I, ______________________ Craig S. Davidson ______________________, hereby submit this work as part of the requirements for the degree of:

Master of Science

in:

Environmental Science

It is entitled:

Efficacy of selected N95 respirators, surgical masks, and transparent mesh netting against airborne, non-pathogenic Bacillus anthracis strain Sterne 34F2 vegetative cells and endospores

This work and its defense approved by:

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Dr. Christopher F. Green
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Efficacy of selected N95 respirators, surgical masks, and transparent mesh netting against airborne, non-pathogenic *Bacillus anthracis* strain Sterne 34F2 vegetative cells and endospores

A thesis submitted to the
Division of Research and Advanced Studies of the University of Cincinnati

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By Craig S. Davidson

B.A. Indiana University, 2000

Pasquale V. Scarpino, Ph.D.
Committee Chair
Abstract

Aerosol droplet- and airborne-transmitted diseases are an important concern in healthcare settings. Because respiratory protection is an important component of protection against airborne-transmitted diseases, there is a need to better assess the respiratory protection provided by respirators and surgical masks available to healthcare workers. A new method for quantitatively assessing relative efficiency of respirators and surgical masks was developed and implemented in the evaluation of five surgical masks, three N95 respirators, three surgical mask/N95 respirator hybrids, and two types of transparent mesh netting (a possible low-cost alternative). Relative efficiency of the 11 surgical masks and respirators ranged from 34% to 69% against vegetative cells and from 34% to 65% against endospores. The netting with the largest pore size (0.8mm) provided no arrestance in bioaerosols, while the 0.25 mm pore size netting provided an 18% arrestance in non-pathogenic Bacillus anthracis strain Sterne 34F2 vegetative cell aerosols and a 19% arrestance in non-pathogenic B. anthracis strain Sterne 34F2 endospore aerosols.
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List of Acronyms

ABS  Automated Breathing Simulator
AGI  All-glass Impinger
AHH  American Home & Habitat
ANOVA  Analysis of Variance
CDC  Centers for Disease Control and Prevention
CI  Confidence Interval
CFU  Colony Forming Unit
CMAD  Count Median Aerodynamic Diameter
CMD  Count Median Diameter
DOP  Dioctylphthalate
GM  Geometric Mean
GSD  Geometric Standard Deviation
HCP  Health Care Personnel
HEPA  High Efficiency Particulate Air (filter)
MMAD  Mass Median Aerodynamic Diameter
MMD  Mass Median Diameter
MRV  Minute Respiratory Volume
NIOSH  National Institute for Occupational Safety and Health
NPPTL  National Personal Protective Technology Laboratory
OSHA  Occupational Safety and Health Administration
PBW  Phosphate Buffered Water
PSDVB  Polystyrene divinylbenzene
PSL  Polystyrene Latex
QNFT  Quantitative Fit Test
RPE  Respiratory Protective Equipment
SAS  Statistical Analysis Software
SWPF  Simulated Workplace Protection Factor
TSA  Tryptic Soy Agar
TSB  Tryptic Soy Broth
TSR  Tropical Safety Research
TV  Tidal Volume
WHO  World Health Organization
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Chapter 1: Introduction and Literature Review

A review of the literature pertaining to respiratory protective equipment (RPE) shows a diverse array of documents covering the following topics: the theory, development, and history of RPE use; RPE performance, efficiency, and fit-tests; studies investigating re-aerosolization of droplets collected on RPE after repeated handling of the equipment; and guidelines for RPE use.

Rockwood and O’Donoghue (1960) describe different stages of development concerning the basic theory, preliminary testing and the initial implementation of surgical mask use that occurred during the first part of this century. Specifically, they cite 1899 as being the hallmark year for the onset of facemask use as this was the year in which the theory of droplet infection was first presented. During this period, rudimentary facemasks consisting of rolled gauze placed over the mouth were first put into use. The authors then go on to describe the development of new masks, the great deal of importance that was placed on their use, and the subsequent period of what they describe as “masking unimportance” due the introduction of newly discovered antibiotics.

The discussion by Rockwood and O’Donoghue focuses primarily on surgical-type facemasks used in health care settings to prevent the spread of airborne pathogens released by the wearer of the mask. In contrast, Harris (2000) points out that in 1937 the Bureau of Mines put forth regulations (U. S. Department of the Interior, 1937) as a result of the study it conducted to evaluate the performance of supplied air respirators based on inward leakage. These regulations stipulated the conditions under which the RPE should be evaluated. The parameters involved testing the RPE in an atmosphere containing a specified concentration of airborne coal-dust particles having specific diameters. The regulations also indicated a series of varied
movements that the subject wearing the test RPE should perform during a certain time period. Additionally, Harris (2000) goes on to describe further studies conducted by the Bureau of Mines (U.S. Department of the Interior, 1965) in which the concept of the RPE “Decontamination Factor” (i.e. “protection factor”) was first introduced. The determination of this parameter was based on the inside facemask contaminant concentration versus the outside facemask contaminant concentration.

After the Bureau of Mines work, RPE research challenging surgical facemasks against the release of bacterial aerosols became more common. Citing some of the faults of and a need to modify previous efficiency studies with updated equipment, Greene and Vesley (1962) presented their method for evaluating surgical mask efficiency based on the masks’ ability to contain the contaminants expelled from the test subject wearing the mask. In the paper, they detail the use of an “isolation box” supplied with filtered air in order to provide a controlled testing environment.

As disposable surgical masks and masks made of different materials became more widely used, additional work was completed to measure efficiency based on the masks’ stopping capacity. Ford and Peterson (1963) examined efficiencies of the popular, filtering type (as opposed to non-filtering type) surgical masks. They were able to show a wide range of efficiency among different types of disposable, filtering type masks (15 to 99 percent). Madsen and Madsen (1967) also evaluated an assortment of masks made with a variety of materials and attempted to do so in a manner which simulated the conditions under which a surgeon wears a mask. Their results showed differences in efficiencies for masks composed of different materials. Quesnel (1975) expanded on this by testing even more masks with varying designs (presence or absence of pleating) and compositions (cotton or various synthetic materials). The
study provided additional evidence of varying efficiencies related to mask design and composition.

A study by Ha’eri and Whiley (1980) further investigated the effectiveness of surgical masks by simulating bacteria with sterile human albumin microspheres (HAM) that were of diameters (10-35 µm) similar to particles and droplets that carry airborne bacteria. The investigators showed that exposure increased with operating time by quantifying the HAM in samples taken from test patients’ wounds. They also showed that the transmission of the HAM occurred mainly because of leakage around the edges of the mask and not through the mask.

During recent years, RPE research has largely been dedicated to conducting respirator efficiency and leakage tests with bacterial aerosols and non-biological surrogates. In contrast to earlier studies with surgical masks, the more current work has been focused toward evaluating the protection afforded to workers wearing RPE. Weber et al. (1993) looked at the protection surgical masks afford HCWs against the penetration of aerosol particles in the sub-micrometer size range. They concluded that surgical masks might provide insufficient protection to HCWs working in environments containing aerosols with particles in this size range. In the following year Johnson et al. (1994), tested a range of RPE (air-purifying respirators, surgical masks, dust/mist respirators, and high-efficiency respirators) against a bio-aerosol of *Bacillus subtilis* subsp. *niger*. Under simulated breathing conditions, they showed that the various RPE ranged from 67 to 99.95 percent efficacious, and inefficiency could be mostly attributed to face seal leakage rather than penetration of the RPE filter material. Wake et al. (1997) also performed tests on various types of RPE (surgical, dust, nuisance, and resuscitation masks along with respirator filter cartridges) against bacterial aerosols, but repeated the same tests with non-biological aerosols of sodium chloride. This study showed that masks and filter material with
electrically charged surfaces performed better than non-charged counterparts. Also, comparable efficiencies were found when challenging masks with both types of aerosols having equivalent particle sizes. Similarly, Qian et al. (1998) presented work comparing N95 respirator efficiencies when challenged with both bio-aerosols and inert aerosol particles. The researchers concluded that N95 respirators provide exceptional protection against bio-aerosol surrogates for *Mycobacterium tuberculosis*. Additionally, it was shown that respirators made by different manufacturers exhibit a degree of variability in efficiency when challenged with sodium chloride aerosols containing particles in the size range of the most penetrating sizes (0.1 to 3 µm).

Expanding on the respirator efficiency and leakage studies, another category of RPE research has involved work to evaluate various techniques used in respirator fit-tests. Subsequent to quantitative fit-testing (QNFT) methods first developed in the 1960’s and 1970’s (Burgess et al., 1961; Hyatt et al., 1972), Willeke et al. (1981) proposed a new method for the evaluation of respirator fit-tests. The work demonstrated the efficacy of utilizing a variety of non-hazardous, non-biological aerosols measured by a condensation nuclei counter compared to conventional methods that used potentially carcinogenic aerosols measured by a photometer.

Since then, a great amount of other work investigating fit-test methods has become available. During 1998 and 1999, the results of a series of investigations aimed at comparing six respirator fit-test methods were put forth (Coffey et al., 1998a; Coffey et al, 1998b; Coffey et al., 1999a). These studies included the development of an evaluation protocol (Coffey et al, 1998a), actual testing of the methods being compared (Coffey et al., 1998b), and validation of the comparison tests (Coffey et al., 1999a). Through preliminary studies, the protocol development work established acceptable testing parameters for use in the ensuing evaluation of the various fit-test methods being compared. The comparison tests of the fit-test methods confirmed that
several of the methods actually prove that a relationship exists between QNFT and respirator performance while the validation study substantiated the results of these tests.

Following these conclusions, the efforts of Coffey et al. (1999b) provided the results of research aimed at simulating workplace performance of the nine classes of particulate respirators certified by the National Institute for Occupational Safety and Health (NIOSH) along with determining the effect of respirator fit-tests and the associated respirator fit-test pass/fail criteria. The results of the study yielded the conclusions that fit-tests are necessary to guarantee adequate performance, more rigorous pass/fail criteria improve performances, and not all N95 respirators perform at the same level.

Building on the previous fit-test method evaluation study, Coffey et al. (2002) imparted more information concerning the accuracy of various QNFT methods. The study judged the PortaCount® Plus by TSI (TSI, Inc., St. Paul, MN) to be “…perhaps the best method for identifying poorly fitting respirators.” However, it was determined that fit test method accuracy still needed to be improved along with the fit characteristics of respirators.

Many other RPE fit-test studies have also featured the PortaCount® Plus. An example is field tests that proved a relationship between respirator fit and protection in an actual workplace environment (Zhuang et al., 2003). The PortaCount® Plus has also been used in work examining the fit characteristics of a number of filtering-facepiece respirators (Coffey et al., 2004; Coffey et al., 2002).

Other novel RPE research efforts have been focused towards exploring the re-
aerosolization capacity of microbial particles collected on RPE. The main focus of this work is in quantifying the amount of microbial particles collected on RPE that can be expected to be released during repeated donning or handling of the equipment. Qian et al. (1997) found that re-
aerosolization of both biological and non-biological surrogate particles of Mycobacterium tuberculosis is trivial under environmental conditions characteristic of normal respirator use (relative humidity less than 35%). In a more in depth study examining the use of respirators to control tuberculosis, Willeke and Qian (1998) came to the same conclusion the following year.

More recently, Kennedy and Hinds (2004) used inert, non-biological surrogates to simulate the release of anthrax particles from disposable respirators. They too demonstrated that only a small fraction of the collected particles were usually released from the RPE. However, citing that little is still known about how anthrax particles react in this context they caution that additional investigation is necessary and that RPE should be handled carefully after used in an environment possibly contaminated by anthrax.

Since the introduction of the nine new classes of particulate respirators by NIOSH, many usage recommendations and guidelines have been put forth. Guidance and opinions concerning RPE use have been imparted by agencies both public (NPPTL, 2005; OSHA, 1998) and private (ANSI, 2001; ANSI, 1992) as well as by researchers in the field (Colton and Nelson, 1997; Lange, 2004; Lange, 2003a; Lange, 2003b).

Particularly worth noting is the recent Centers for Disease Control and Prevention (CDC) sponsored “Workshop on Respiratory Protection for Airborne Infectious Agents” (NPPTL, 2005). According to the record of the proceedings, the main focus of the discussions included the state of existing scientific understanding of the transmission of airborne pathogens, tactics to advance respiratory protection, and the needs of future RPE research. The convening of this forum and its stated goals serves as a prime example of the need to advance the scientific knowledge base regarding the protection that RPE affords against infectious bio-aerosols.
Chapter 2:

Method for evaluating the relative efficiency of selected N95 respirators and surgical masks in preventing the inhalation of airborne, non-pathogenic *Bacillus anthracis* strain Sterne 34F2 vegetative cells by healthcare personnel
2.1 Abstract

Aerosol droplet- and airborne-transmitted diseases are an important concern in healthcare settings. The anthrax attacks of 2001, SARS outbreaks which resulted in transmission to numerous HCP, concerns about the possible use of smallpox as a bioterrorist agent, and historical reports of airborne transmission of smallpox have contributed to heightened concern about airborne infectious agents. Because respiratory protection is an important component of protection against airborne-transmitted diseases, there is a need to better assess the respiratory protection provided by respirators and surgical masks.

