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NIOSH HEALTH HAZARD EVALUATION REPORT

HETA #2002-0109-2927
NIOSH Evaluation of Air Sampling Methodologies for *Bacillus anthracis* in a United States Postal Service Processing and Distribution Center
Trenton, New Jersey

February 2004

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health

PREFACE

The Hazard Evaluation and Technical Assistance Branch (HETAB) of the National Institute for Occupational Safety and Health (NIOSH) conducts field investigations of possible health hazards in the workplace. These investigations are conducted under the authority of Section 20(a)(6) of the Occupational Safety and Health (OSHA) Act of 1970, 29 U.S.C. 669(a)(6) which authorizes the Secretary of Health and Human Services, following a written request from any employers or authorized representative of employees, to determine whether any substance normally found in the place of employment has potentially toxic effects in such concentrations as used or found.

HETAB also provides, upon request, technical and consultative assistance to federal, state, and local agencies; labor; industry; and other groups or individuals to control occupational health hazards and to prevent related trauma and disease. Mention of company names or products does not constitute endorsement by NIOSH.

ACKNOWLEDGMENTS AND AVAILABILITY OF REPORT

This report was prepared by Robert E. McCleery, MSPH; Kenneth F. Martinez, MSEE, CIH; Gregory A. Burr, CIH; and Dino A. Mattorano, MS of HETAB, Division of Surveillance, Hazard Evaluations and Field Studies (DSHEFS). Field assistance was provided by Donnie Booher, John Cardarelli, Richard Hartle, and Bradley King. Analytical support was provided by a Centers for Disease Control and Prevention (CDC) contract laboratory. Additional report review was provided by Paul Baron, Beth Reh, and Teresa Seitz. Desktop publishing was performed by Robin Smith. Review and preparation for printing were performed by Penny Arthur.

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Highlights of the NIOSH Health Hazard Evaluation

NIOSH Evaluation of Air Sampling Methodologies for *Bacillus anthracis* in a United States Postal Service Processing and Distribution Center

On January 16, 2002, NIOSH received a request for a health hazard evaluation (HHE) from the United States Postal Service (USPS) regarding *Bacillus anthracis* in the Trenton Processing and Distribution Center (TPDC) located in Trenton, New Jersey. The USPS requested assistance in determining the most appropriate method(s) of air sampling for *B. anthracis* spores. In response to this request, NIOSH investigators conducted an environmental investigation at the site on February 4-7, 2002.

What NIOSH Did

- We took surface wipe samples on a Delivery Bar Code Sorter (DBCS) before and after turning it on.
- We took air samples using mixed-cellulose, polytetrafluoroethylene, and gelatin filters; Andersen samplers with sheep blood agar; and dry filter units with polyester felt filters.
- We took air samples around the DBCS before, during, and after turning it on.
- We tested the filter samples two ways. First, the samples were tested as normal (not all the sample is used). Second, any negative results (no *B. anthracis*) had the remaining sample tested to make sure the sample was negative.

What NIOSH Found

- All of the wipe samples were positive, indicating the DBCS was heavily contaminated with *B. anthracis*.
- Some of the Andersen samples collected before operating the DBCS were positive, while most of the samples collected after DBCS operation were positive.
- The first test of the filter samples collected before operating the DBCS resulted in no positive samples.

What NIOSH Found *(Cont'd)*

- The first test of the filter samples collected after operating the DBCS found more positive samples.
- Testing of the remaining sample of the negative filters from before and after operating the DBCS indicated that all filter types had more positive samples.
- The results suggest that the entire filter sample should be used to analyze for *B. anthracis*.
- The results suggest that the dry filter units and their high flow rate could have influenced the way the other samplers collected *B. anthracis*.
- *B. anthracis* can become airborne by walking or lightly working in a contaminated area.

What NIOSH Recommends

- The Andersen sampler should be **one** of the instruments utilized when screening, characterizing, and/or clearing a *B. anthracis* contaminated facility for re-occupancy.
- When taking air samples for *B. anthracis* using filter media, the entire sample extract must be tested.
- More information is needed on how well these and other methods work when sampling for *B. anthracis*.

What To Do For More Information:
We encourage you to read the full report. If you would like a copy, either ask your health and safety representative to make you a copy or call 1-513-841-4252 and ask for HETA Report #2002-0109-2927

Health Hazard Evaluation Report 2002-0109-2927
Trenton Processing and Distribution Center
Trenton, New Jersey
February 2004

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SUMMARY

On January 16, 2002, NIOSH received a request for a health hazard evaluation (HHE) from the United States Postal Service (USPS) regarding *Bacillus anthracis* (*B. anthracis*) contamination in the Trenton Processing and Distribution Center (TPDC) located in Trenton, New Jersey. The USPS requested assistance in determining the most appropriate method(s) of air sampling for *B. anthracis* spores.

In response to this request, NIOSH investigators conducted an evaluation of sampling methods at the TPDC on February 4-7, 2002. NIOSH investigators collected 106 surface wipe samples on the jogger/sorter, feeder, reader, and all final stacker (bin) sections of a Delivery Bar Code Sorter (DBCS), 130 general area (GA) air samples using Andersen samplers with sheep blood agar, 24 GA air samples using mixed-cellulose ester filter media, 24 GA air samples using polytetrafluoroethylene filter media, 72 GA air samples using gelatin filter media, and 6 GA air samples using a dry filter unit with polyester felt filter media.

Wipe and air samples were collected before and after operating the DBCS. Operating the DBCS provided a means of re-aerosolization of spores resulting in enhanced capture potential for air sampling media. All of the wipe samples were positive for *B. anthracis*. The initial analysis of air samples (using 10% of the sample extract) collected before DBCS operation resulted in no detectable *B. anthracis* colonies (negative sample), except for some Andersen samples. All of the negative filter samples were re-analyzed using the remaining sample, which resulted in each type of filter media having one or more false negative samples. All air sample media had detectable *B. anthracis* colonies subsequent to DBCS operation.

Based on the surface wipe and air sample data, NIOSH investigators conclude the following: (1) walking and light work may be sufficient to re-aerosolize *B. anthracis* spores; (2) all air sampling methods used were capable of collecting *B. anthracis* spores, albeit some more efficiently than others; (3) not plating the entire sample during analysis may result in false negative sample results; (4) the Andersen sampling method seems to be the most sensitive for *B. anthracis* spore collection; (5) because of its high flow rate the dry filter unit may have reduced the number of available spores for collection; (6) the dry filter unit may be the least sensitive when considering the volume of air passing through the sampler. Further laboratory and field evaluation of these and other methods is necessary to understand their practical uses and limitations for collection of *B. anthracis* in contaminated facilities.

Keywords: SIC 4311 (United States Postal Service), *Bacillus anthracis*, *B. anthracis*, anthrax, air sample, wipe sample, filter cassette, mixed cellulose ester, MCE, polytetrafluoroethylene, PTFE, gelatin, GEL, Andersen sampler, dry filter unit, DFU

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INTRODUCTION

On January 16, 2002, the National Institute for Occupational Safety and Health (NIOSH) received a request for a health hazard evaluation (HHE) from the United States Postal Service (USPS) regarding *Bacillus anthracis (B. anthracis)* contamination in the Trenton Processing and Distribution Center (TPDC) located near Trenton, New Jersey. The USPS requested assistance in determining the most appropriate method(s) for air sampling for *B. anthracis* spores. In response to this request, a collaborative research project between NIOSH and the USPS was conducted during the week of February 4-7, 2002.

The site visit began with an opening conference on February 4, 2002, with representatives from NIOSH, USPS, Environmental Protection Agency (EPA), New Jersey Department of Health and Senior Services (NJDHSS), The Shaw Group Incorporated™ (formally the IT Group), and URS Corporation personnel (USPS contractors). Discussion topics included the sampling objectives, the sampling protocol for composite surface wipe sampling and air sampling, the previous decontamination efforts, the needs and concerns of the various groups involved with on-going efforts in and around the site, and the safety requirements of the site. Environmental sampling began on the afternoon of February 4 and continued until the afternoon of February 7.

BACKGROUND

The roughly 300,000 square foot (ft^2) TPDC is a facility that employs approximately 850 people. At least four letters containing *B. anthracis* were processed through this facility: two on September 18, 2001, intended for New York City media outlets, and two on October 9, 2001, to U.S. Senators in Washington, D.C.[1] The facility was closed on October 18, 2001, after cutaneous anthrax was confirmed in a TPDC postal worker. Seven anthrax cases (three confirmed cutaneous, two suspect cutaneous, and two confirmed inhalational) were eventually identified. Five cases were associated with TPDC, one with a downstream post office, and one with a private business. On October 25, 2001, all TPDC employees were offered the 60-day post-exposure prophylaxis.[2,3]

After the initial cutaneous anthrax case was confirmed, the Federal Bureau of Investigation conducted the first directed environmental anthrax sampling at TPDC on October 18, 2001. Twenty-three wet swab samples were collected, which traced the mail paths of the four suspected letters; fourteen of these samples were positive for *B. anthracis*. Subsequently, a joint Centers for Disease Control and Prevention (CDC)/ NJDHSS team collected an additional 95 wet swab and surface vacuum samples on October 21, 2001, and November 11, 2001, to characterize the extent of *B. anthracis* contamination throughout the mail processing areas. Of these 95 samples, 40 were positive, including several from the TPDC air handling systems, which suggested that the contamination was not limited to specific sorting machines or work areas. Since November 2001, hundreds of additional wet swabs, wipes, and surface vacuum samples have been collected by the USPS as part of their clean-up effort.

