Department of Dental Hygiene Old Dominion University, Norfolk, Virginia.

Research

June – August 2006	Co-Op Dental Hygiene Intern – Oral Care R & D Pfizer Consumer Healthcare, Morris Plains, New Jersey.

Private Practice

January 2007 – present	Dental Hygienist (General Dentistry) part-time

GRANTS AWARDED:

2006	McCombs GB (Principal Investigator) **Morris AD**, Laroussi M and Hynes WL. Bactericidal Effects of Cold Plasma Technology on *Geobacillus stearothermophilus* and *Bacillus cereus* Microorganisms. American Dental Hygienists' Association Institute of Oral Health, $6,506.

HONORS, AWARDS & SCHOLARSHIPS:

February 2005	Gene W. Hirschfeld School of Dental Hygiene Outstanding Student of the Month
2005	Induction into Alpha Eta National Honor Society for Allied Health Professionals
2005	Induction into Phi Kappa Phi Honor Society
2005 – 2006	Recipient of the ADHA Institute Baccalaureate Scholarship
2006	ODU School of Dental Hygiene *Proctor & Gamble* Academic Achievement Award
2006	Induction into Sigma Phi Alpha National Dental Hygiene Honor Society
2006	Graduated Summa Cum Laude
2006 – 2007	Recipient of the Friends of Dental Hygiene Scholarship
2007	Recipient of the first *CareerFlow Retreat* Scholarship