This paper describes a method developed for quantitatively assessing the relative efficiency of selected respirators and surgical masks. The method includes a procedure for bioaerosol generation, recovery using a horizontal bioaerosol chamber, as well as appropriate methods for data analysis. The chamber used is a stainless steel 0.56 m² cross-sectional area duct approximately 3.7 m long that was constructed as a secure environment for bioaerosol testing and evaluation. Using this method, we evaluated five surgical masks, three N95 respirators, and three surgical mask/N95 respirator hybrids. All are commercially available and used in U.S. healthcare settings. Bacterial aerosols of non-pathogenic Bacillus anthracis strain Sterne 34F2 (a surrogate for pathogenic B. anthracis) were generated with a six-jet Collison nebulizer. To mimic human respiratory breathing, an automated breathing simulator (ABS) calibrated to normal tidal volume and active breathing rate (500 mL/breath and 20 breaths/min, respectively) was used. Respirators were placed on manikin head forms designed for use in CPR training and used in our investigation as surrogates for humans.

This method showed that a Collison nebulizer could generate monodisperse bacterial aerosol from a monoculture to effectively test respiratory protective equipment (RPE) total inward leakage. The method also showed that the AGI-30 air samplers, combined with the
ABS, provided an accurate representation of RPE relative efficiency. A manikin head form was used in this study as a surrogate for healthcare personnel (HCP).

Relative efficiency of the 11 surgical masks and respirators ranged from 34% to 69%. The within RPE standard deviation was 7% while the between RPE standard deviation was 5%. A one-way analysis of variance (ANOVA) revealed statistically significant differences between several of the RPE models. On the basis of the ANOVA pair-wise analysis, the RPE models could be grouped into four categories based on relative efficiency, A through D. The RPE models in group A had a mean relative efficiency of 65% and standard deviation of 3% while those in group D had a mean relative efficiency of 35% and a standard deviation of 1%. Neither RPE type (N95, surgical mask or hybrid) nor brand name (3M, Moldex, Kimberly Clark, and Medline) was an indicator of RPE relative efficiency. Future studies should be conducted to compare filter media type, manikin head forms with different anthropometric characteristics, and most important, each RPE’s ability to be fitted to the HCP’s face.

2.2 Introduction

Aerosol droplet- and airborne-transmitted diseases are an important concern in healthcare settings. The anthrax attacks of 2001, SARS outbreaks which resulted in transmission to numerous healthcare personnel (HCP), concerns about the possible use of smallpox as a bioterrorist agent, and historical reports of smallpox transmission among patients and HCP have contributed to heightened concern about airborne infectious agents. These incidents have highlighted the need for a better understanding of transmission of aerosol producing droplets and the efficacy of methods used in providing respiratory protection in healthcare settings, including surgical masks and respirators. Because respiratory protection is a key element of protection against airborne-transmitted diseases, there is a need to better assess the respiratory protection provided by respirators and surgical masks.
Johnson et al.\textsuperscript{(4)} tested a range of respiratory protective equipment (RPE) (air-purifying respirators, surgical masks, dust/mist respirators, and high-efficiency respirators) against a bioaerosol of \textit{Bacillus subtilis} subsp. \textit{niger}. To simulate normal breathing, an automated breathing simulator (ABS) connected to an anthropometric head form placed in an aerosol chamber was used to produce breathing patterns similar to “light exertion.” The RPE were tested both sealed to the anthropomorphic head form and unsealed. Wake et. al.\textsuperscript{(5)} performed tests on filter media from various types of RPE (surgical, dust, nuisance, and resuscitation masks along with respirator filter cartridges) against bacterial aerosols, but repeated the same tests with aerosols of sodium chloride. Wake’s study addressed only the efficiency of filter media, and did not address leakage occurring between the RPE and face of the wearer. Similarly, Qian et. al.\textsuperscript{(6)} compared the efficiencies of N95 respirator filter media when challenged with both bioaerosol and inert aerosol. All filter media on the respirators tested were at least 95\% efficient when challenged with sodium chloride aerosol containing particles in the most penetrating size range (0.1 to 0.3 \textmu m). Filter media were 99.5\% or more efficient against particles about 0.75 \textmu m in size. The researchers concluded that, while filter media from N95 respirators provide exceptional protection against bioaerosol surrogates for \textit{Mycobacterium tuberculosis} when a “proper face seal” is achieved, significant amounts of bacteria could enter the respirator-wearer’s breathing space if the respirator is inadequately sealed to the wearer’s face.

The absence of a standard method for simultaneously evaluating the relative efficiency of the filter media and fit when challenged with a bioaerosol is noted. The objective of our study was to develop and evaluate a standard method for use in testing the relative efficiency of commercially available RPE for reducing exposure to a bioaerosol, taking into account previously published studies.\textsuperscript{(7-20)} We selected a sampling method based on work by Green and Scarpino\textsuperscript{(7)} and Scarpino et. al.\textsuperscript{(8)} in a controlled bioaerosol chamber. We used different types of
bioaerosol samplers described by Jensen et. al.\textsuperscript{(9)} and Gillespie et. al.\textsuperscript{(10)} and the Collison nebulizer described by May\textsuperscript{(11)} when developing the method. Coffey et al. measured respirator performance with several methods, including the 5\textsuperscript{th} percentile simulated workplace protection factor (SWPF) value.\textsuperscript{(17)} The SWPF is a measurement of protection provided by an RPE, considering filter penetration and face seal leakage. The 5\textsuperscript{th} percentile SWPF is the value at which 95\% of the SWPF values will be greater than or equal to this number.\textsuperscript{(16)} The 5\textsuperscript{th} percentile SWPF for each RPE was calculated using the geometric mean (GM) and the geometric standard deviation (GSD), as GM/GSD\textsuperscript{1.645}. They first calculated 5\textsuperscript{th} percentile SWPF without regard to the results of fit tests, then in separate groups. The values were then separately calculated for subjects passing a fit-test and for subjects failing that fit-test.\textsuperscript{(12)} A more detailed account of 5\textsuperscript{th} percentile SWPF calculations can be found in Coffey et al.\textsuperscript{(16)}

We attempted to simulate HCP use of RPE for exposure to airborne infectious agents. Unlike the research described above, we assessed total inward leakage which is a function of both filter media efficiency and face seal leakage. This investigation improves on methods by Johnson et. al.\textsuperscript{(4)} by being the first to describe the use of an ABS connected to a biological aerosol sampler as well as testing with surrogates for humans. These methods have been developed to better mimic respiratory parameters for use in quantifying RPE relative efficiency.

\textbf{2.3 Materials and methods}

\textbf{2.3.1 Bioaerosol generation}

Non-encapsulated, culturable \textit{Bacillus anthracis} strain Sterne 34F2 (Colorado Serum Company, Denver, CO) vegetative cells were used as a surrogate for \textit{Bacillus anthracis} because of its lack of pathogenicity in humans.\textsuperscript{(21)} The aerobic, non-pathogenic \textit{B. anthracis} strain Sterne 34F2 cells are gram-positive rods with a diameter of 1.0-1.5 µm, a length of 1-8 µm and a cell diameter greater than 1µm.\textsuperscript{(22)}
Vegetative cell cultures were made prior to each testing day. Suspensions of non-pathogenic \textit{B. anthracis} Sterne 34F2 were inoculated into approximately 100 mL of tryptic soy broth (TSB) and incubated for 24 h at 35°C in Erlenmeyer flasks on a shaker. The broth was centrifuged for 15 min, 3°C at 1000g on a Beckman TH-4 Rotor (Beckman Coulter, Fullerton CA) forming a soft pellet of bacterial cells. The supernatant was then decanted and the pellet resuspended in Phosphate Buffered Water (PBW). This process was repeated two times. The concentration of bacterial suspensions was then adjusted to approximately \(10^{6-7}\) colony-forming units (CFU)/mL suspension for aerosol generation.

The test bioaerosol was generated with a modified MRE-type six-jet Collison nebulizer with a full-sized bottle (CN-25, BGI Inc., Waltham, MA) filled with a 100 mL aliquot.\(^{(23)}\) The compressed air from the clean air source to the nebulizer was passed through a Balston Type 100-18-DX filter (Parker Hannifin Corporation, Haverhill MA). The Balston filter has a reported efficiency of 93% for particles greater than or equal to 0.01 \(\mu\)m.\(^{(24)}\)

Aerosol size distributions, and their changes over time, were measured and compared to published results to determine the feasibility of utilizing the nebulizer to create culturable single-celled bioaerosol from a monoculture.\(^9\),\(^{23}\) Run-to-run variations were also evaluated using both bioaerosol data collected from all-glass impinger-30 samplers (AGI-30, Ace Glass, Inc., Vineland, NJ) and particle data collected from an ARTI Hand Held Particle Counter (HHPC-6, HACH Ultra Analytics, Houston TX). In addition, the ARTI particle counter was used to determine how long it took to achieve a steady state bioaerosol concentration inside the chamber. (It took less than 8 minutes to reach equilibrium according to the ARTI particle counter.)

The count median diameter (CMD), mass median diameter (MMD) and geometric standard deviation (GSD) of the nebulizer output were determined using measurements by the Andersen six-stage impactor (Andersen Instruments, Waltham MA) for culturable bioaerosol,
and the ARTI HHPC-6 for chamber particle concentration. Airflow rate from the nebulizer was measured by a Dwyer RMB Series Rate-Master Flowmeter, model RMB-53 (Dwyer Instruments, Inc., Michigan City, IN). Velocity of air exiting the nebulizer was measured by an Extech Hot-wire Thermo-Anemometer model 407123 (Extech Instruments Corporation, Waltham, MA).

Eleven RPE models were selected for testing because of their commercial availability and use in healthcare settings. Several of these models have been evaluated in respirator fit-test studies.\textsuperscript{(16,17,25)} The 11 models included 5 surgical masks, 3 filtering facepiece disposable N95 respirators, and 3 surgical mask/N95 respirator hybrids. The order in which the RPE were tested was randomized. The RPE evaluated, grouped according to manufacturer, model number, and type (surgical mask, N95, or hybrid), are presented in Table 2.1. Manikin head forms of the type used for CPR training were chosen as surrogates for HCP. The Simulaids\textsuperscript{®} Brad\textsuperscript{TM} CPR torso (Simulaids, Inc. Woodstock, NY) was selected because it is a commercially available, “adult” size manikin\textsuperscript{(26)}, and was able to be easily modified for the purposes of this investigation. In addition to its life-like facial features, the Brad\textsuperscript{TM} model was also selected because the mouth orifice of the manikin head form allowed for attachment of an air sampler.
Table 2.1: List of the RPE models evaluated

<table>
<thead>
<tr>
<th>Brand</th>
<th>Model</th>
<th>(Surgical Mask, N95, Hybrid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3M</td>
<td>9210</td>
<td>N95</td>
</tr>
<tr>
<td>3M</td>
<td>1818FS</td>
<td>Surgical Mask</td>
</tr>
<tr>
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<td>1818</td>
<td>Surgical Mask</td>
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<td>3M</td>
<td>1860</td>
<td>Hybrid</td>
</tr>
<tr>
<td>3M</td>
<td>1860s</td>
<td>Hybrid</td>
</tr>
<tr>
<td>Kimberly Clark</td>
<td>48201</td>
<td>Surgical Mask</td>
</tr>
<tr>
<td>Kimberly Clark</td>
<td>PFR95</td>
<td>Hybrid</td>
</tr>
<tr>
<td>Kimberly Clark</td>
<td>48208</td>
<td>Surgical Mask</td>
</tr>
<tr>
<td>Medline</td>
<td>NON27501</td>
<td>N95</td>
</tr>
<tr>
<td>Medline</td>
<td>NON27402</td>
<td>Surgical Mask</td>
</tr>
<tr>
<td>Moldex</td>
<td>2200</td>
<td>N95</td>
</tr>
</tbody>
</table>

All test, control, and blank bioaerosol samples were taken with AGI-30 air samplers. The AGI-30 is a high-velocity impinger designed to collect airborne particles with a diameter of 0.8 µm to 15.0 µm into 20 mL of a collection fluid (PBW) at a nominal airflow rate of 12.5 L/min (12.3-12.6 L/min, Ace Glass, Inc., 2004) when the vacuum exceeds 55 kPa (41 cm Hg). The nominal airflow through the sampler cannot be greater than 12.5 L/min as the stem of the AGI-30 acts as a critical orifice. Increasing the magnitude of vacuum merely increased the rate of evaporation of liquid within the AGI-30.