CONDUCT OF FIELD STUDY

Site Selection

For this evaluation of various air sampling methods, investigators selected Delivery Bar Code Sorter #70 (DBCS) for study since it was in the direct mail path for several of the contaminated letters. A DBCS is divided into the following sections: jogger/sorter, feeder, reader, and stackers (bins). The unit had been spot-cleaned in November 2001 using a four-step process: (1) high-efficiency particulate air vacuuming; (2) application of a 10% bleach solution with a contact time of 10 minutes; (3) application of a neutralizing solution (sodium thiosulfate); and (4) final rinse with clean water. Following cleaning, the machine was loosely covered in plastic. For the purpose of our study,

in January 2002 a plastic-walled enclosure (approximately 70' x 15' x 7') was constructed around the machine. Prior to sampling in the enclosure, the loose plastic covering over the machine was cleaned and removed. The enclosure was constructed to improve the chances of collecting *B. anthracis* spores and to avoid contaminating surrounding equipment.

Sample Collection

CDC/NIOSH investigators collected air and surface wipe samples for *B. anthracis* spores before and after operating the DBCS. (**Note:** when referring to air sampling, the phrase "after DBCS operation" means during **and** after DBCS operation.) Wipe samples were collected to gain insight into whether the DBCS was contaminated and the degree of contamination in the following areas: the jogger/sorter, feeder, reader, and all final stacker (bin) sections of the DBCS. In the bin area, most wipe samples were collected from a column of four bins (referred to as composite wipe samples). There were two exceptions where a column consisted of three bins and a printer.

Figure 1 presents the locations where mixed-cellulose ester (MCE), polytetrafluoroethylene (PTFE), gelatin (GEL), dry filter unit (DFU) (developed by the Joint Program Office for Biological Defense [JPO-BD]), and Andersen air samples were collected. Locations were selected to encompass the area around each section of the DBCS and maximize the possibility of spore collection. The overall sample collection period lasted approximately 8 hours each day, with additional sample collection during the first 2 hours of this 8-hour period. Investigators conducted this additional sampling for the following reasons: (1) to address the susceptibility of GEL filters to dessication, (2) to balance the air sample volumes for comparison of the methods, (3) to investigate whether 2- and 8-hour sampling periods result in positive samples, and (4) to mirror air-sampling periods used in prior anthrax investigations. MCE and PTFE filter samples were collected for 2- and 8-hour sessions. GEL filter samples were collected for 1-hour each during the initial 2-hour session and then over 2-hour intervals each during the 8-hour session. During the first two hours, Andersen samples were collected in succession approximately every ten minutes. One 10-minute sample was then collected every two hours over the remaining 6-hour period. DFU filter samples were collected for both the 2- and 8- hour sessions.

On February 6, a USPS employee entered into the building with the CDC/NIOSH team to energize and operate the DBCS. At issue was whether operation of the DBCS would aerosolize *B. anthracis* spores. The employee started the DBCS and its computer program, configured it to operational status, and set the parameters of the computer program to deposit the "test deck" sheets to specific bins. The program was then run and the test deck began distributing through the system. The employee gathered the sheets, set the computer program to release the sheets at another set of bins, and ran the sheets through again. At times, the DBCS jammed which required the employee to re-set the computer program and re-initiate the test run. In total, the machine was operated for 45 minutes. The DBCS computer program indicated that 6,521 "test deck" sheets had passed through the system during that time period.

Sampling Methods

Surface Wipe Sampling

Composite wipe samples were collected according to CDC guidelines.[4] The procedure included the use of 4" x 4" sterile polyester/ rayon pads moistened with sterile water, placement of samples in sterile conical vials, a change of gloves after each sample collection, decontamination of sample containers with bleach solution, and shipment according to established regulations.

Air Sampling

Andersen Sampler

Air samples for *B. anthracis* were collected using trypticase soy agar (TSA) with five percent sheep blood (referred to as sheep

blood agar [SBA]) in Andersen single-stage cascade impactors at a calibrated sampling rate of 28.3 liters per minute (lpm). The Andersen single-stage cascade impactor has a calculated d_{50} of 0.57 micrometer (µm) (the d_{50} of an impactor is the diameter at which theoretically 50% of the particles are collected and 50% of the particles pass through).[5] Agar plates were loaded into the Andersen sampler outside the containment area at an approximate height of three feet using aseptic techniques (e.g., the cleaning and sanitization of working surfaces, the wiping of Andersen sampler collection surfaces with alcohol between samples, and the inversion of sample plates before and after sampling). Samplers were then moved inside the containment and placed in their predetermined location. At that time, the Andersen samplers which had operated for their 10-minute sampling period were moved outside of containment, their agar plates removed and covered, sealed with tape around the circumference, and placed into individual sealed bags. After sampling was complete, the individual sealed bags containing the samples went through the decontamination process, were packed into containers according to existing transportation regulations, and sent to the CDC contract laboratory.

Mixed Cellulose Ester, Polytetrafluoroethylene, and Gelatin Filters

Air samples for *B. anthracis* were collected using MCE, PTFE, and GEL filters in a closed-faced cassette at a calibrated sampling rate of 2.0 lpm. MCE and PTFE filters were 37-millimeter (mm) diameter, 0.8-µm pore size, while GEL filters were 37-mm diameter, 3.0-µm pore size. After sampling was complete, each filter cassette was capped and placed into a plastic cup with a screw-on top. The cups were then placed into a large sealed bag for decontamination and shipping.

Dry Filter Unit

Air samples for *B. anthracis* were collected using a 47-mm diameter, 1.0 µm pore size polyester felt filter disk (American Felt and Filter Company, New Windsor, New York) at an approximate (no specific calibration device available) sampling rate of 400 lpm. Samples were collected using single filter DFUs, one of which was modified to potentially reduce the amount of by-pass air-flow around the filter (provided by URS personnel). After sampling was complete, filters were removed from the DFU and placed into a plastic conical vial with a screw-on top. These vials were then placed into a large sealed bag and were treated the same as the above agar plates from the decontamination process forward.

Test Deck Sheets

Test deck sheets are currently used by the USPS to test the operation of a DBCS or other mail sorting machinery. These sheets are shaped to resemble a typical envelope in dimension and are thicker than a normal sheet of paper. Ten sheets (not chosen in any particular order) of the "test deck" that went through the DBCS during the 45-minute operation time were sent to the CDC contract laboratory for analysis.

Sample Analysis

A CDC contract laboratory (provisionally approved Level B in the Laboratory Response Network) analyzed the samples by using a wet extraction for wipes and wet extraction with modifications for other sample collection matrices,[6] and it presumptively identified *B. anthracis* (Level A screening) according to CDC protocol.[7] The confirmation of presumptive positive samples was accomplished by Gamma Phage Lysis (GPL) and Direct Fluorescence Antibody (DFA) analyses.

Statistical Strategy

A statistical analysis of the Andersen sampling results was done to assess the effects of time and sampling location on *B. anthracis* concentration. Individual air sample concentrations from Andersen samplers were compiled into a STATGRAPHICS Plus® database. Histograms and the Shapiro-Wilks test for normality of the log-transformed data indicated that the data were approximately lognormally distributed. Thus, *B. anthracis* concentrations were log-transformed for statistical analyses using multiple linear regression. Factors including

time, location, and their interaction term, were added to the model and eliminated using a backwards elimination process. Only factors with P-values less than 0.05 were kept in the final model.

Theoretical Decay Curves

Theoretical *B. anthracis* concentration decay curves were established for each type of sampler inside containment (i.e., DFU, Andersen, and other pumps). This information was used to investigate the operating sampler contribution to the decrease of airborne *B. anthracis* inside the containment. Specific interest was placed on the theoretical curve of the DFU due to its high flow rate (≈400 lpm). The sampler decay curves were combined to form one overall theoretical *B. anthracis* decay curve, which is presented with the actual Andersen sampler decay curve to investigate the consistency between theoretical and actual data. The cureves were also subjectively evaluated to determine the extent to which the samplers potentially "filtered" the air of *B. anthracis* as well as affected the sampling collection potential of the other samplers.

EVALUATION CRITERIA

As a guide to the evaluation of the hazards posed by workplace exposures, NIOSH field staff employ environmental evaluation criteria for the assessment of a number of chemical and physical agents. These criteria are intended to suggest levels of exposure to which most workers may be exposed up to 10 hours per day, 40 hours per week for a working lifetime without experiencing adverse health effects. It is, however, important to note that not all workers will be protected from adverse health effects even though their exposures are maintained below these levels. A small percentage may experience adverse health effects because of individual susceptibility, a pre-existing medical condition, and/or a hypersensitivity (allergy). In addition, some hazardous substances may act in combination with other workplace exposures, the general environment, or with medications or personal habits of the worker to produce health effects even if the occupational exposures are controlled at the level set by the criterion. These combined effects are often not considered in the evaluation criteria. Also, some substances are absorbed by direct contact with the skin and mucous membranes, and thus potentially increases the overall exposure. Finally, evaluation criteria may change over the years as new information on the toxic effects of an agent become available.

The primary sources of environmental evaluation criteria for the workplace are: (1) NIOSH Recommended Exposure Limits (RELs),[8] (2) the American Conference of Governmental Industrial Hygienists' (ACGIH®) Threshold Limit Values (TLVs®),[9] and (3) the U.S. Department of Labor, OSHA Permissible Exposure Limits (PELs).[10] Employers are encouraged to follow the OSHA limits, the NIOSH RELs, the ACGIH TLVs, or whichever are the more protective criterion.

OSHA requires an employer to furnish employees a place of employment that is free from recognized hazards that are causing or are likely to cause death or serious physical harm [Occupational Safety and Health Act of 1970, Public Law 91-596, sec. 5(a)(1)]. Thus, employers should understand that not all hazardous chemicals have specific OSHA exposure limits such as PELs and short-term exposure limits (STELs). An employer is still required by OSHA to protect their employees from hazards, even in the absence of a specific OSHA PEL.

A time-weighted average (TWA) exposure refers to the average airborne concentration of a substance during a normal 8- to 10-hour workday. Some substances have recommended STEL or ceiling values which are intended to supplement the TWA where there are recognized toxic effects from higher exposures over the short-term.

Bacillus anthracis

B. anthracis is a rod-shaped, gram-positive, non-motile, spore-forming bacterium which is

present globally. The sporulated form of *B. anthracis* is typically found in the soil and is known to inhabit environments for decades. The bacteria are known to produce a variety of toxins. Anthrax, also referred to as Woolsorter's disease and Ragpicker's disease, is the clinical term for the disease caused by *B. anthracis*. The word originates from the Greek word for coal, which probably refers to the skin appearance of the cutaneous form of the disease. Anthrax has an extensive human history and is referred to in the following: (1) Homer's *Iliad*,[11,12] (2) biblical passages describing the fifth Egyptian plague affecting livestock and the sixth plague called the "plague of boils"[12,13] (although a study conducted by John Marr and Curtis Malloy presents a different explanation for the fifth and sixth biblical plagues[14]) and (3) Virgil's *The Georgics*.[12,15,16] Robert Koch showed that *B. anthracis* spores caused anthrax disease in mice[17] and Lewis Pasteur developed an effective immunization against anthrax in animals.[18] Several countries have experimented with anthrax as a biological warfare agent. A large outbreak in Sverdlovsk, Russia, killed at least 60 people and is thought to be the result of spores from a Soviet Army biological research facility upwind of the town.[19]

Humans can naturally contract anthrax disease through exposure to infected livestock or wild animals, or contaminated animal products such as hides or hair. There are three clinical forms of anthrax: cutaneous (the most common), gastrointestinal, and inhalational. The cutaneous form begins when *B. anthracis* enters the skin through a cut or abrasion forming a black lesion. Once the bacterium enter the blood stream it can cause septicemia (blood poisoning) and ultimately death. The gastrointestinal form of anthrax is caused by the consumption of contaminated animal meat. Symptoms usually begin with nausea, vomiting, and fever which can progress to septicemia. Inhalational anthrax is the most deadly form. The number of spores needed to cause disease and the incubation period are both unknown. Symptoms may begin with a flu-like feeling and mild fever and progress to respiratory failure. Anthrax-related information, publications, and guidance for post-exposure prophylaxis and treatment are available on the CDC website, http://www.cdc.gov/.
There are currently no numeric criteria against which to compare environmental measurements for anthrax.

RESULTS

The following information provides summary results for the composite wipe samples and each type of air sampling media used in this study. Results are described as positive (detectable colonies) or negative (no detectable colonies). Appendices A-G present the individual sample results before and after DBCS operation (in tabular form) for the composite wipe samples, each type of air sampling media (Andersen sampler, MCE, PTFE, GEL, DFU), and test kit sheet samples, respectively.

In Appendices B-F, the air samples "after DBCS operation" were started at the same time the machine was started and ran for their allotted time periods. Table 1 presents the "test deck" information from the DBCS machine operation period. Table 2 presents the sample results from the initial sample analysis by individual sample location. Table 3 provides a summary of all the sample results before and after DBCS operation including initial analysis and analysis of the remaining sample results.

The sample *B. anthracis* concentration of each initially positive filter, assuming a homogeneous mixture, was calculated by extrapolating from the plated portion of the sample concentrate. Filter media that were initially positive did not have the remaining sample analyzed to establish an overall *B. anthracis* colony concentration.

Surface Wipe Samples

Wipe sample results before and after DBCS operation are presented in Appendix A. A total of 106 wipe samples were collected on the DBCS machine (53 samples were collected before and 53 after the machine was in operation). All stackers (bins) were included in the wipe sampling. All of the wipe samples were positive. *B. anthracis* concentrations before

the machine was operated ranged from 50 to 100 colony forming units per wipe (CFUs/wipe) in the feeder, jogger, and reader sections of the DBCS, with a mean of 80 CFUs/wipe. *B. anthracis* concentrations in the bin section of the DBCS ranged from 4 to 8 x 10^8 CFUs/wipe, with a mean of 1.7 x 10^7 CFUs/wipe. The highest concentration (8 x 10^8 CFUs/wipe) was found on the wipe sample collected from bins 167-170.

B. anthracis concentrations after the machine was operated ranged from 12 to 3500 CFUs/ wipe in the feeder, jogger, and reader sections of the DBCS, with a mean of 724 CFUs/wipe. *B. anthracis* concentrations in the bin section of the DBCS ranged from 1 to 2.5 x 10^4 CFUs/ wipe, with a mean of 5052 CFUs/wipe. The highest concentration (2.5 x 10^4 CFUs/wipe) was found on the wipe samples collected on bins 155-158 and 167-170.

Andersen Sampler

Andersen sampler results before and after DBCS operation are presented in Appendix B. Sixty-five Andersen samples were collected before DBCS operation. During the first 2-hour session, sample locations 1, 2, 4, and 5 each had positive samples. During the 6-hour period, sample locations 1 and 5 had a positive sample. Sixty-four Andersen air samples were collected after DBCS operation. During the first 2-hour session, all samples collected were positive. During the 6-hour period, all sampling locations had some positive samples.

B. anthracis concentrations before DBCS operation ranged from no detectable colonies to 7 colony forming units per cubic meter (CFUs/m^3). The highest concentration (7 CFUs/m^3) was at location 3 during the 6-hour period and location 5 during the first 2-hour period. *B. anthracis* concentrations after the DBCS was operated ranged from no detectable colonies to 360 CFUs/m^3. The highest concentration (360 CFUs/m^3) was at location 2 during the 2-hour session.

Filter Media

Initial Results

MCE filter sample results before and after DBCS operation are presented in Appendix C. All 12 MCE air samples collected before DBCS operation were initially negative. Of the 12 MCE air samples collected after DBCS operation, four were initially positive (locations 4, 5, and 6 during the 2-hour period and location 5 during the 8-hour period).

PTFE filter sample results before and after DBCS operation are presented in Appendix D. All 12 PTFE air samples collected before DBCS operation were initially negative. Of the 12 PTFE air samples collected after DBCS operation, six were initially positive (locations 3, 4, and 5 during the 2-hour period and locations 2, 3, and 4 during the 8-hour period).

GEL filter sample results before and after DBCS operation are presented in Appendix E. All 36 GEL air samples collected before DBCS operation were initially negative. Of the 36 GEL air samples collected after DBCS operation, 13 were initially positive (locations 2-6 with location 3 having two positive samples during the 2-hour period and locations 1-4 and 6 with locations 3 and 4 each having two positive samples during the 8-hour period).

DFU filter sample results before and after DBCS operation are presented in Appendix F. All three DFU air samples collected before DBCS operation were initially negative. All three of the DFU air samples collected after DBCS operation were initially positive.

Results from the Analysis of the Remaining Sample

Mixed Cellulose Ester Filter

All 12 MCE air samples collected before DBCS operation were initially negative. Analysis of the remaining portion of these samples indicated 1 of 12 was positive (at location 5 during the 2-hour session). Of the eight initially negative MCE air samples collected after DBCS

operation, analysis of the remaining sample indicated all eight were positive.

Figure 2 presents the 2- and 8-hour MCE filter data by location for both days of sampling. *B. anthracis* concentrations before DBCS operation ranged from no detectable colonies to 4 CFUs/m^3. The highest concentration (4 CFUs/m^3) was at location 5 during the 2-hour session. *B. anthracis* concentrations after DBCS operation ranged from 7 to 400 CFUs/m^3. The highest concentration (400 CFUs/m^3) was at location 6 during the 2-hour session.

Polytetrafluoroethylene Filter

All 12 PTFE air samples collected before DBCS operation were initially negative. Analysis of the remaining portion of these samples indicated 1 of 12 was positive (at location 4 during the 8-hour session). Of the six initially negative PTFE air samples collected after DBCS operation, analysis of the remaining sample indicated all six were positive.

Figure 3 presents the 2- and 8-hour PTFE filter data by location for both days of sampling. *B. anthracis* concentrations before DBCS operation ranged from no detectable colonies to 1 CFU/m^3. The highest concentration (1 CFU/m^3) was at location 4 during the 8-hour session. *B. anthracis* concentrations after DBCS operation ranged from 10 to 120 CFUs/m^3. The highest concentration (120 CFUs/m^3) was at location 6 during the 8-hour session.

Gelatin Filter

All 36 GEL air samples collected before DBCS operation were initially negative. Analysis of the remaining portion of these samples indicated 1 of 36 was positive (at location 3 during one of the two 1-hour sessions). Of the 23 initially negative GEL air samples collected after DBCS operation, analysis of the remaining sample indicated 14 were positive (locations 1-2 and 4-6 with location 1 having two positive samples during the 2-hour session and locations 1-6 with location 5 having three positive samples during the 8-hour period).