However, the AGI-30 sampler was not operated at a constant flow; rather, flow rate was regulated by an ABS to mimic human respiratory breathing rate. The apparatus consists of a 2-liter syringe (Hamilton Super Syringe Model S2000, Hamilton Company, Reno, NV) with the plunger connected to a variable speed motor via an adjustable cam (Figure 2.1). Air is drawn into and forced out of the syringe as the cam moves the plunger into and out of the syringe chamber. The motor speed and syringe volume were adjusted to achieve a tidal volume of 500 mL/ breath\(^{27,28}\) and a breath rate of 20 breaths/ min to simulate an active HCP. The resulting
minute respiratory volume (MRV) was 10 L/min. Because the ABS mimics both MRV inhalation and exhalation, slippage of the RPE may occur resulting in variable effects on relative efficiencies.

**Figure 2.1:** Automated breathing simulator

![Diagram of Automated breathing simulator](image)

All sampling was completed in the same bioaerosol chamber utilized in previous studies.\(^{(7,8,9)}\) The chamber is a stainless steel duct approximately 3.7 m long with a cross-sectional area of 0.56 m\(^2\). An exhaust fan draws room air into the chamber through a high-efficiency particulate air (HEPA) filter. The air is then moved to the generation area where aerosols are generated and mixed with clean air. Next, air is moved to the sampling area where experiments are conducted. Finally, aerosol-laden air is passed through a second HEPA filter before being pushed outside of the building by the exhaust fan (Figure 2.2). The airflow rate through the chamber was set at 9.9 L/sec (594 L/min) to simulate nearly still air.\(^{(29)}\) At this flow rate, air stream velocity was below the limit of detection of a conventional thermo-anemometer. The average air stream velocity in the chamber was calculated to be approximately 0.016 m/s, based on the airflow rate and cross-sectional area. There was negligible sampling error from particle settling velocity and particle inertia while sampling in still or nearly still air based on criteria established by Davies.\(^{(30)}\)
Section 1: Air inlet and prefilter

Section 2: HEPA filter to remove any outside contaminants

Section 3: Pipe inlet from nebulizer (encased in polycarbonate housing) where aerosol mix with clean air

Sections 4, 5: Sampling areas

HEPA filter to remove remaining aerosols and exhaust fan in the rear

Penetrations into sampling areas (for utilities and tubing)

Figure 2.2: Bioaerosol chamber description of sections

16
The nebulizer, encased in air-tight polycarbonate housing, was attached to an inlet on the bioaerosol chamber (Figure 2.3). Once sealed, the housing protected researchers from possible exposure during aerosolization. The neck of the nebulizer was firmly fitted into the cored opening of a rubber stopper, and placed into the 25 mm diameter inlet tubing to the chamber. The air flow and pressure to nebulizer were adjusted to generate an aerosol of predominantly single bacterial cells at the selected chamber flow rate of 9.9 L/sec.\(^{(9)}\)

**Figure 2.3:** Placement of manikin head forms in bioaerosol chamber sections 3 through 5

2.3.2 RPE assessment

An AGI-30 sampler was connected to the “test” manikin head form wearing RPE, and another AGI-30 sampler was connected to the “control” manikin head form without RPE. Both manikin head forms were placed in the testing area of the bioaerosol chamber as shown in Figure
2.3. The AGI-30 sampler was fitted to the inside of the manikin head form’s mouth in a manner similar to the nebulizer’s attachment to the chamber inlet. A cored rubber stopper fixed to the opening of the sampler “neck” ensured a tight seal between the sampler neck and the mouth of the manikin head form. This design was used to simulate the use of an RPE, by an HCP, as protection from exposure to airborne vegetative cells.

Compressed air to the nebulizer was turned on, and the nebulizer was run for 8 minutes to achieve a uniform bacterial distribution and a steady state condition in the chamber. The average time for a particle to travel from the nebulizer to the manikin head form was estimated to be 2.6 minutes, based on an estimated air velocity of 0.016 m/s and a travel distance of 2.5 meters. This value was increased to 8 minutes to ensure a steady state. The ABS attached to the AGI-30 samplers was turned on and samples collected for 15 minutes, similar to the procedure described in Jensen et. al. After the ABS and compressed air flow to the nebulizer were turned off, the chamber exhaust fan was left on for an additional 8 minutes to remove any remaining airborne microorganisms from the chamber testing area.

2.3.3 Biological analysis

To quantify the bacterial CFUs in the AGI-30 samplers, 0.1 mL of PBW suspension was transferred to a Tryptic Soy Agar (TSA) plate for a heterotrophic plate count. Membrane filtration with 1.0 mL aliquots and 0.45 µm pore size with a 47mm diameter filter (Fisherbrand Water-Testing Membrane Filters, Fisher Research, Pittsburgh PA) was used to concentrate the AGI-30 solution when necessary. Three test and three control plates were obtained for each run. The plates were grown and enumerated using standard methods.

The relative efficiency of each RPE was calculated. RPE relative efficiency is the ratio of the AGI-30 concentration (CFU/L) of the test manikin head form with RPE to the AGI-30
concentration for the control manikin head form without RPE. There were five replicates for each of the 11 RPE models. Each replicate consisted of the mean of three test plates divided by three control plates which equates to one data point. The data set for this analysis consisted of 55 relative efficiency ratios. Each data point was calculated by dividing the average AGI-30 collection medium concentration from the test manikin head form (in triplicate from each of five replicates for all 11 RPE models) by the average of the corresponding AGI-30 collection medium concentration from the control manikin head form.

2.3.4 Statistical analysis

The dependent variable (% relative efficiency) was plotted against two possible independent variables (relative humidity and temperature). The plots were checked for any patterns suggestive of a nonlinear trend. Independent variables were then compared together to the dependent variable using multiple regression. The multiple linear regression analysis was performed using Analyze-it® for Microsoft Excel®.\(^{(32)}\)

A power calculation of the minimum number of runs needed to detect statistically significant differences between the RPE models and Shapiro-Wilk test to confirm a normal distribution of the relative efficiencies were performed using Statistical Analysis Software (SAS) (PROC GLMPOWER and PROC UNIVARIATE, respectively).\(^{(33)}\) To detect any variability in efficiencies between RPE models, and within the trials for each RPE model, a one-way analysis of variance (ANOVA) test was also performed using SAS (PROC GLM).\(^{(33)}\)

Efficiencies of the RPE models were further evaluated using an ANOVA pair-wise analysis (PROC GLM).\(^{(33)}\) In this statistical analysis, each RPE model was compared to every other RPE model to determine whether significant differences exist between their relative efficiency ratios. Since the results of a multiple comparison of this type can be highly influenced
by random error, a fairly conservative error protection (Tukey’s method) was applied to the input instructions for the one-way ANOVA pair-wise test.\(^{(34)}\)

### 2.3.5 Quality control

The bioaerosol chamber was tested using selected procedures from NSF-49 for the certification of Class II biological safety cabinets (BSCs), including supply air velocity, exhaust air velocity, supply HEPA filter leak test (<0.01%), and exhaust HEPA filter leak test (<0.01%) (Agape instruments, Cincinnati OH).\(^{(35)}\)

Prior to the nebulizer being turned on, a sample was taken with the AGI-30 to ensure no environmental bacteria were leaking into the chamber and bypassing the HEPA filter. After the final test each day, another sample was taken to ensure no residual bacteria remained in the chamber. Media blanks were conducted during each experiment to confirm proper media preparation and handling.

Since work was performed with a Biosafety Level 2 microorganism, all investigators wore examination gloves whenever handling potentially infectious material. To minimize potential exposure to aerosols, safety goggles and N95 respirators were worn whenever investigators worked inside the bioaerosol chamber. All inoculations and transfers were performed in a certified Class II BSC to contain splatters and aerosols and to minimize contamination of the samples or stock solutions.\(^{(36)}\)

### 2.4 Results

Airflow from the nebulizer was measured as 10.4 L/min while velocity was measured as 1.9 m/sec out of the nebulizer. According to the ARTI HHPC-6, initial chamber aerosol concentration had a CMD of 0.27, an MMD of 0.78 µm and a GSD of 1.82. According to the Andersen 6-stage impactor data, initial culturable bioaerosol had a count median aerodynamic...
diameter (CMAD) of 1.2, a mass median aerodynamic diameter (MMAD) of 2.1 and a GSD of 1.5. After 150 minutes chamber aerosol concentration had a CMD of 0.30, a MMD of 1.3 and GSD of 2.0. Culturable bioaerosol had a CMAD of 1.0, MMAD of 1.5 and GSD of 1.5.

Microscopic analysis of the vegetative cells from the nebulizer suspension indicated vegetative cell diameters ranging from approximately 0.8 to 1.0 µm and lengths reaching 3.0 µm.

Measurements obtained using the ARTI HHPC-6 confirmed the Balston Type 100-18-DXE filter removed 98% of all particles ≥0.3 µm and 99% of all particles ≥1.0 µm.

During nebulization, chamber culturable bioaerosol concentration decreased while chamber particle concentration increased. After 150 minutes of nebulization, the initial 100 mL aliquot in the nebulizer was reduced to 50 mL. Over the course of 150 minutes, the suspension concentration in the nebulizer dropped from an initial $6.9 \times 10^7$ CFU/mL to $3.9 \times 10^7$ CFU/mL (a 43% reduction). Simultaneously, chamber particle concentration increased over the same 150-minute period from $3.5 \times 10^5$ particles/L of air to $4.5 \times 10^5$ particles/L of air (a 29% increase). Culturable bioaerosol concentration decreased over the 150-minute time period from 600 CFU/L to 440 CFU/L (a 27% decrease). Figure 2.4 shows the reduction in chamber culturable bioaerosol concentration as well as the increase in chamber particle concentration.
Figure 2.4: Comparison of chamber culturable bioaerosol/L to chamber particle concentration/L ≥ 3 µm

The AGI-30 samplers attached to the 12.5 L/min constant flow pump collected 172 CFU/L of air, while the AGI-30 samplers attached to the variable flow ABS collected 163 CFU/L of air (a 6% reduction). Based on the t statistic (α=0.05, p=0.2666) there was no significant difference between sampling with the variable flow ABS and the 12.5 L/min constant flow pump. Maximum flow (Q_{max}) for the ABS reached 0.3 L/sec (18 L/min), greater than the 12.5 L/min limit set by the critical orifice of the AGI-30. Average flow (Q_{average}) during inhalation or exhalation for the ABS was below 0.2 L/sec (12 L/min).

Using AGI-30 samplers, no culturable microorganisms were found inside the chamber before the nebulizer was turned on. After 8 minutes with the nebulizer on, the chamber reached
a steady state of approximately 600 CFU/L. After 8 minutes with the nebulizer off, the air inside the chamber contained no airborne culturable microorganisms.

Using the ARTI HHPC-6, the air inside the chamber prior to nebulization contained an average of 46 particles/L ranging in size from 0.3 µm in size to 0.7 µm, and no detectable particles 1.0 µm or greater in size. After 8 minutes with the nebulizer on, the air inside the bioaerosol chamber reached a concentration of $4.9 \times 10^5$ particles/L ($2.6 \times 10^4$ particles $\geq 1.0$ µm). After 8 minutes with the nebulizer off, the air inside the chamber contained an average of 63 particles/L ranging in size from 0.3 µm to 0.7 µm, and no detectable particles 1.0 µm or greater in size.

We tested for normal distribution and could not reject equality of variance using the Shapiro-Wilk test ($p = 0.1633$). Based on this test there was no transformation of the original data. The within RPE model standard deviation was determined to be 7% while the between RPE standard deviation was 5%. We tested for equal variance and could not reject equality of variance using Levene’s test ($p = 0.20$). For this investigation with 11 different RPE models, a sample size of 3 had a power of 0.60 while a sample size of 5 had a power of 0.92. These results showed that a sample size of 5 for each of the 11 RPE models allowed for the detection of a minimum statistically significant difference of 12% ($\alpha = 0.05$). The results of the multiple linear regression analysis did not show any significant influence on relative efficiency by either slight variations in indoor temperature readings (18°C to 23°C) or moderate relative humidity fluctuations (25% to 58% RH).