Figure 4 presents the 2- and 8-hour GEL filter data by location for both days of sampling (samples averaged). *B. anthracis* concentrations before DBCS operation ranged from no detectable colonies to 5 CFU/m^3. The highest concentration (5 CFU/m^3) was at location 3 during the 2-hour session. *B. anthracis* concentrations after DBCS operation ranged from 11 to 84 CFUs/m^3. The highest concentration (84 CFUs/m^3) was at location 5 during the 2-hour session.

Dry Filter Unit Filter

All three initially negative DFU air samples collected before DBCS operation were positive after analysis of the remaining portion of the sample.

Figure 5 presents the 2- and 8-hour DFU filter data by location for both days of sampling. *B. anthracis* concentrations before DBCS operation were all less than 1 CFU/m^3. *B. anthracis* concentrations after DBCS operation ranged from 5 to 15 CFUs/m^3. The highest concentration (15 CFUs/m^3) was from the filter in the modified DFU, which operated for a 2-hour session.

Test Deck Sheets

"Test deck" information is presented in Table 1 and results are provided in Appendix G. One of the ten sheets was positive (1 CFU) from the initial analysis. Of the nine initially negative sheets, analysis of the remaining portion of these samples indicated all six were positive (range of 1 to 5 CFUs).

Statistical Analysis

The airborne *B. anthracis* concentrations collected with Andersen samplers were log-transformed to perform statistical analyses. A multiple regression analysis was performed on the data to describe the relationship between log-transformed concentration, time of sample collection, and location of sample collection. Results indicate that the only statistically significant covariate was time (*P*-value < 0.0001). The final mathematical model is presented graphically in Figure 7. Since the

factors ("location" and the interaction term [location x time]) were not statistically significant, regression lines grouped by location were not statistically different from each other and the parameter estimates (slope and intercept) from individual regression lines were not statistically different from each other. Therefore, grouping data from all locations into one regression model was appropriate and the final model can be used to describe *B. anthracis* concentrations.

Figures 6 and 7 show the decay curves, including the regression curve, of the Andersen sampler results before and after DBCS operation (raw data and log-transformed data, respectively). The regression model (subsequently referred to as the Andersen decay curve) above was used to calculate 2- and 8-hour TWA concentrations based upon Andersen sampler data from five different locations in containment. The 2- and 8-hour predicted concentrations based on this model were 122 CFU/m^3 and 15 CFU/m^3 TWAs, respectively.

DISCUSSION

The wipe sampling data should be viewed as semi-quantitative since there was not a quantitatively defined area of collection. However, persons collecting wipe samples were instructed to wipe the bin and its surrounding surfaces in a manner which created a relatively consistent collection area. The initial round of wipe samples (before DBCS operation) may have reduced the surface loading of *B. anthracis* spores resulting in the observed reduction in concentrations. While it is unknown how this reduction may have impacted the air concentrations, NIOSH investigators believe it was minimal since spore dissemination was probably due to mechanical action of the belts and pinch rollers as opposed to test envelopes collecting in receiving bins.

The positive air samples before DBCS operation suggest that walking and light work may be sufficient to re-aerosolize *B. anthracis* spores. Additionally, there does not appear to be a single event that re-aerosolized spores since the positive air samples were found at various locations in the beginning, middle, and end of the 8-hour sampling period.

Analysis of the remaining portion of the initially negative filter samples resulted in additional positive samples. Therefore, if the entire sample is not analyzed, there is a potential for false negative results (recalling that the initial analysis of filter media samples used only 10% of the sample). This can play a significant role in the selection of air sampling methodologies when considering the intent of sampling, e.g., screening, characterization, or clearance.

The positive air samples collected before and after DBCS operation demonstrate that all evaluated sample methods are capable of collecting *B. anthracis* spores and that re-aerosolization of *B. anthracis* spores by mechanical disruption is possible. Additionally, the positive results of the initial air sampling suggest that all evaluated methods are capable of collecting *B. anthracis* spores at low concentrations. Of the samples collected before DBCS operation, the Andersen appeared to be more sensitive than other air sampling methods. Additional advantages of the Andersen sampler include lower risk processing for the laboratory, quicker turn-around time, and less laboratory bias due to reduced sample processing. A disadvantage of the Andersen sampler is that the maximum sample collection time is limited to 20 minutes, depending upon the concentration. Operating this instrument is a labor-intensive, time-consuming process when compared to sampling with filters where one sample can be collected over long time periods, (hours).

The objective of the statistical analysis of Andersen sampler results was to assess the effect of sample location and time on *B. anthracis* concentration. The decline in *B. anthracis* concentration over time can be attributed to the influence of the pumps in effectively filtering the air inside the containment and the contribution of spore settling.

As part of the investigation into the pump influence in filtering containment air, theoretical

B. anthracis concentration curves were developed for the Andersen sampler, DFU, and the other filter media (combination of MCE, PTFE, and GEL) based on a purging equation for dilution ventilation, factoring in time, containment volume, and ventilation rate (in this case a collection rate).[20] *B. anthracis* concentrations and a decay curve were also established for the particle loss (spore settling) contribution.[21] For this calculation, it was assumed that there was uniform stirring of spores in the containment, spores inside the containment were 1 µm in diameter,[22,23] and the spore density was 1.1 g/cm^3.[24]

The 8-hour decay curve (Figure 6) reflects two distinct periods: (1) the first 2-hrs inside the containment when five Andersen samplers, three DFUs, and 36 pumps for MCE, PTFE, and GEL filter media were operating, totaling 1414 lpm and (2) the next 6-hour period when one DFU, 18 pumps for MCE, PTFE, and GEL filter media, and five Andersen samplers (one ten-minute session every two hours) were operating, totaling 578 lpm and 436 lpm when the Andersen samplers were in use and not in use, respectively. Additionally, it is assumed that the Andersen data (Figure 6) reflects the *B. anthracis* concentration inside the containment at the start of sampling (1st event) and at the 2-hour point (start of 2nd event). Specifically, the initial *B. anthracis* concentrations used for the first and second event were 245 CFU/m^3 and 60 CFU/m^3, respectively.

Figures 8 and 9 present the theoretical curves for each set of air sampling media (Andersen, DFU, other media), the settling curve, as well as a curve which combines all the sampling media representing the overall removal rate (the reduction or capture of *B. anthracis* in containment). The decay curves in both figures indicate that based on removal rate, the DFU would be expected to have more of an impact on the reduction of *B. anthracis* inside the containment than the other sampling media and may have influenced the capture potential of the other sampling media inside the containment.

Using the decay curves (Figures 8 and 9) and the data generated from the calculations above, an additional decay curve was generated for the two events and is presented in Figures 10 and 11. This curve is the combined effect that each set of air sampling media and spore settling provide to the overall decrease in *B. anthracis* concentration inside containment. The Andersen decay curve and the theoretical DFU decay curve are also provided to offer a visual reference for comparison. Assuming that the Andersen decay curve provides a time indication of the *B. anthracis* concentration in containment, the combined decay curve reflects the general trend shown by the Andersen sampler.

In the investigation of the Andersen sampler decay curve, the assumption was made that the *B. anthracis* spores were 1 µm in diameter. However, there is the possibility of agglomeration of spores into a particle greater than 1 µm in diameter. The larger particle size would result in a settling curve showing a sharper decrease in overall *B. anthracis* concentration inside containment over time. Subsequently, the combined decay curve would also indicate this decrease in concentration. Previous study into *B. anthracis* reaerosolization in the Hart Senate Office Building, Washington, D.C. suggests that 80% of *B. anthracis* spores ranged from 0.95 µm to 3.5 µm.[25] It is reasonable to suspect that *B. anthracis* spores in the TPDC also fall into this size range. However, size discrimination inside the TPDC is unknown.

Figures 8-11 suggest that the removal rate of the DFU (which is presumed high based on the large volume of air that is run through these samplers) would have a "cleansing" effect (removal of material) inside the containment. However, the *B. anthracis* concentrations were considerably lower than any of the other sampling methods. This may be indicative of spores passing through the filter due to the flow rate, spores passing around the filter due to a poor seal, and/or spores becoming imbedded in the filter to a degree where typical laboratory sample preparation was unable to extract them. This raises a concern with the reliability of quantitative data from the polyester felt filter. A second concern stems

from the extraction efficiency associated with each type of filter media. In general, as the extraction efficiency decreases so does the precision of the data, which can significantly impact the limit of detection for that analytical method. For example, a low airborne concentration may not provide enough spores for collection to overcome the limit of detection, resulting in the possibility of false negative results.

Further laboratory and field evaluation of these and other methods is necessary to understand their practical uses and limitations for the collection of *B. anthracis* in facilities contaminated from bioterrorist events. Analytical methods must be validated, including studies of the extraction efficiencies of sample collection matrices, to ensure consistent and reliable results from response laboratories. Greater confidence in environmental measurements can result in better informed public health decisions.

CONCLUSIONS

1. Although all types of air sampling media were capable of collecting *B. anthracis* spores before and after DBCS operation, the Andersen sampler appeared to be the most sensitive.

2. The DFUs were capable of sampling a substantial volume of air over extended time periods. However, compared to the *B. anthracis* concentrations found by the other sampling media, the low DFU concentrations suggest that spores may have passed through the instrument uncollected and/or spores were imbedded in the filter too deeply to be extracted.

3. Re-aerosolization of *B. anthracis* spores is possible and may occur from merely walking or conducting light work in a contaminated area.

4. The positive results for the ten "test deck" sheets suggests that it is possible for *B. anthracis* spores to adhere to sheets moving through an operating DBCS.