Seven out of eleven RPE models (3M models 9210 and 1818, Kimberly Clark models 48201, 48208 and PFR95, Medline models NON27501 and Moldex model 2200) showed relative efficiency above 60%, with the Medline model NON27501 surgical mask and Moldex
model 2200 N95 respirator showing the highest efficiencies at 69%. The least efficient RPE was the Medline NON27402 surgical mask with a relative efficiency of 34% (Table 2.2). Table 2.2 also shows the 5th percentile SWPF value for each RPE. Figure 2.5 compares the relative efficiencies of the eleven RPE models with 95% confidence interval (CI).
Table 2.2: Summary of relative efficiencies of 11 RPE models in stopping non-pathogenic *Bacillus anthracis* strain Sterne 34F2 vegetative cells

<table>
<thead>
<tr>
<th></th>
<th>Moldex 2200</th>
<th>Medline NON27501</th>
<th>3M 9210</th>
<th>3M 1818FS</th>
<th>Kimberly Clark 48201</th>
<th>3M 1818</th>
<th>3M 1860</th>
<th>3M 1860s</th>
<th>Kimberly Clark PFR95</th>
<th>Kimberly Clark 48208</th>
<th>Medline NON27402</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>Mean</td>
<td>1782</td>
<td>1647</td>
<td>928</td>
<td>99</td>
<td>170</td>
<td>86</td>
<td>48</td>
<td>71</td>
<td>24</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td>175</td>
<td>198</td>
<td>88</td>
<td>147</td>
<td>6</td>
<td>18</td>
<td>13</td>
<td>7</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>558</td>
<td>506</td>
<td>317</td>
<td>777</td>
<td>33</td>
<td>64</td>
<td>38</td>
<td>31</td>
<td>27</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td>75</td>
<td>84</td>
<td>59</td>
<td>76</td>
<td>3</td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>% Efficiency</td>
<td></td>
<td>69%</td>
<td>69%</td>
<td>66%</td>
<td>52%</td>
<td>67%</td>
<td>63%</td>
<td>56%</td>
<td>35%</td>
<td>62%</td>
<td>62%</td>
</tr>
<tr>
<td>5th % SWPF</td>
<td></td>
<td>3.7</td>
<td>4.0</td>
<td>3.4</td>
<td>2.6</td>
<td>3.2</td>
<td>2.9</td>
<td>3.0</td>
<td>1.7</td>
<td>2.9</td>
<td>3.0</td>
</tr>
</tbody>
</table>


Figure 2.5. Comparison of the relative efficiencies of the eleven RPE models.*

* Error bars denote 95% confidence interval about the mean

The ANOVA pair-wise analysis using Tukey’s method for multiple comparisons allowed for a partitioning of the 11 models into four groups based on relative efficiency. Both N95 respirators and surgical masks were found in all four categories. Table 2.3 shows the mean relative efficiency and standard deviation of the four groups.
### Table 2.3: ANOVA pair-wise analysis groupings

<table>
<thead>
<tr>
<th>Group</th>
<th>RPE</th>
<th>Mean Relative Efficiency</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Moldex 2200</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medline 27501</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3M9210</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>Kimberly Clark 48201</td>
<td>65%</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>3M 1818</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kimberly Clark PFR95</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kimberly Clark 48208</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3M9210</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kimberly Clark 48201</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3M 1818</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kimberly Clark PFR95</td>
<td>63%</td>
<td>4%</td>
</tr>
<tr>
<td></td>
<td>Kimberly Clark 48208</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3M 1860</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>3M 1818</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kimberly Clark PFR95</td>
<td>59%</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>Kimberly Clark 48208</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3M 1860</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>3M 1818 F/S</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>Medline NON27402</td>
<td>35%</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>3M1860s</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Several RPE models in groups A, B, and C are in multiple groups, while the Medline NON27402 and 3M 1860s, the two with the lowest relative efficiencies, are the only models in group D and are in no other groups. Each model in group A, B, C and D can be considered similar to all other models within the same group even if the model is in multiple groups. The two models in group D are statistically different from all other RPE models while not being significantly different from each other.
2.5 Discussion

A bioaerosol of non-pathogenic *B. anthracis* strain Sterne 34F2 vegetative cells was used in a unique experimental simulation of normally-respiring HCP wearing RPE. Some of the models of surgical masks tested had relative efficiencies greater than or equal to those of some N95 or hybrid respirators. The methods and experimental designs used previously to quantify RPE efficiency differ from ours.\(^{(4,5,6)}\) The test apparatus utilized by Wake et. al.\(^{(5)}\) quantified the penetration of bacterial aerosol and monodisperse urea aerosol when passed through RPE filter media in a continuous flow air stream rather than in a simulation of human breathing. Sampling and subsequent enumeration of the penetrated bacteria was achieved through the use of AGI-30 samplers like those used for this investigation. Qian et. al.\(^{(6)}\) also quantified the penetration of bacterial aerosol as well as NaCl aerosol and monodisperse inert latex particles when passed through RPE filter media by sealing the RPE to an anthropometric manikin head form. The investigation measured filtration efficiency by using a laser aerosol size spectrometer and aerodynamic size spectrometer instead of AGI-30 samplers.

Rather than a continuous flow air stream for sampling we utilized an ABS. This system allowed for simulation of RPE slippage from inhalation and exhalation by the manikin head form, providing a more accurate representation of RPE relative efficiency. The data from the AGI-30 air samplers show that while the ABS draws less air per minute than the 12.5 L/min constant flow, comparable numbers in CFU/ L of air sampled make the AGI-30 attached to the ABS feasible as an inhalation sampling device. The ABS briefly exceeded the \(Q_{\text{max}}\) as controlled by the critical orifice of the AGI-30. An estimated 1.2 L from the original draw of 10L/min was lost due to the critical orifice. This drop of flow rates from 10 L/min vs. 8.8 L/min (a 12% reduction in volume of air samples) is suggested as the reason we observed a 6% lower CFU/L.
between AGI-30 samplers attached to the 12.5 L/min constant flow and those attached to the ABS. While exceeding $Q_{\text{max}}$ is a limitation with the procedure, the ABS should still be considered an acceptable approximation of human breath rate.

The methods utilized by Johnson et. al.\(^{(4)}\) more closely resemble those used in this investigation. Johnson et. al.\(^{(4)}\) also made use of anthropometric head forms, an aerosol testing chamber, and an ABS to mimic respiratory parameters (breath rate, TV, and MRV), in an effort to simulate a normal RPE usage scenario. The investigation by Johnson et. al.\(^{(4)}\) used a sampling flow rate of 6 L/min for a different AGI sampler adjacent to the ABS. The goal of our investigation was to demonstrate the feasibility of an experimental design in which an ABS is connected to AGI-30 samplers.

This investigation’s measured nebulizer output for chamber particle concentration (CMD=0.27 µm, MMD=0.78 µm, GSD=1.82 µm) and for culturable bioaerosol (CMAD=1.2 µm, MMAD=2.1 µm, GSD=1.5 µm) differs significantly from previously reported results of non-biological aerosols. Gussman reported that the BGI CN-25 Collison nebulizer with the large bottle generates dioctylphthalate (DOP) droplets with MMD of 2.90 and 3.00 µm with a GSD of 3.17 and 3.33.\(^{(23)}\) The difference in the results of Collison nebulizer particle distributions achieved by Gussman\(^{(23)}\) with those found in this investigation, can be explained by considering the following factors: type of aerosol, sampling device, and experimental design. First, the type of aerosol Gussman produced was non-volatile DOP.\(^{(23)}\) The bioaerosol in this investigation was produced from culturable bacteria suspended in volatile PBW. May\(^{(37)}\) reported a 10 µm water droplet in air at 20°C and 80% RH to have a wet lifetime of 0.6 sec, while Jensen et. al.\(^{(9)}\) reported that a 2.9 µm water droplet had a wet lifetime of 0.03 sec. Second, the analytical device used by Gussman\(^{(23)}\) to characterize the aerosol was a May / Research Engineers’ Cascade
Impactor (MRE) (BGI, Inc., Waltham, MA). The MRE has seven stages that sample 30, 16, 8, 4, 2, 1, 0.5 µm respectively.\(^{(37)}\) This investigation made use of the ARTI HHPC-6. Lastly, Gussman\(^{(23)}\) sampled the aerosol he produced by placing the MRE 0.025 m from the outlet of the nebulizer and sampling for a 10 second period. For this investigation, the bioaerosol was sampled in 1-L aliquots by the ARTI HHPC-6 approximately 0.75 m away from the outlet of the nebulizer. Overall, the data show the feasibility of utilizing the nebulizer to create a culturable single-celled bioaerosol from a monoculture with a CMD of 0.27 µm and a GSD of 1.82.

The reduction of nebulizer suspension volume, nebulizer suspension concentration, and nebulizer-generated bioaerosol over time changed both the bioaerosol concentration within the chamber and the amount of bioaerosol collected. The increase in the number of particles 0.3 µm or greater in size, as well as the decrease in culturable bioaerosol concentration, may be attributed to lysed cells or cell fragments being generated as the bacterial suspension was physically stressed by the Collison nebulizer. Microscopic analysis confirmed an increase in cell fragments over time. Terzieva et. al.\(^{(38)}\) microscopically confirmed increases in bacterial cell fragments after prolonged nebulization (t=60 min).

The head form dimensions of the Brad™ model fall within the range of the dimensions included in the conventional respirator fit test panel developed by Los Alamos National Laboratory.\(^{(39)}\) Thus, according to the fit test panel the Brad™ represents approximately 20% of the test panel. Measurements of the characteristic dimensions used in respirator fit testing discussed by Harris\(^{(40)}\) were taken of the head form and are listed in Figure 2.6, showing where they fit on the Hyatt panel.\(^{(39)}\) More recently, Zhuang et. al.\(^{(41)}\) developed an anthropometric database detailing the face size distributions of RPE users in the United States using both traditional measurement methods and three-dimensional scanning systems taking into account
user age, race, and gender. Table 2.4 compares the Brad™ head form to the database developed by Zhuang et. al.\textsuperscript{(40)} It also shows where the Brad™ anthropometric features fit by gender compared to Zhuang et. al.’s sample population.\textsuperscript{(41)} The mean face length on the Hyatt panel was measured at 114 mm while Zhuang measured a mean face length (Menton-Sellion length) of 123 mm for males and 113 mm for females.\textsuperscript{(39,41)} The Brad™ face length was measured at 114 mm. The median lip length on the Hyatt panel was measured at 48 mm while Zhuang measured a median lip length of 51 mm for males and 48 mm for females.\textsuperscript{(39,41)} The Brad lip length was measured at 46 mm.

**Figure 2.6.** Simulaid® Brad™ Manikin head form comparison with Los Alamos National Laboratory panel for testing half-mask respirators\textsuperscript{(39)}

<table>
<thead>
<tr>
<th>Lip Length (mm)</th>
<th>34.5</th>
<th>43.5</th>
<th>52.5</th>
<th>61.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>133.5</td>
<td>Box 9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>123.5</td>
<td>Box 6</td>
<td>Box 7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>113.5</td>
<td>Box 3</td>
<td>Box 4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>103.5</td>
<td>Box 1</td>
<td>Box 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>93.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Manikin measurements:
- Lip length 46 mm,
- Face length 122 mm.
(Fits in Box 7)
Table 2.4: Simulaids® Brad™ Manikin head form measurement comparison

<table>
<thead>
<tr>
<th>Dimension</th>
<th>Description</th>
<th>Brad head form measurement (mm)</th>
<th>Male/Female average(^{(41)})</th>
<th>Male/Female percentile(^{(41)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bigonial breadth</td>
<td>Straight-line distance measured with a spreading caliper between the right and left Gonion landmarks on the corners of the jaw.</td>
<td>105</td>
<td>120 / 110</td>
<td>5 / 30</td>
</tr>
<tr>
<td>Bizygomatic breadth</td>
<td>Maximum horizontal breadth of the face as measured with a spreading caliper between the zygomatic arches.</td>
<td>115</td>
<td>144 / 135</td>
<td>&lt; 1 / &lt; 1</td>
</tr>
<tr>
<td>Head breadth</td>
<td>Maximum horizontal breadth of the head as measured with a spreading caliper above the level of the ears.</td>
<td>159</td>
<td>153 / 147</td>
<td>80-90 / 98</td>
</tr>
<tr>
<td>Interpupillary distance</td>
<td>Distance as measured with a pupillometer at the center of the right and the center of the left pupil.</td>
<td>68</td>
<td>65 / 62</td>
<td>80 / 95</td>
</tr>
<tr>
<td>Menton-Sellion length</td>
<td>Distance as measured with a sliding caliper in the midsagittal plane between the Menton landmark and the Sellion landmark.</td>
<td>114</td>
<td>123 / 113</td>
<td>10 / 40-50</td>
</tr>
<tr>
<td>Minimum frontal breadth</td>
<td>Straight-line distance as measured with a spreading caliper between the right and left Frontotemporale landmarks.</td>
<td>109</td>
<td>106 / 103</td>
<td>70 / 80-90</td>
</tr>
<tr>
<td>Nasal root breadth</td>
<td>Horizontal breadth of nose as measured with a sliding caliper at the Sellion landmark and a depth equal to one-half the distance from the bridge of the nose to the eyes.</td>
<td>20</td>
<td>17 / 16</td>
<td>95 / 95</td>
</tr>
<tr>
<td>Nose breadth</td>
<td>Straight-line distance as measured with a sliding caliper between the right and left Alare landmarks.</td>
<td>27</td>
<td>37 / 33</td>
<td>&lt; 1 / 2</td>
</tr>
<tr>
<td>Nose protrusion</td>
<td>Straight-line distance as measured with a sliding caliper between the Pronasale landmark and the Subnasale landmark.</td>
<td>25</td>
<td>21 / 20</td>
<td>90 / 95*</td>
</tr>
<tr>
<td>Subnasale-Sellion length</td>
<td>Straight-line distance as measured with a sliding caliper between the Subnasale landmark and the Sellion landmark.</td>
<td>49</td>
<td>52 / 48</td>
<td>25 / 60</td>
</tr>
<tr>
<td>Lip Length</td>
<td>Straight-line distance as measured with a sliding caliper using the Pronasale landmark</td>
<td>46</td>
<td>51 / 48</td>
<td>10 / 30*</td>
</tr>
</tbody>
</table>

*With the exception of lip length and nose protrusion, all other measurements were significantly different between genders based on t-test (p < 0.05).
The 5\textsuperscript{th} percentile SWPF values ranged from 1.7 (3M 1860s) to 4.0 (Medline NON27501) while the average for all 11 RPE models tested was 2.8. Coffey et al.\textsuperscript{(17)} compared 18 models of N95 filtering-facepiece respirators randomly selected from the over 165 commercially available N95s at the time the investigation began. Their investigation found an average 5\textsuperscript{th} percentile SWPF value of 2.9 for all 18 RPE models. Their 5\textsuperscript{th} percentile SWPF values for Moldex 2200 and 2201 models’ were significantly higher than ours (11.4 vs. 3.7). The same is true for their 5\textsuperscript{th} percentile SWPF values for the 3M1860 and 3M1860s models (17.0 vs. 3.0 and 17.0 vs. 1.7, respectively). It should be noted, however, that our investigation treated all RPE models separately while the Coffey investigation only used the RPE which best fit the subject. This distinction stresses the importance of proper fit and its effect on RPE relative efficiency and also supports Zhuang et al.’s conclusions that N95 respirator size had significant impact on respirator fit with only one or two sizes being available.\textsuperscript{(40)}

Of the 11 RPE models tested under these experimental conditions, 3M 1860s and Medline NON27402 were the least efficient with respect to non-pathogenic \textit{B. anthracis} strain Sterne 34F2, vegetative cells, followed by 3M 1818FS.

From the ANOVA pair-wise analysis groupings, there were discernible differences among RPE relative efficacies when challenged with aerosols of non-pathogenic \textit{B. anthracis} Sterne 34F2 vegetative cells. Our investigation was restricted to a limited sample of RPE models attached to one manikin head form challenged by one microorganism. Future studies are needed to determine if differences exist among the RPE models when challenged with different bioaerosols and used on different head forms.
2.6 Conclusions

We described a method for assessing the relative efficiencies of a sample of RPE using a bioaerosol chamber, Collison nebulizer, manikin head form, and ABS. The method may also be modified for testing RPE with different types of bioaerosol (endospores, viruses, fungi, endotoxins, etc.).

This method showed that a Collison nebulizer could generate monodisperse bacterial aerosol from a monoculture to effectively test RPE total inward leakage. The Brad™ model manikin head form seemed to be a reasonable surrogate for HCP. For future research, the relative efficiency of RPE should be assessed using other anthropometric manikin head forms of sizes and shapes that fit on the Hyatt or other panels.\(^{(38,40)}\) Also, this method should be correlated with quantitative fit-testing methods and may be used to measure differences in relative efficiencies of RPE. For future research, the ABS could be modified to more closely mimic normal human breath rate.

Neither RPE type (i.e., N95, surgical mask or hybrid) nor brand (i.e., 3M, Moldex, Kimberly Clark, or Medline) was an indicator of RPE relative efficiency. Future studies should be conducted to assess selected filter media type, manikin head forms with different anthropometric characteristics, and most important, each RPE’s ability to be fitted to the HCP’s face. In addition, such studies could include challenges with different bioaerosol types, the effect of RPE re-use and of different breath rates on RPE relative efficiency. Our method described in this study may be helpful in selection of more efficient and better fitting RPE.
2.7 References


Chapter 3:
Performance testing of selected N95 respirators and surgical masks when challenged with airborne, non-pathogenic Bacillus anthracis strain Sterne 34F2 endospores and inert particles
3.1 Abstract

This is the second in a series of articles that present results from work to develop, evaluate, and apply a method aimed at quantifying relative efficiencies of N95 respirators and surgical masks. The first article details the methodology (i.e. bioaerosol generation, respiratory protective equipment [RPE] assessment, biological and statistical analysis, and quality control), and presents results validating the procedure along with relative efficiency data from experiments with challenge aerosols of non-pathogenic *Bacillus anthracis* Sterne 34F2 vegetative cells.\(^{(1)}\) Presented here are results from experiments using aerosols of non-pathogenic *Bacillus anthracis* Sterne 34F2 endospores and from replicates of the experiments with inert aerosols comprised of polystyrene latex (PSL).

This results of these experiments validates earlier work by Davidson et al., including a procedure for bioaerosol generation, recovery using a horizontal bioaerosol chamber, along with the appropriate methods for data analysis.\(^{(1)}\) The chamber used is a stainless steel 0.56 m\(^2\) cross-sectional area duct approximately 3.7 m long that was constructed as a secure environment for bioaerosol testing and evaluation. As in the previous study, we evaluated five surgical masks, three N95 respirators, and three surgical mask/N95 respirator hybrids. Non-encapsulated, viable *Bacillus anthracis* strain Sterne 34F2 endospores were used as a surrogate for pathogenic *Bacillus anthracis*. AGI-30 samplers attached to an automated breathing simulator (ABS) were used to collect bioaerosol samples. Six different polystyrene latex (PSL) suspensions, containing particles with mean diameters of 0.1, 0.43, 0.6, 1.3, 3.2, and 8.0\(\mu\)m were used to create a PSL challenge solution. The ARTI Hand Held Particle Counter (HHPC-6, HACH Ultra Analytics, Houston, TX) was used to measure total particle concentration.
Of the eleven RPE models, the 3M 1818 surgical mask, Moldex 2200 N95 respirator, and 3M 9210 N95 respirator demonstrated mean relative efficiencies greater than 60% against the challenge bioaerosol (65%, 62%, and 62%, respectively). The least efficient RPE model was the Medline NON27402 surgical mask with a relative efficiency of 34%. For the PSL experiments, the Kimberly Clark 18201 surgical mask, which demonstrated a mean relative efficiency of 64% was the only RPE model greater than 60%. Using a viable, practical approach to determining relative efficiency of RPE used by healthcare personnel (HCP), differences in mean relative efficiencies of 11 RPE models were shown for experiments conducted with a challenge bioaerosol of non-pathogenic *B. anthracis* Sterne 34F2 endospores and an aerosol comprised of PSL spheres with known particle size characteristics.

The results also further validate the use of a bioaerosol chamber and ABS to assess relative efficiencies between RPE models worn by HCP. Results were supplemented by use of bioaerosol challenges comprised of non-pathogenic *B. anthracis* Sterne 34F2 endospores and PSL. Certain RPE were shown to perform better than other models. Results from these experiments may, therefore, be taken into consideration during selection of RPE for protection against airborne infectious agents.
3.2 Introduction

This is the second in a series of articles that present results from work to develop, evaluate, and apply a method aimed at quantifying relative efficiencies of N95 respirators and surgical masks. The first article details the methodology (i.e. bioaerosol generation, respiratory protective equipment [RPE] assessment, biological and statistical analysis, and quality control), and presents results validating the procedure along with relative efficiency data from experiments with challenge aerosols of non-pathogenic Bacillus anthracis Sterne 34F2 vegetative cells.\(^{(1)}\) Presented here are results from experiments using aerosols of non-pathogenic Bacillus anthracis Sterne 34F2 endospores and from inert aerosols comprised of polystyrene latex (PSL). Specifically, tests were conducted to determine relative efficiencies of the respiratory protective equipment (RPE) in an experiment to simulate healthcare personnel (HCP) exposure to airborne B. anthracis endospores.

Recent efforts have focused on characterizing the protection afforded to workers wearing RPE. Examples include Weber et al. which evaluated the protection afforded by eight different surgical masks and a dust/mist fume respirator against aerosol particles in the sub-micrometer and micrometer size range.\(^{(2)}\) Both face seal leakage and filter penetration were measured with a corn oil challenge aerosol by conducting experiments with the mask or respirator sealed and unsealed to mannequins. Weber et al. simulated penetration through face seal leaks at different diameters and determined that the difference in penetration increased with particle size. It was concluded that surgical masks might provide insufficient protection to HCP working in environments containing aerosols with particles in this size range. Chen et al. used aerosols of Mycobacterium
cheloneae (surrogate for Mycobacterium tuberculosis) and PSL in a study to determine efficiency of selected RPE when completely sealed to a sampling plate.\textsuperscript{(3)} By taking upstream and downstream samples, mean efficiencies of a surgical mask, particulate respirator, dust/mist respirator, dust/welding fume respirator, and a high-efficiency particulate air (HEPA) respirator were determined to be between 97\% (surgical mask) and 99.99\% (HEPA respirator). Additional results from other similar studies, also using both biological and inert challenge aerosols, are reviewed in detail by Davidson et al.\textsuperscript{(1,4-6)}

It is commonly understood that surgical masks are not intended for use as a primary means of respiratory protection. However, as suggested by the SARS outbreak of 2003, pandemics of airborne infections may lead to severe shortages of respirators and necessitate the use of surgical masks.\textsuperscript{(7)} Economic factors in developing areas of the world can further exacerbate this scenario. Materials published by the Regional Offices for Southeast Asia and the Western Pacific of the World Health Organization (WHO) make specific recommendations for use of surgical masks by HCP when working within 1-2 meters of a patient infected with SARS.\textsuperscript{(8)} Similarly, a SARS fact sheet on the Centers for Disease Control and Prevention (CDC) website recommends use of surgical masks for protection against airborne droplets greater than 5 µm.\textsuperscript{(9)} Thus, the utility of assessing the protection afforded by both surgical masks and respirators is evident. Results presented here expand on those previously collected by Davidson et al. during the method development and validation phases of the study, and by those from the aforementioned studies evaluating RPE against biological and inert challenge aerosols.\textsuperscript{(1)} Methods of RPE testing described by Davidson et al. were conducted to determine whether differences could be detected in relative efficiencies of the RPE when challenged
with a different bioaerosol and an inert aerosol containing particles in a known size range. (1) By introducing the application of an automated breathing simulator (ABS) connected to a bioaerosol sampler, the use of this method allows for an accurate determination of the amount of protection HCP can expect to receive when properly wearing an RPE. Therefore, when considered along with and in the context of previous studies, the results presented in this article will be valuable to HCP in making decisions about wearing various types of RPE.

3.3 Materials and methods

Materials used in the development of the method followed in this study are detailed by Davidson et al. (1) For a thorough discussion on characterization of the performance and output of a Collison nebulizer, AGI-30 sampler bioaerosol collection efficiency when connected to an ABS, evaluation of the bioaerosol chamber parameters (particle counts and bioaerosol samples to test chamber filter integrity; relative humidity and temperature), comprehensive statistical analysis, and quality control measures Davidson et al. can be referred to. (1)

3.3.1 Bioaerosol generation

Viable Bacillus anthracis strain Sterne 34F2 endospores were cultured from Anthrax Spore Vaccine (Colorado Serum Company, Denver, CO) as a surrogate for pathogenic Bacillus anthracis. (10) The aerobic, non-pathogenic B. anthracis Sterne 34F2 cells are gram-positive rods with a diameter of 1.0-1.5 µm, a length of 1-8 µm, and a spore size of approximately 1µm. (11) Sterne 34F2 strain was chosen as the experimental surrogate because of its lack of pathogenicity in humans and its use as a surrogate in previous studies. (12)
Endospore preparation was conducted according to methods adapted from Rose et al.\textsuperscript{13} Spores obtained from the Anthrax Spore Vaccine culture were inoculated into 100 ml of Tryptic Soy Broth (TSB) and incubated for 7 days at 35°C in Erlenmeyer flasks on a rotary shaker. Sporulation was verified microscopically with malachite green stain. The suspension of TSB and spores was centrifuged for 15 min, 3°C at 1000g on a Beckman TH-4 Rotor (Beckman Coulter, Fullerton CA) to form a pellet of bacterial endospores and remove vegetative cell debris. The supernatant of cell debris and TSB was decanted, and the remaining spore pellet was washed with sterile, reverse osmosis water and re-centrifuged. After decanting any additional debris in the supernatant, the final spore pellet was re-suspended in 50% Phosphate Buffered Water (PBW) and stored at 4°C as the stock spore suspension.