5. Further investigation into air sampling and analytical methods, specifically sample collection and extraction efficiencies, is necessary to have confidence in their reliability when used in potential future bioterrorism events.

RECOMMENDATIONS

NIOSH investigators provide the following recommendations based on findings from this investigation.

1. When air sampling is to be utilized for screening, characterizing, and/or clearing a *B. anthracis*-contaminated facility for re-occupancy, the Andersen sampler should be **one** of the instruments used.

2. When collecting *B. anthracis* using filter media, the entire filter should be analyzed to obtain the greatest sensitivity.

3. Laboratory and field evaluation of the methods used in this investigation and other methods available, as well as research into future air sampling methodologies, must be continued to provide bioterrorism responders the best available instruments to detect not only *B. anthracis* but also other bioterrorism agents.

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TABLES

Abbreviations and Symbols Used in the Following Tables, Figures, and Appendices

DBCS	=	delivery bar code sorter
MCE	=	mixed-cellulose ester filter
PTFE	=	polytetrafluorethylene filter
GEL	=	gelatin filter
DFU	=	dry filter unit
pos	=	positive sample (detectable colonies)
neg	=	negative sample (no detectable colonies)
tot	=	total number of samples
min	=	minutes
hr	=	hour
n/a	=	not applicable
CFUs	=	colony forming units
CFU/m^3	=	colony forming units per cubic meter of air
*	=	two sequential samples @ 60 min each
**	=	four sequential samples @ 120 min each
$	=	10% of the sample extract was analyzed
■	=	sample(s) were initially positive; therefore, no further analysis
†	=	ten sequential samples @ 10 min each @ five locations
††	=	one ten minute sample every 2 hours @ five locations
n/a	=	not applicable (100% of the sample was initially analyzed)
theo	=	theoretical curve (see Discussion Section)
calc	=	calculated curve (see Discussion Section)

Table 1. DBCS Operation and Test Kit Information (bins sheets sent to)

Start Time	Bins	Start Time	Bins
0927	Machine Start - 1, 2, 3	0956	32, 33, 34, 35
0934	Machine Jam	0958	64, 65, 66, 67, 190
0938	32, 33, 34	1000	96, 97, 98, 99
0940	32, 33, 34, 35	1004	1, 2, 3
0941	64, 65, 66, 67	1006	32, 33, 34, 35
0943	96, 97, 98, 99	1008	64, 65, 66, 67
0944	Machine Jam	1010	96, 97, 98, 99
0945	1, 2, 3	1012	1, 2, 3
0947	32, 33, 34, 35	1013	32, 33, 34, 35, 187, 188, 190
0948	64, 65, 66, 67	1017	64, 65, 66, 67
0949	96, 97, 98, 99	1019	96
0954	1, 2, 3	1020	Machine Stop
Total Sheets Run Through Machine = 6521			

Table 2. Initial Sampling Results for *B. anthracis* Presented By Location Before and After DBCS Operation

Test Description: Air Sampling Before DBCS Operation (Test Duration: 60, 120, and 480 minutes)

Sample Location	Gel Filters										MCE Filter				PTFE Filter				Andersen				DFU Sampler				Totals per Location	
	60 min		120 min		120 min		120 min		120 min		120 min		480 min		120 min		480 min		†120 min		††360 min		120 min		480 min			
	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot
1	0	2	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	2	10	1	3	—	—	—	—	3	23
2	0	2	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	2	10	0	3	—	—	—	—	2	23
3	0	2	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	10	2	3	—	—	—	—	2	23
4	0	2	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	1	10	0	3	0	1	—	—	1	24
5	0	2	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	3	10	1	3	0	1	—	—	4	24
6	0	2	0	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	—	—	—	—	—	—	0	1	0	11
Totals per Test	0	12	0	6	0	6	0	6	0	6	0	6	0	6	0	6	0	6	8	50	4	15	0	2	0	1	12	128

Test Description: Air Sampling After DBCS Operation (Test Duration: 60, 120 and 480 minutes)

Sample Location	Gel Filters										MCE Filter				PTFE Filter				Andersen				DFU Sampler				Totals per Location	
	60 min		120 min		120 min		120 min		120 min		120 min		480 min		120 min		480 min		†120 min		††360 min		120 min		480 min			
	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot	Pos	Tot
1	0	2	1	1	0	1	0	1	0	1	0	1	0	1	0	1	0	1	9	9	1	3	—	—	—	—	11	22
2	1	2	0	1	1	1	0	1	0	1	0	1	0	1	0	1	1	1	10	10	3	3	—	—	—	—	16	23
3	2	2	1	1	1	1	0	1	0	1	0	1	0	1	1	1	1	1	10	10	2	3	—	—	—	—	18	23
4	1	2	1	1	1	1	0	1	0	1	1	1	0	1	1	1	1	1	10	10	2	3	1	1	—	—	19	24
5	1	2	0	1	0	1	0	1	0	1	1	1	1	1	1	1	0	1	10	10	2	3	1	1	—	—	17	24
6	1	2	1	1	0	1	0	1	0	1	1	1	0	1	0	1	0	1	—	—	—	—	—	—	1	1	4	11
Totals per Test	6	12	4	6	3	6	0	6	0	6	3	6	1	6	3	6	3	6	49	49	10	15	2	2	1	1	85	127

†Sample media changed every 10 min for the 120 min sampling period ††360 min sampling period, samples were collected every two hours for 10 min.

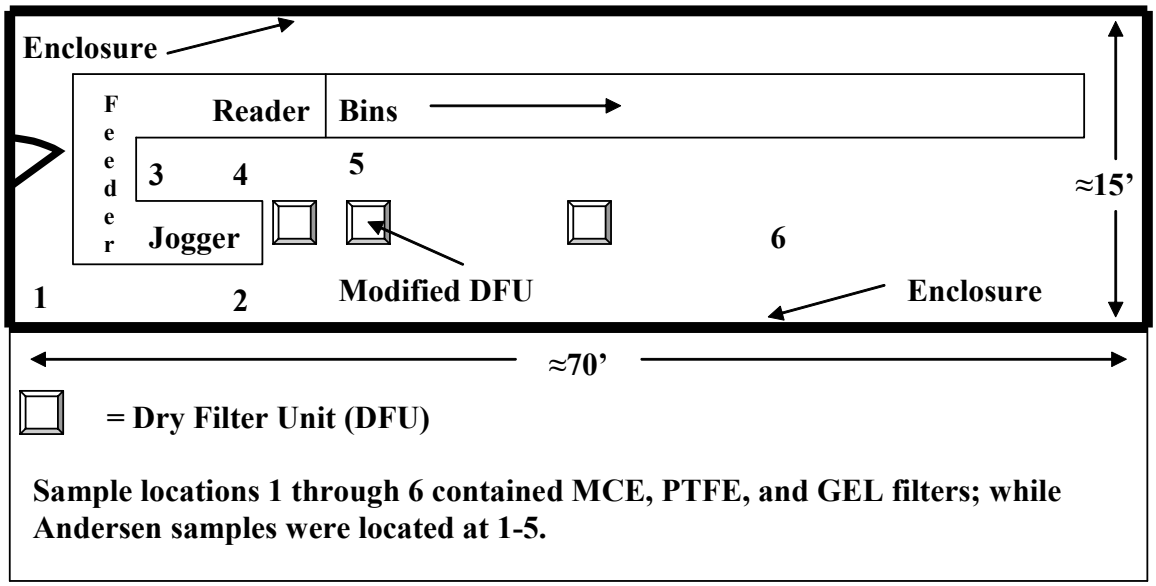
Table 3. Sample Results for *B. anthracis* Before and After DBCS Operation Per Sample

Sample Media	Time Period	Before DBCS Operation (10% of sample analyzed)	Before DBCS Operation (remaining sample analyzed)	After DBCS Operation (10% of sample analyzed)	After DBCS Operation (remaining sample analyzed)
		% Positive	% Positive	% Positive	% Positive
MCE	120 min	0% (0 of 6)	17% (1 of 6)	50% (3 of 6)	100% (3 of 3)
	480 min	0% (0 of 6)	0% (0 of 6)	17% (1 of 6)	100% (5 of 5)
PTFE	120 min	0% (0 of 6)	0% (0 of 6)	50% (3 of 6)	100% (3 of 3)
	480 min	0% (0 of 6)	17% (1 of 6)	50% (3 of 6)	100% (3 of 3)
Gel	120 min*	0% (0 of 12)	8% (1 of 12)	50% (6 of 12)	100% (6 of 6)
	480 min**	0% (0 of 24)	0% (0 of 24)	29% (7 of 24)	47% (8 of 17)
DFU	120 min	0% (0 of 2)	100% (2 of 2)	100% (2 of 2)	■
	480 min	0% (0 of 1)	100% (1 of 1)	100% (1 of 1)	■
Andersen	120 min†	16% (8 of 50)	n/a	100% (49 of 49)	n/a
	360 min††	27% (4 of 15)	n/a	67% (10 of 15)	n/a

* = two sequential samples @ 60 min each
** = four sequential samples @ 120 min each
■ = all samples were initially positive; therefore, no further analysis
† = ten sequential samples @ 10 min each @ five locations
†† = one sample every 2 hours @ five locations
n/a = not applicable (100% of the sample was initially analyzed)