Using serial dilution and standardized plate enumerations, aliquots were taken from the stock suspension to confirm a concentration of $10^6$-7 colony-forming units (CFU) per milliliter in the suspension. Adjustments were made by dilution with 50% PBW, if necessary. One-hundred milliliters of the stock spore suspension were prepared for bioaerosol generation. Concentrations of aliquots used in experimentation were also confirmed by serial dilution and standardized plate enumerations.

Test bioaerosol were generated with a modified MRE-type six-jet Collison nebulizer (CN-25 with full-size bottle, BGI Inc., Waltham, MA) operated at a pressure of 140 kilopascals (kPa) Compressed air to the nebulizer was passed through a Balston Type 100-18-DXE filter (Parker Hannifin Corporation, Haverhill, MA). The count median aerodynamic diameter (CMAD), mass median aerodynamic diameter (MMAD) and geometric standard deviation (GSD) of particles produced by the nebulizer were
determined using measurements by the Andersen six-stage impactor (Andersen Instruments, Waltham MA) for culturable bioaerosol. The count median diameter (CMD), mass median diameter (MMD) and GSD were determined using measurements by the ARTI Hand Held Particle Counter (HHPC-6, HACH Ultra Analytics, Houston, TX) for total particle concentration.

### 3.3.2 Bioaerosol collection

Sampling was completed in the bioaerosol chamber utilized by Davidson et al. and previous studies. The chamber is a stainless steel duct approximately 3.7 m in length with a cross-sectional area of 0.56 m². Room air is drawn in through a high-efficiency particulate air (HEPA) filter, moved through the testing area and passed through a second HEPA filter before being exhausted. Flow through the chamber was set at 9.9 L/sec (594 L/min) to simulate nearly still air (air stream velocity ≈ 0.016 m/s, determined from flow and chamber cross-sectional area). This provided for negligible sampling error that normally results from particle settling velocity and particle inertia.

The 11 RPE models assessed by Davidson et al. were evaluated in this study in a randomized order. The RPE evaluated, grouped according to manufacturer, model number, and type (surgical mask, N95, or surgical mask/N95 hybrid), are presented in Table 3.1. CPR training manikin head forms described by Davidson et al. were used as test and control surrogates for HCP (Simulaids® Brad™ CPR torso; Simulaids, Inc. Woodstock, NY).
Table 3.1: RPE evaluated

<table>
<thead>
<tr>
<th>Brand</th>
<th>Model</th>
<th>(Surgical Mask, N95, Hybrid)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3M</td>
<td>9210</td>
<td>N95</td>
</tr>
<tr>
<td>3M</td>
<td>1818FS</td>
<td>Surgical Mask</td>
</tr>
<tr>
<td>3M</td>
<td>1818</td>
<td>Surgical Mask</td>
</tr>
<tr>
<td>3M</td>
<td>1860</td>
<td>Hybrid</td>
</tr>
<tr>
<td>3M</td>
<td>1860s</td>
<td>Hybrid</td>
</tr>
<tr>
<td>Kimberly Clark</td>
<td>48201</td>
<td>Surgical Mask</td>
</tr>
<tr>
<td>Kimberly Clark</td>
<td>PFR95</td>
<td>Hybrid</td>
</tr>
<tr>
<td>Kimberly Clark</td>
<td>48208</td>
<td>Surgical Mask</td>
</tr>
<tr>
<td>Medline</td>
<td>NON27501</td>
<td>N95</td>
</tr>
<tr>
<td>Medline</td>
<td>NON27402</td>
<td>Surgical Mask</td>
</tr>
<tr>
<td>Moldex</td>
<td>2200</td>
<td>N95</td>
</tr>
</tbody>
</table>

Bioaerosol samples were taken with all-glass impinger-30 samplers designed to collect airborne particles with a diameter ranging from 0.8µm to 15.0 µm into 20 mL of a collection fluid (PBW) at a nominal airflow rate of 12.5 L/min (12.3-12.6 L/min, Ace Glass, Inc., 2004) when the vacuum exceeds 55 kPa (41 cm Hg). The AGI-30 sampler was not operated at a constant flow, but regulated by an ABS to approximate human respiratory breathing rate.\(^{(1)}\)

### 3.3.3 PSL aerosol generation and measurement

Six different polymer microsphere suspensions containing particles with mean diameters of 0.1, 0.43, 0.6, 1.3, 3.2, and 8.0µm were used to create a PSL challenge solution. The four suspensions containing microspheres with diameters of 0.1, 0.43, 0.6, and 1.3 µm were comprised of particles made from polystyrene latex, while the two suspensions containing particles with diameters of 3.2 and 8.0µm were comprised of polystyrene divinylbenzene (PSDVB)(General Purpose Latex Particles 5000 and 7000 series, respectively; Duke Scientific Corporation, Palo Alto, CA). These sizes were
chosen to so there would be one particle size covered in each range as measured by the ARTI HHPC-6 which are read from the instrument as ≥0.3, ≥0.5, ≥0.7, ≥1.0, ≥3.0, and ≥5.0µm. One milliliter aliquots of each suspension were diluted by 5:1 with sterile, reverse osmosis water. All six of the diluted suspensions were combined into one challenge solution and adjusted to a final volume of 100 mL. The final suspension was mixed in an ultrasonic water bath (Haver USC 200-78 Ultrasonic Bath, Haver & Boecker, Oelde, Germany) and placed into the same MRE-type six-jet Collison nebulizer used for generation of bioaerosol. PSL concentration was measured from the ARTI HHPC-6. Particle CMD, MMD, and GSD were also calculated for the PSL using these measurements.

3.3.4 Chamber arrangement

A test manikin head form with RPE, and control manikin head form without RPE were placed in the testing area of the bioaerosol chamber.\(^{(1)}\) For bioaerosol experiments, an AGI-30 sampler was fitted behind the mouth opening of each manikin head form. To ensure a complete seal between the sampler and manikin head form, silicone caulk was applied to form a complete seal between the sampler and head form. Compressed air to the nebulizer was turned on and the nebulizer was run for 8 minutes to reach a steady state condition in the chamber. The ABS attached to the AGI-30 samplers was turned on, and samples were taken for fifteen minutes—similar to the procedure described in Jensen et al.\(^{(14)}\) The fan and exhaust were left on for an additional 8 minutes after the ABS and compressed air flow to the nebulizer were turned off.

For PSL experiments, the same experimental design was utilized except for modifications to the test and control manikin head forms in order to allow them to receive
the ARTI HHPC-6 sampling tube near the opening of the fitted AGI-30 samplers. The tubes were connected to the ARTI HHPC-6 outside the chamber where instrument readings were recorded. An alternating sequence of tests, followed by control readings was utilized since only one instrument was available.

3.3.5 Assessment

To quantify the bacterial CFU in the AGI-30 samplers, 0.1 mL of PBW suspension was transferred to a Tryptic Soy Agar (TSA) plate for spread plate enumeration. Membrane filtration, with 1.0 mL aliquots and 0.45 µm pore size with 47mm diameter (Fisherbrand Water-Testing Membrane Filters, Fisher Research, Pittsburgh PA), was used to concentrate the AGI-30 solution when necessary. All test and control plates were grown and enumerated in triplicate using standard methods.\(^{(19)}\)

For both endospore and PSL experiments, the relative efficiency of each RPE was calculated for both endospore and PSL experiments with the following equation:

\[
1 - \frac{\text{test CFU} / L}{\text{control CFU} / L} \times 100\%
\]

Statistical Analysis Software® 9.1.3 (SAS) (SAS® Institute, Cary, NC) was used for statistical analysis of the data. A power calculation of the minimum number of runs needed to detect statistically significant differences between the RPE models was performed based on results of preliminary data.\(^{(1)}\)

For bioaerosol experiments, each of the eleven RPE models was evaluated in five replicates. Individual data points for statistical analysis were calculated as the ratio of AGI-30 CFU of test manikin head form with RPE to control manikin head form without RPE. This model yielded a total sample size of 55 relative efficiency ratios (i.e., 11 RPE
models x 5 trials for each model for each pair of test and control AGI-30 sampler aliquots). Each sample was taken in triplicate. Ratios were log-transformed before further analysis.

For PSL experiments, there were three replicates for each of the eleven RPE models. Each replicate consisted of the mean of five test measurements divided by three control measurements which equates to one data point. Individual data points were calculated from ARTI HHPC-6 measurements as the ratio of all particles $\geq 1 \mu m$ measured from the test manikin head form to all particles $\geq 1 \mu m$ measured from the control manikin head form. This model provided for a total sample size of 33 relative efficiency ratios (i.e., 11 RPE models x 3 trials for each model for each paired test and control reading). Ratios were not log-transformed, but two outliers were removed before further analysis.

Statistical Analysis Software® was used to test for normality and repeated measures analysis of variance (ANOVA). A Shapiro-Wilk test for normality was performed using the univariate procedure (PROC UNIVARIATE). A repeated measures ANOVA was performed with the mixed linear model procedure (PROC MIXED).

The Shapiro-Wilk test, where the dependent variable was the log-transformed ratios for the bioaerosol experiments, and the untransformed ratios for the inert aerosol experiments, was performed to confirm a normal distribution of the relative efficiency data. The repeated measures ANOVA with a pair-wise analysis comparing each mask to all other masks (Tukey’s method for multiple comparisons) was conducted to determine whether significant differences exist between relative efficiency ratios of
different RPE models. As with a usual one-way ANOVA, the assumptions of a normally distributed data set and equality of variances must be met.

For the repeated measures ANOVA, each RPE model was treated as a fixed factor. Trial nested within the RPE models was the random (or subject) factor. Replicate was the repeated factor. Independence was assumed across trials. A compound symmetric covariance structure was assumed. Repeated measures ANOVA was used for analysis as opposed to one-way ANOVA used for previous results. Repeated measures ANOVA more robustly accounts for variations in the data between runs and replicates. In addition, the use of PROC MIXED for the repeated measures ANOVA accommodates the removal of outliers from a data set without imposing limitations on the results of the analysis.

3.4 Results

Since non-pathogenic *B. anthracis* Sterne 34F2 endospores are physiologically different from cells in the vegetative state, data were collected to establish any differences in particle size characteristics of the challenge bioaerosol from findings reported by Davidson et al. Calculations from data collected by the ARTI HHPC-6 showed that initial total particle concentration had an MMD of 1.6 µm, a CMD of 0.25 and a GSD of 2.2 while calculations from samples taken with the Andersen 6-stage impactor showed an initial MMAD of 2.1, a CMAD of 1.1 and a GSD of 1.6 for culturable endospore bioaerosol. Figure 3.1 compares the aerodynamic profile of initial viable bioaerosol comprised of non-pathogenic *B. anthracis* Sterne 34F2 endospores to those comprised of vegetative cells as described by Davidson et al. Microscopic analysis of the endospore stock solution confirmed documented spore sizes of
approximately 1 µm.\textsuperscript{(11)} After 150 minutes of nebulization total particles in the chamber had an MMD of 0.89, a CMD of 0.24 and a GSD of 1.9. Culturable endospore bioaerosol had an MMAD of 1.2, a CMAD of 0.9 and a GSD of 1.4. Since the PSL was comprised of particles of known standard sizes, confirmation of size characteristics through determination of MMD, CMD, and GSD was not necessary.

**Figure 3.1:** Aerodynamic profile of culturable bioaerosol with 95% confidence interval

Nebulizer volume was recorded along with liquid suspension concentration and chamber particle concentrations during 150 minutes of nebulization. Data were collected to verify any differences in endospore liquid suspension concentration and chamber particle concentrations from those reported for the vegetative cell suspension by Davidson et al.\textsuperscript{(1)} The suspension concentration in the nebulizer increased from an initial 2.2 x 10\textsuperscript{7} CFU/mL to a final reading of 3.0 x 10\textsuperscript{7} CFU/mL (a 34% increase) and the
chamber culturable bioaerosol concentrations increased from $7.1 \times 10^3$ CFU/mL to $1.2 \times 10^4$ CFU/mL (a 65% increase). Concurrently, the chamber total particle concentration increased from $1.28 \times 10^5$ particles/L of air to $1.58 \times 10^5$ particles/L of air (a 23% increase). Figure 3.2 summarizes the changes in chamber concentration over time.