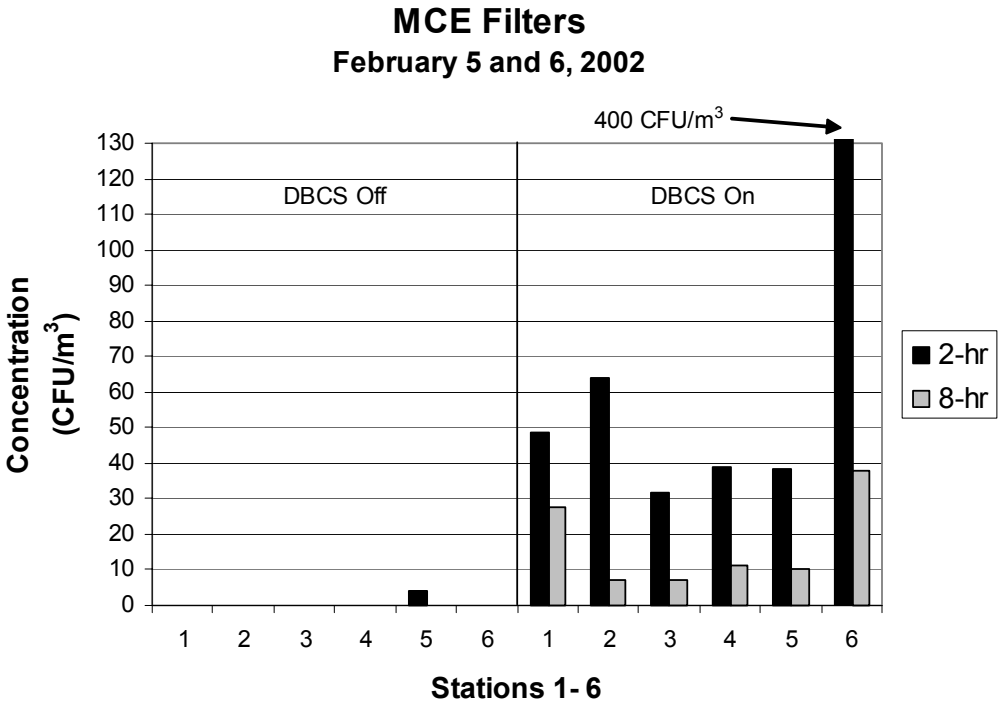
FIGURES

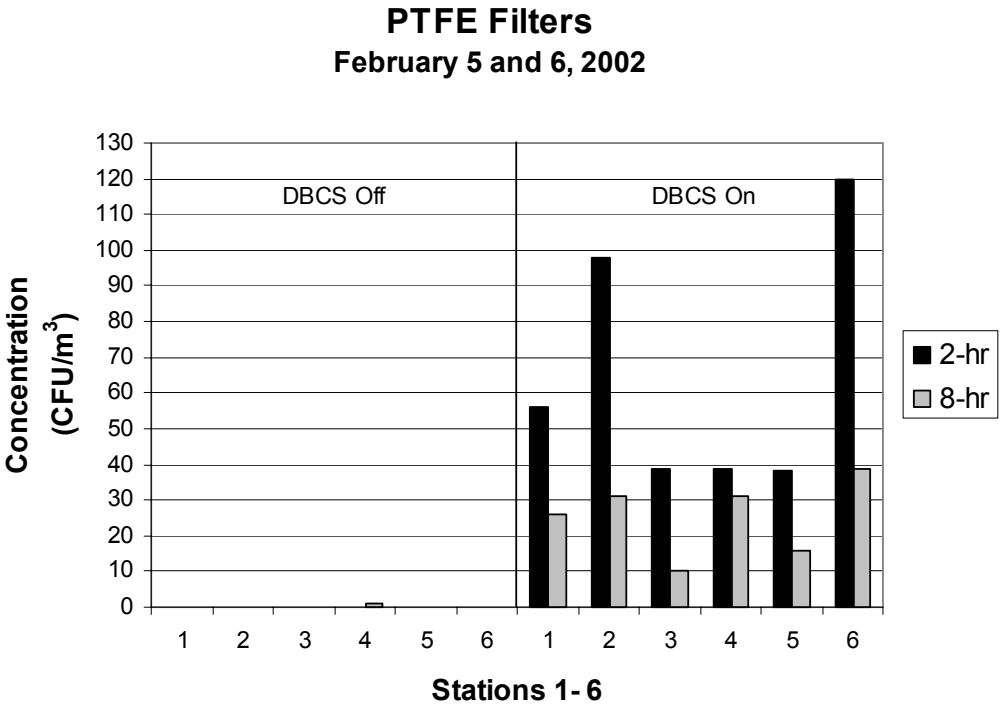
Figure 1. Diagram of the Air Sampling Locations Surrounding the DBCS



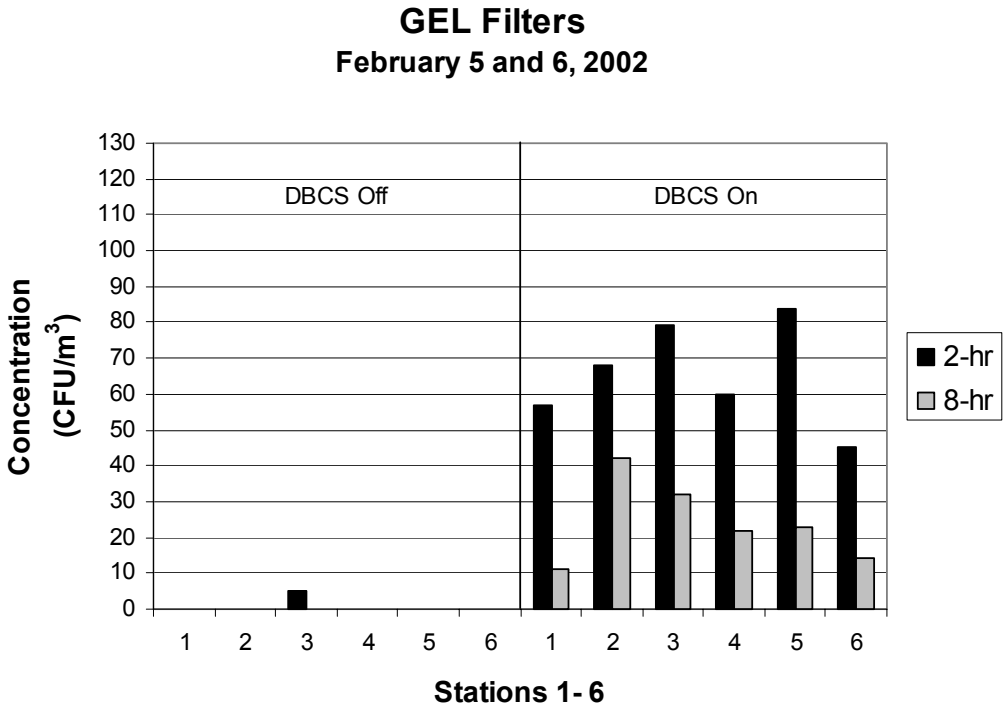
Sample locations 1 through 6 contained MCE, PTFE, and GEL filters; while Andersen samples were located at 1-5.

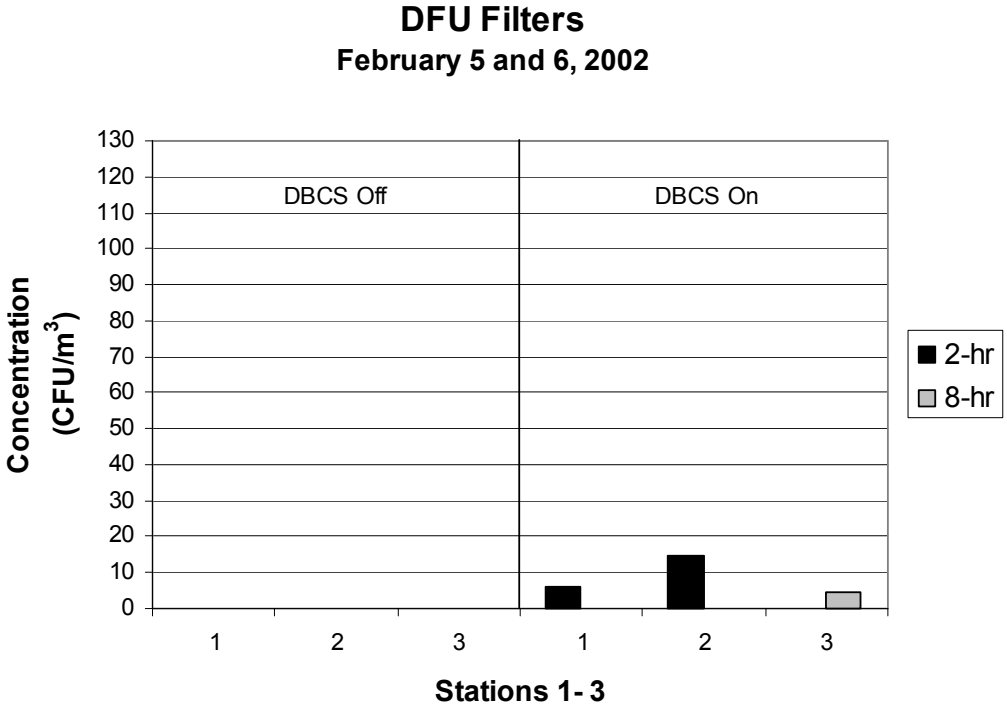
Figures 2 and 3. 2-hour and 8-hour Filter Sample *B. anthracis* Concentrations