**Figure 3.2:** Comparison of chamber culturable endospores/L to chamber particle concentration/L $\geq 3$ µm

As previously mentioned, ratios from the bioaerosol experiments were log-transformed while two ratios from the inert aerosol experiments were removed as outliers before statistical analysis was performed. This was done in order to offset marginally unequal variances in the data. The Shapiro-Wilk test for normality provided an
acceptable p-value greater than 0.05 for both the bio- and inert aerosol experiments (p = 0.9718 and p = 0.9863, respectively).

Of the eleven RPE models, the 3M 1818 surgical mask, Moldex 2200 N95 respirator, and 3M 9210 N95 respirator demonstrated mean relative efficiencies greater than 60% against the challenge bioaerosol (65%, 62%, and 62%, respectively). The least efficient RPE model was the Medline NON27402 surgical mask with a relative efficiency of 34% (Table 3.2). For the inert aerosol experiments, the Kimberly Clark 18201 surgical mask with a mean relative efficiency of 64% was the only RPE model greater than 60% (Table 3.3). Figure 3.3 compares the mean relative efficiencies with 95% confidence intervals (CI) of the eleven RPE models against the bio- and inert aerosol challenges described in this study, along with those from similar experiments conducted with bioaerosol of non-pathogenic *B. anthracis* Sterne 34F2 vegetative cells.¹
### Table 3.2: Summary of results of efficiency of 11 RPE models in stopping non-pathogenic *Bacillus anthracis* strain Sterne 34F2 endospores

<table>
<thead>
<tr>
<th></th>
<th>3M 9210</th>
<th>3M 1818FS</th>
<th>3M 1818</th>
<th>3M 1860</th>
<th>3M 1860s</th>
<th>Kimberly Clark 48201</th>
<th>Kimberly Clark PFR95</th>
<th>Kimberly Clark 48208</th>
<th>Medline NON27501</th>
<th>Medline NON27402</th>
<th>Moldex 2200</th>
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</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>Mean</td>
<td>1699</td>
<td>1483</td>
<td>4367</td>
<td>1434</td>
<td>1562</td>
<td>1619</td>
<td>1594</td>
<td>1665</td>
<td>4213</td>
<td>2231</td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td>109</td>
<td>145</td>
<td>497</td>
<td>69</td>
<td>130</td>
<td>64</td>
<td>126</td>
<td>91</td>
<td>902</td>
<td>167</td>
</tr>
<tr>
<td><strong>Test</strong></td>
<td>Mean</td>
<td>656</td>
<td>711</td>
<td>1529</td>
<td>638</td>
<td>1199</td>
<td>686</td>
<td>1099</td>
<td>753</td>
<td>2016</td>
<td>1211</td>
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<tr>
<td></td>
<td>Std Dev</td>
<td>69</td>
<td>75</td>
<td>63</td>
<td>65</td>
<td>125</td>
<td>76</td>
<td>158</td>
<td>91</td>
<td>247</td>
<td>194</td>
</tr>
<tr>
<td><strong>% Efficiency</strong></td>
<td>61%</td>
<td>53%</td>
<td>65%</td>
<td>55%</td>
<td>22%</td>
<td>58%</td>
<td>30%</td>
<td>55%</td>
<td>49%</td>
<td>45%</td>
<td>62%</td>
</tr>
</tbody>
</table>

*Data shown as untransformed test and control CFU/mL
† Calculated from log-transformed ratios

### Table 3.3: Summary of results of efficiency of 11 RPE models in stopping PSL ≥1 µm

<table>
<thead>
<tr>
<th></th>
<th>3M 9210</th>
<th>3M 1818FS</th>
<th>3M 1818</th>
<th>3M 1860</th>
<th>3M 1860s</th>
<th>Kimberly Clark 48201</th>
<th>Kimberly Clark PFR95</th>
<th>Kimberly Clark 48208</th>
<th>Medline NON27501</th>
<th>Medline NON27402</th>
<th>Moldex 2200</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>Mean</td>
<td>234742</td>
<td>158335</td>
<td>201694</td>
<td>406418</td>
<td>369564</td>
<td>228427</td>
<td>200341</td>
<td>337446</td>
<td>250179</td>
<td>287597</td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td>5306</td>
<td>6201</td>
<td>6502</td>
<td>12461</td>
<td>11944</td>
<td>8053</td>
<td>9329</td>
<td>17260</td>
<td>3182</td>
<td>9104</td>
</tr>
<tr>
<td><strong>Test</strong></td>
<td>Mean</td>
<td>95493</td>
<td>81772</td>
<td>94676</td>
<td>199437</td>
<td>241590</td>
<td>82128</td>
<td>104686</td>
<td>158183</td>
<td>102047</td>
<td>167032</td>
</tr>
<tr>
<td></td>
<td>Std Dev</td>
<td>3994</td>
<td>3640</td>
<td>4651</td>
<td>10216</td>
<td>7433</td>
<td>2413</td>
<td>4949</td>
<td>8870</td>
<td>2927</td>
<td>5190</td>
</tr>
<tr>
<td><strong>% Efficiency</strong></td>
<td>59%</td>
<td>48%</td>
<td>53%</td>
<td>51%</td>
<td>35%</td>
<td>64%</td>
<td>48%</td>
<td>53%</td>
<td>59%</td>
<td>42%</td>
<td>58%</td>
</tr>
</tbody>
</table>

*Data shown as test and control particles
The repeated measures ANOVA of the relative efficiencies demonstrated the effect of RPE type was significant for the 11 models for both bioaerosol and inert aerosol experiments with $p < 0.001$ for both analyses. The pair-wise analysis using Tukey’s method for multiple comparisons allowed for a partitioning of the 11 models into four groups for the bioaerosol experiments, and five groups for inert aerosol experiments based on mean relative efficiency. For the former, Table 3.2 shows the groups along with group mean relative efficiency and standard deviation. Table 3.3 shows the same for the latter. Each model in group A, B, C and D can be considered similar to all other models within the same group even if the model is in multiple groups. As a result of the 3M 1860s showing the lowest mean relative efficiency for both sets of experiments, it can be
considered statistically different from all other RPE models for the inert aerosol experiments, and statistically different from all other models but the Kimberly Clark PFR95 for the bioaerosol experiments. Table 3.4 compares the ANOVA groups for each RPE when challenged with the three different aerosols.

**Table 3.4:** ANOVA pair-wise analysis groupings for aerosol challenge experiments

<table>
<thead>
<tr>
<th></th>
<th><em>Bacillus anthracis</em> vegetative cells(^{(1)})</th>
<th>Sterne 34F2 endospores(^{*})</th>
<th>PSL(^{*})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moldex 2200</td>
<td>A</td>
<td>A</td>
<td>A, B</td>
</tr>
<tr>
<td>Medline 27501</td>
<td>A</td>
<td>A, B</td>
<td>A</td>
</tr>
<tr>
<td>3M9210</td>
<td>A, B</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Kimberly Clark 48201</td>
<td>A, B</td>
<td>A, B</td>
<td>A</td>
</tr>
<tr>
<td>3M 1818</td>
<td>A, B, C</td>
<td>A</td>
<td>B, C</td>
</tr>
<tr>
<td>Kimberly Clark PFR95</td>
<td>A, B, C</td>
<td>C, D</td>
<td>C, D</td>
</tr>
<tr>
<td>Kimberly Clark 48208</td>
<td>A, B, C</td>
<td>A, B</td>
<td>B, C</td>
</tr>
<tr>
<td>3M 1860</td>
<td>B, C</td>
<td>A, B</td>
<td>C</td>
</tr>
<tr>
<td>3M 1818 F/S</td>
<td>C</td>
<td>A, B</td>
<td>C</td>
</tr>
<tr>
<td>Medline NON27402</td>
<td>D</td>
<td>B, C</td>
<td>D</td>
</tr>
<tr>
<td>3M1860s</td>
<td>D</td>
<td>D</td>
<td>E</td>
</tr>
</tbody>
</table>

\(^{*}\)Repeated Measures Analysis

### 3.5 Discussion

Using a viable, practical approach to determining relative efficiency of RPE used by HCP, differences in mean relative efficiencies of 11 RPE models were shown for experiments conducted with a challenge bioaerosol of non-pathogenic *B. anthracis* Sterne 34F2 endospores and an aerosol comprised of inert particles with known particle size characteristics. These results expand on and support those collected from previous experiments during validation of the method.\(^{(1)}\)

As described by Davidson et al., the method used in this study differs from methodologies applied in earlier work with similar objectives, as well as those previously
discussed in this paper.\textsuperscript{(1-6)} The results from Weber et al. can be considered comparable to the results presented in this article. Because, in part, an inert aerosol with similar particle size characteristics was used to determine total inward leakage, due to filter penetration and face seal leakage, out of a sample of surgical masks and one type of respirator when placed on a surrogate for HCP.\textsuperscript{(2)} However, implementation of an ABS to more closely approximate inward air flow through the filter material and face seal from a normally-respiring human was not used in the previously mentioned study. Moreover, Chen et al. also sampled with a constant flow apparatus, but only measured efficiency due to filter penetration, not accounting for face seal leakage.\textsuperscript{(3)} In this study, mean relative efficiencies were determined for both bio- and inert aerosol challenges produced by the same aerosol generator.

Particle size characteristics of the challenge aerosol produced by the nebulizer used in this study that differ from those previously documented have been described and accounted for.\textsuperscript{(1,22)} Even though the same organism was used to produce the challenge bioaerosol, it was to be expected that differences would be found in the particle size characteristics of the bioaerosol used in this study compared to the one used in experiments following the same method. Disparities can be accounted for by considering that a different physiological form of the organism was used with different particle size distribution.

The reduction of nebulizer suspension volume was in accordance with previous results using a similar suspension fluid.\textsuperscript{(1)} The increase in nebulizer suspension concentration along with corresponding increase in nebulizer-generated culturable bioaerosol and total particle concentration over time was, however, in contrast to
previously reported results. Explanation for this disparity can likely be accounted for by considering the use of endospores rather than vegetative cells in producing the bioaerosol. Endospores are a much more resilient physiological form of the vegetative cell and more able to withstand extreme environmental conditions. They should, therefore, be more resistant to lysing which can occur during extended periods of nebulization.\textsuperscript{(23)}

The results presented here, and those from Davidson et al. clearly demonstrate that the 3M 1860s N95 respirator is least efficient with respect to both non-pathogenic \textit{B. anthracis} Sterne 34F2, vegetative cells and endospores, as well as PSL \(\geq 1\) µm (Figure 3.3). Neither RPE type (N95, surgical mask or hybrid) nor brand name (3M, Moldex, Kimberly Clark, and Medline) could be considered an indicator of relative efficiency.

RPE relative efficiency when challenged with non-pathogenic \textit{B. anthracis} Sterne 34F2 vegetative cells were, on average, slightly higher than when challenged with non-pathogenic \textit{B. anthracis} Sterne 34F2 endospores or PSL.\textsuperscript{(1)} The average RPE relative efficiency when challenged with endospores was 7% (\(\pm 7\)% less than when challenged with vegetative cells. This reduction can be attributed to the larger CMAD of the vegetative cell bioaerosol (1.2) compared to the endospore bioaerosol (1.1). Figure 3.1 shows the slight variations in the aerodynamic profile between the different morphological forms of non-pathogenic \textit{B. anthracis} Sterne 34F2. The average RPE relative efficiency when challenged with PSL was 5% (\(\pm 4\)% less than when challenged with vegetative cells. The reduction in RPE efficiency when challenged with PSL can be attributed to the smaller CMD of the PSL (1.1). These results fall closely in line with those from work by Holton et al. that showed little difference in the inside mask aerosol
concentration for particle sizes in the range of 0.2 to 1.0 µm.\textsuperscript{(24)} Therefore, the argument could be made that all particles in this size range behave aerodynamically the same whether inert or biological.\textsuperscript{(24)} From the ratings by group analysis for the two experiments (Davidson et al. and current results), statistically significant differences exist among RPE relative efficacies when challenged with aerosols of non-pathogenic \textit{Bacillus anthracis} Sterne 34f2 vegetative cells and endospores. Our study was restricted to a limited sample of RPE models and one microorganism. Future studies are needed to determine if differences exist among the RPE models when challenged with bioaerosol of different size, morphology, or physiology.

Future studies should be conducted to compare filter material type, attachment of RPE to head, and possibly most important, each RPE model’s ability to be fitted to the wearer’s face.

3.6 Conclusions

The results further validate the use of a bioaerosol chamber and ABS to assess relative efficiencies of RPE models worn by HCP. Previous RPE relative efficiency results were supplemented by use of bioaerosol challenges comprised of non-pathogenic \textit{B. anthracis} Sterne 34F2 endospores and inert particles. For the specific size range of 1.0 to 5.0 µm, it was demonstrated that the biological and inert particles behaved similarly in terms of the computed relative efficiency results from each experiment. Certain models RPE were shown to perform better than other models. Results may be taken into consideration when selecting RPE for protection against airborne infectious agents.

Limitations of the study should be considered. RPE models used in this study were not selected via random sample. Efforts were not made to assure the selection of the
RPE models was a random sample of models currently available or representative of the market share of RPE. Therefore, the study was restricted to a limited sample of RPE models attached to one manikin head form. Furthermore, our investigation was restricted to a limited sample of RPE models attached to one manikin head form. Further studies with different manikin head forms would be required before conclusions about RPE efficiencies can be drawn from these results.

Future work should include experimental modifications to separately account for efficiency influenced by filter penetration as well as that influenced by face seal leakage. In doing so, considerations should be made to assure a proper fit of RPE to the manikin head form in accordance with established fit-test criteria.

3.7 References

1. Davidson, C.S., C.F. Green, A.L. Panlilio, P.A. Jensen, and P.V. Scarpino: Method for evaluation the relative efficiency of selected N95 respirators and surgical masks in preventing the inhalation of airborne, non-pathogenic Bacillus anthracis Sterne 34F2 vegetative cells by healthcare personnel. (in preparation)


Chapter 4:

Feasibility of selected prophylactic barriers in stopping non-pathogenic

*Bacillus anthracis* strain Sterne 34F2 vegetative cells and endospores
4.1 Abstract

Transmission of infection with airborne infectious agents such as Mycobacterium tuberculosis and other bacteria in healthcare settings is a risk for healthcare personnel. Outbreaks of TB in the late 80s and early 90s highlighted these risks. We assessed the ability of prophylactic barriers (transparent mesh netting) on the arrestance of bioaerosols.

Using a bioaerosol chamber and methods adapted from those we used to assess the protective efficacy of respiratory protective equipment, we tested two brands of prophylactic barriers, with pore sizes of 0.8 mm and 0.25 mm respectively. Bacterial aerosols of non-pathogenic *B. anthracis* Sterne 34F2 (both endospores and vegetative cells) were generated with a modified MRE-type six-jet Collison nebulizer. All test, control and blank samples were taken with all-glass impinger-30 samplers. The prophylactic barriers were placed 1.0 meter from the nebulizer with the AGI-30 samplers placed at distances of 0.25, 0.8, 1.2 and 1.75 m from the bioaerosol source.

The prophylactic barrier with the largest pore size (0.8mm) provided no arrestance in bioaerosol concentrations while the 0.25 mm pore size barrier provided an 18% arrestance in non-pathogenic *B. anthracis* Sterne 34F2 vegetative cell concentrations and a 19% arrestance in non-pathogenic *B. anthracis* Sterne 34F2 endospore concentrations. Although the level of arrestance provided by the prophylactic barriers may not justify their use as the only intervention for preventing transmission of airborne infectious agents, our study suggests that these barriers might be feasible as low cost supplements to existing environmental controls.
4.2 Introduction

Transmission of infection with airborne infectious agents such as Mycobacterium tuberculosis and other bacteria in healthcare settings is a risk. For healthcare personnel outbreaks of TB in the late 80s and early 90s highlighted these risks.

Crowding of soldiers in barracks, at the mess table and recreation rooms were suspected as means of facilitating transmission of respiratory diseases in 1919.\(^{(1)}\) Steps the U.S. Army used in preventing of the spread of respiratory illness, included stretching “tent flaps” between beds and suspending a curtain down the center of a mess table. However, the effect of these measures on transmission of respiratory illnesses was not assessed. Although prophylactic barriers (i.e., transparent netting) treated with various insecticides has been demonstrated to reduce incidence of malaria by stopping insect vectors, no studies have been conducted to assess the arrestance of bioaerosols by these prophylactic barriers.\(^{(2-6)}\)

4.3 Materials and methods

We modified techniques developed in assessing the relative efficacy of respiratory protective equipment (RPE) to assess the protective efficacy of prophylactic barriers. This paper adapts procedures using a horizontal bioaerosol chamber, six-jet Collison nebulizer and AGI-30 samplers.\(^{(7,11)}\)

We used bioaerosols of non-pathogenic \(B.\ anthracis\) Sterne 34F2 grown from Anthrax Spore Vaccine (non-encapsulated, viable \(Bacillus anthracis\) strain Sterne 34F2 (Colorado Serum Company, Denver, CO)).\(^{(8)}\) Both vegetative cells and endospores were cultured using similar techniques as those outlined in Davidson et al.\(^{(7,9,10,11)}\)
Two prophylactic barriers were chosen for these experiments. The TSR Traveler’s Mosquito Net prophylactic barrier (Tropical Safety Research, Los Angeles CA) has 0.8 mm diameter perforations while the AHH “No-see-um” prophylactic barrier (American Home and Habitat, Squires, MO) has 0.25 mm perforations. Both prophylactic barriers are transparent.

The test bioaerosols were generated with a modified MRE-type six-jet Collison nebulizer with a full-sized bottle (CN-25, BGI Inc., Waltham, MA). Bioaerosol samples were taken with AGI-30 samplers as per previous studies. Each prophylactic barrier was tested in a bioaerosol chamber set at 9.9 L/sec (594 L/min) utilized in previous studies. Resulting velocity of air through the chamber was calculated to be 0.016 m/s.

After preparation of the bioaerosol suspension the nebulizer was attached to an inlet on the bioaerosol chamber. The air flow and pressure to nebulizer generated an aerosol of predominantly single vegetative cells or endospores. AGI-30 samplers were placed in the bioaerosol chamber at distances of 0.25, 0.8, 1.2 and 1.75 m from the baffle just downwind of the bioaerosol source. The prophylactic barrier was then placed in the bioaerosol chamber at a distance of 0.75 meters from the bioaerosol source. The AGI-30 samplers were turned on and samples collected for 15 minutes similar to the procedure described in Jensen et al. Figure 4.1 shows the positioning of the samplers and prophylactic barriers inside the bioaerosol chamber.
To quantify the bacterial CFU in the AGI-30 samplers, 0.1 mL of PBW suspension was transferred to a Tryptic Soy Agar (TSA) plate for a spread plate count. All test and control samples were cultured in triplicate. The plates were grown and enumerated using standard methods.\(^{(20)}\)

The culturable concentrations (CFU/L) downwind from the bioaerosol source at distances of 0.25, 0.8, 1.2 and 1.75 m were measured. Percent of initial concentration at 0.25 m along with 95% confidence interval were calculated. The process was repeated with the prophylactic barrier affixed to the bioaerosol chamber walls (figure 4.1).

A Power analysis was done using PROC GLMPOWER in SAS 9.1.3 (SAS Institute, Cary, N.C.) to determine the number of replicate runs necessary. A one-way ANOVA model was done using PROC GLM to determine if there was a significant
decrease in particle concentrations downwind of the prophylactic barriers. A two-way ANOVA model (PROC GLM) was considered with the differences between test and control percentages at distances of 1.2 and 1.75 m from the baffle.

To assess the ability of the prophylactic barriers to arrest aerosols at different aerodynamic diameters polystyrene latex (PSL) suspensions (Duke Scientific Corporation, Palo Alto, CA) were utilized. One milliliter aliquots of six PSL suspensions containing particles with aerodynamic diameters of 0.1, 0.43, 0.6, 1.3, 3.2, and 8.0 µm, respectively, were each diluted to a final volume of 100 mL.

PSL aerosols were generated using the same methods as the bioaerosols, employing the Collison nebulizer. Particle size characteristics were calculated for the PSL aerosols using measurements by the ARTI HHPC-6 to determine the prophylactic barriers’ ability to arrest different aerosol sizes. Paired T-tests of upwind and downwind aerosol concentrations were performed using PROC TTEST in SAS 9.1.3.

4.4 Results

Figures 4.2a and 4.2b show the results with the TSR prophylactic barrier. Based on the one-way ANOVA (α=0.05, p=0.778) there was no difference between control and test vegetative cell concentrations downstream of the prophylactic barrier. The same is true for the endospore concentrations (α=0.05, p=0.787).
Figures 4.2a, 4.2b. Efficiency of TSR prophylactic barrier in stopping non-pathogenic *Bacillus anthracis* strain Sterne 34F2 vegetative cells and endospores.

*Error bars denote 95% confidence interval.

Figures 4.3a and 4.3b show the results with the AHH prophylactic barrier. Based on a one-way ANOVA model there was a statistical difference for both the vegetative cells (α=0.05, p=0.001) and endospores (α=0.05, p=0.012) downwind of the prophylactic barrier at distances of 1.2 and 1.75 m from the baffle. The AHH barrier provided an 18%
arrestance in non-pathogenic *B. anthracis* Sterne 34F2 vegetative cells and a 19% arrestance in non-pathogenic *B. anthracis* Sterne 34F2 endospores.

**Figures 4.3a, 4.3b.** Efficiency of AHH prophylactic barrier in stopping non-pathogenic *Bacillus anthracis* strain Sterne 34F2 vegetative cells and endospores.

*Error bars denote 95% confidence interval.

The AHH prophylactic barrier had a power of 0.955 measuring 10% difference ($\alpha=0.05, p=0.001$) when challenged by non-pathogenic *B. anthracis* Sterne 34F2
vegetative cells with six replicate runs. The AHH had a power of 0.744 ($\alpha=0.05$, $p=0.013$) when challenged by endospores with six replicate runs.

The two-way ANOVA was performed to see if the difference between test and control percentage of aerosol concentration changed as air flowed downstream past the prophylactic barrier. Based on this statistic ($\alpha=0.05$, $p=0.227$) there was no difference between test and control at these distances of 1.2 and 1.75 m for the vegetative cells. The same held true for the differences between test and control at the two distances for endospores ($\alpha=0.05$, $p=.254$).

Figure 4.4 shows the percent of arrestance of PSL aerosols by AHH prophylactic barrier by diameter. Based on the t statistic there was no arrestance of 1.0 µm diameter PSL aerosol particles ($\alpha=0.05$, $p=0.280$). The barrier arrested the concentration of 3.0 µm diameter particles by 17±4% ($\alpha=0.05$, $p<0.001$) and 5.0 µm diameter particles by 22±14% ($\alpha=0.05$, $p=0.021$).
Based on the PSL aerosol data (1, 3 and 5µm) a linear regression model was fit using PROC REG in SAS 9.1.3 expressing % aerosol arrestance as a function of aerodynamic diameter. The model can be expressed in the following equation:

\[ \%A = (0.00685 + 0.04552 \times D) \times 100\% \]

Where \( \%A \) is arrestance and \( D \) is aerodynamic diameter (in µm).

4.5 Discussion/Conclusions

The prophylactic barrier with the larger pore size (TSR, 0.8 mm) failed to arrest aerosol concentrations when challenged with either non-pathogenic \textit{B. anthracis} Sterne 34F2 aerosolized vegetative cells or endospores. The prophylactic barrier with the smaller pore size (AHH, 0.25mm) provided a statistically significant arrestance in aerosol
concentration when challenged with both non-pathogenic \textit{B. anthracis} Sterne 34F2 vegetative cells and endospores.

When producing vegetative cell aerosols the nebulizer yielded total aerosols with a mass median diameter (MMD) of 0.78 µm, while culturable colony-forming unit (CFU) bioaerosols had a mass median aerodynamic diameter (MMAD) of 2.1.\textsuperscript{(7)} When producing endospore aerosols the nebulizer yielded total aerosols with a MMD of 1.6, while cultural CFU bioaerosols had an MMAD of 2.1.\textsuperscript{(11)} The linear regression model based on the PSL aerosol data predicted that the previously generated vegetative cell and endospore bioaerosols with an MMAD of 2.1 µm would be arrested by 10% when passed through the AHH prophylactic barrier. Actual data showed an increased arrestance of bioaerosols, with the non-pathogenic \textit{B. anthracis} Sterne 34F2 vegetative cell concentration being arrested by 18% and endospore concentration being arrested by 19%.

This level of protection cannot justify suggesting prophylactic barriers as substitutes for RPE. It does suggest that barriers with low critical pores (≥0.25 mm) might be feasible as low cost supplements to existing environmental controls or possibly in limited resource settings.

4.6 References


challenged with airborne, non-pathogenic *Bacillus anthracis* strain Sterne 34F2 endospores and inert particles. *In Preparation.* (2006b).


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