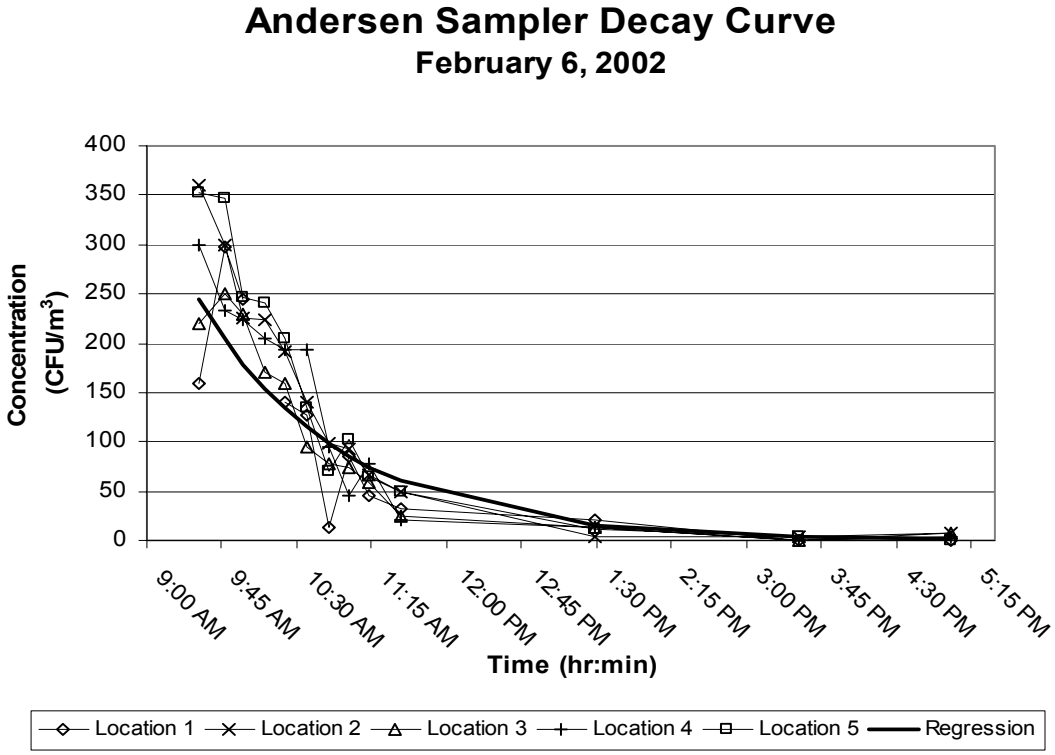


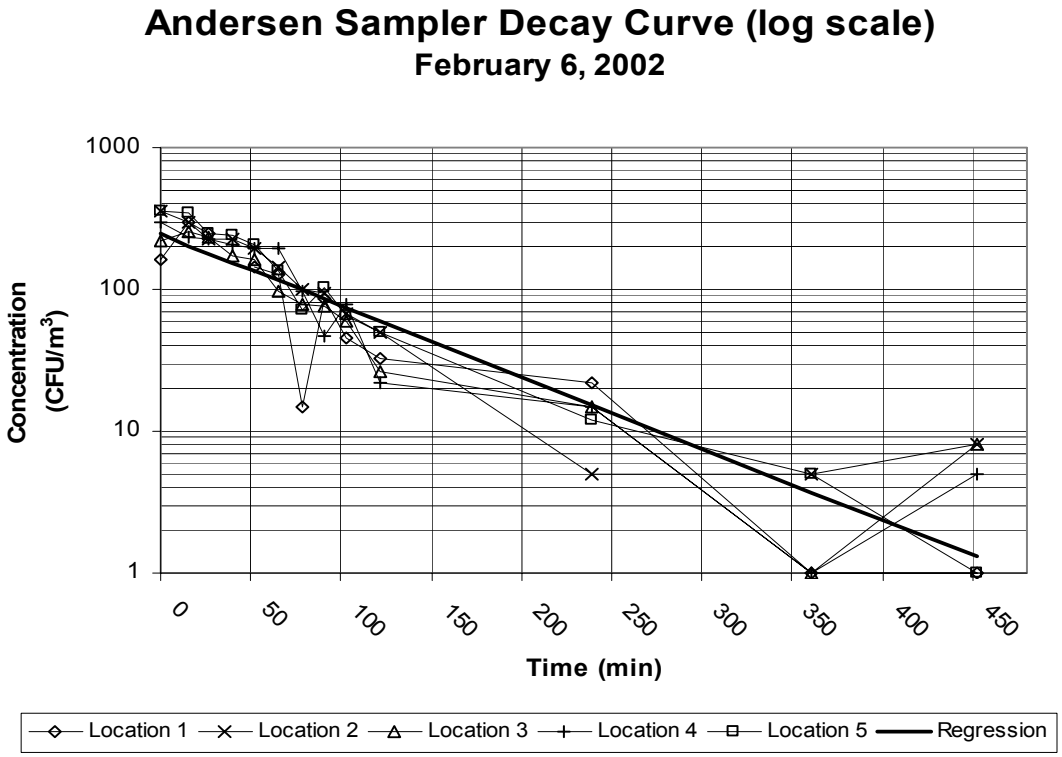
Figures 4 and 5. 2-hour and 8-hour Filter Sample *B. anthracis* Concentrations



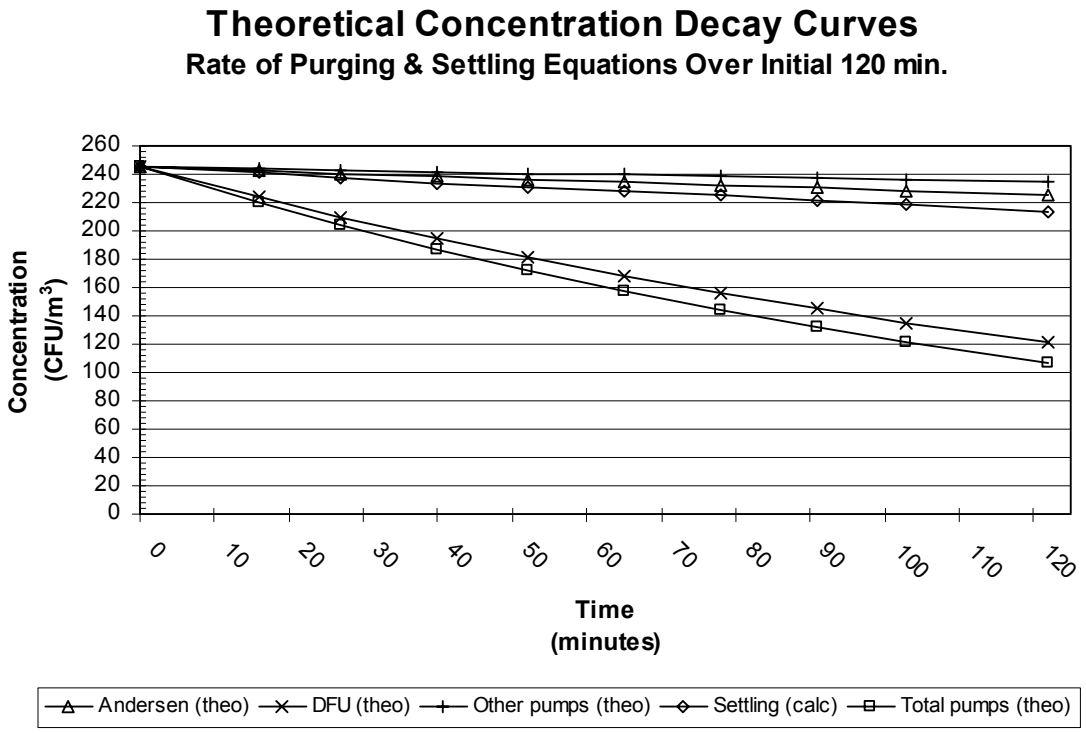


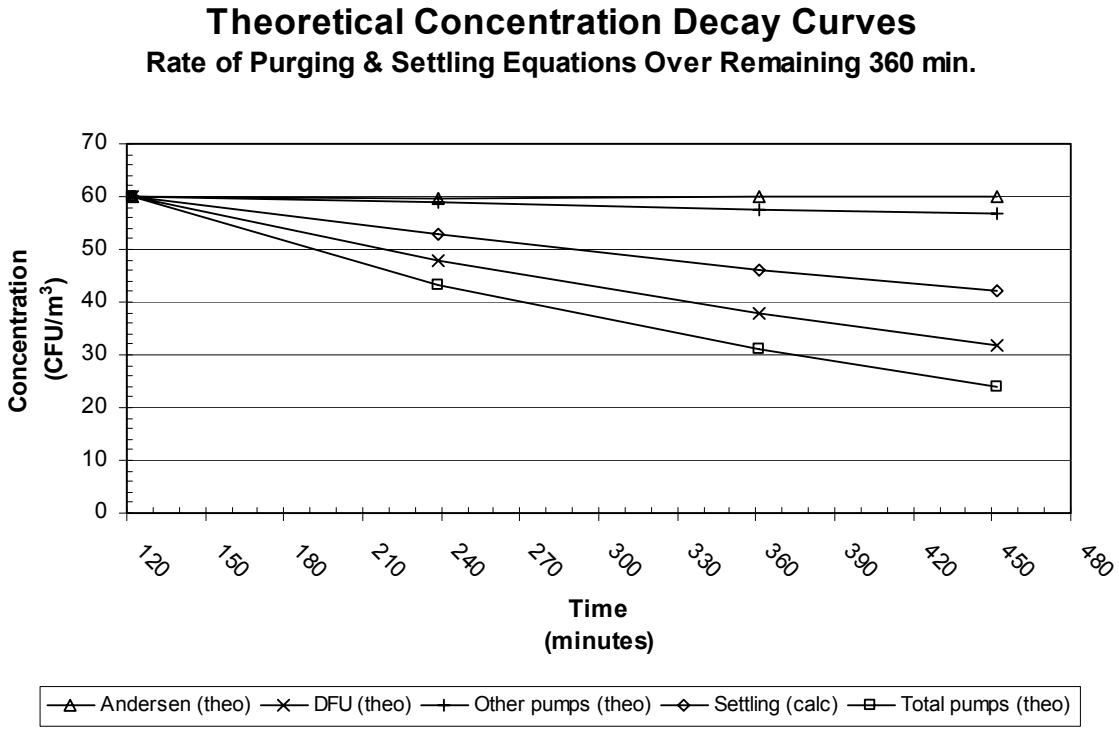
Figures 6 and 7. Andersen Sampler Decay Curves.



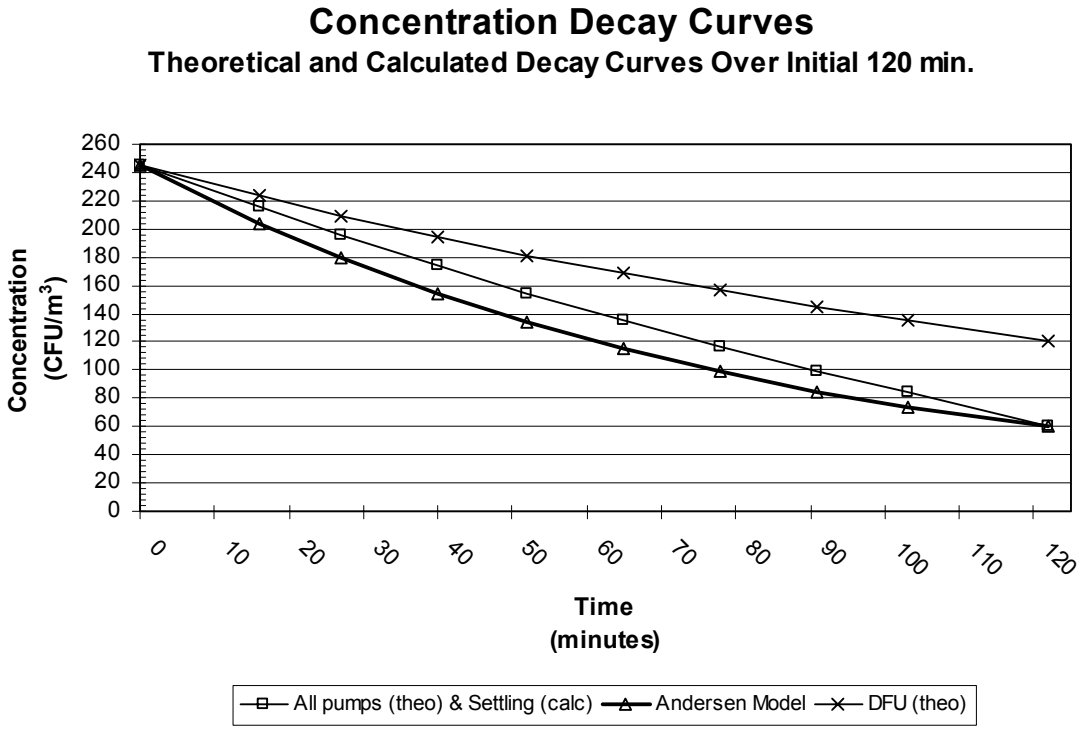


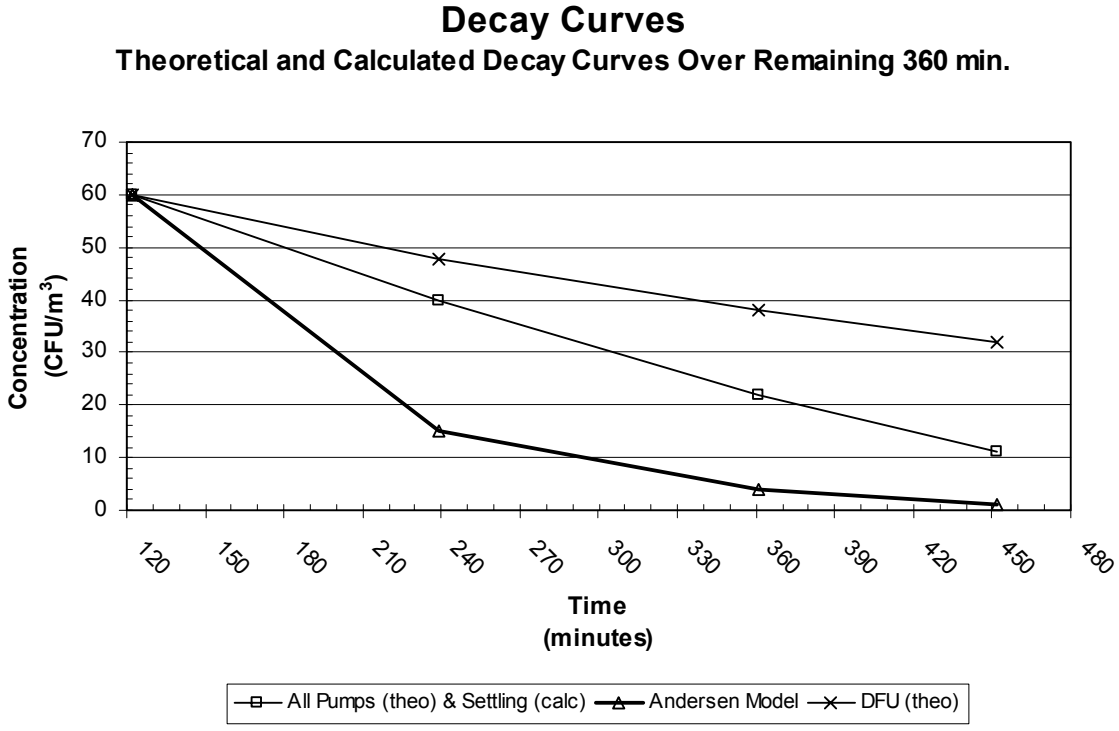
Figures 8 and 9. Rate of Purging and Settling Equations.





Figures 10 and 11. Combined Theoretical Decay Curve and Reference Curves.





APPENDICES

Appendix A. Wipe Sample Results for *B. anthracis*

Before DBCS Machine in Operation		
Sample Location	**Sample #**	**Concentration (CFUs)**
Jogger - table and frame	Wipe - 001	100
Feeder - transport and frame (front)	Wipe - 002	100
Feeder - transport (interior, back)	Wipe - 003	52
Reader (interior) - front section	Wipe - 004	100
Reader (interior) - back section	Wipe - 005	50
Column 1, bins 1-3 and printer	Wipe - 006	11
Column 2, bins 4-7	Wipe - 007	33
Column 3, bins 8-11	Wipe - 008	50
Column 4, bins 12-15	Wipe - 009	4
Column 5, bins 16-19	Wipe - 010	10
Column 6, bins 20-23	Wipe - 011	14
Column 7, bins 24-27	Wipe - 012	22
Column 8, bins 28-31	Wipe - 013	13
Column 9, bins 32-35	Wipe - 014	19
Column 10, bins 36-39	Wipe - 015	23
Column 11, bins 40-43	Wipe - 016	28
Column 12, bins 44-47	Wipe - 017	104
Column 13, bins 48-51	Wipe - 018	68
Column 14, bins 52-55	Wipe - 019	63
Column 15, bins 56-59	Wipe - 020	37
Column 16, bins 60-63	Wipe - 021	40
Column 17, bins 64-67	Wipe - 022	68
Column 18, bins 68-71	Wipe - 023	67
Column 19, bins 72-75	Wipe - 024	124
Column 20, bins 76-79	Wipe - 057	17
Column 21, bins 80-83	Wipe - 058	10

Appendix A. Wipe Sample Results for *B. anthracis*

Before DBCS Machine in Operation		
Sample Location	**Sample #**	**Concentration (CFUs)**
Column 22, bins 84-87	Wipe - 059	19
Column 23, bins 88-91	Wipe - 060	69
Column 24, bins 92-95	Wipe - 056	51
Column 25, bins 96-99	Wipe - 025	180
Column 26, bins 100-103	Wipe - 026	110
Column 27, bins 104-107	Wipe - 027	540
Column 28, bins 108-111	Wipe - 028	320
Column 29, bins 112-114	Wipe - 029	700
Column 30, bins 115-118	Wipe - 030	1,600
Column 31, bins 119-122	Wipe - 031	28,000
Column 32, bins 123-126	Wipe - 032	48,000
Column 33, bins 127-130	Wipe - 033	28,000
Column 34, bins 131-134	Wipe - 034	68,000
Column 35, bins 135-138	Wipe - 035	110,000
Column 36, bins 139-142	Wipe - 036	750,000
Column 37, bins 143-146	Wipe - 037	600,000
Column 38, bins 147-150	Wipe - 038	690,000
Column 39, bins 151-154	Wipe - 039	1,500,000
Column 40, bins 155-158	Wipe - 040	2,000,000
Column 41, bins 159-162	Wipe - 041	6,300,000
Column 42, bins 163-166	Wipe - 042	690,000
Column 43, bins 167-170	Wipe - 043	800,000,000
Column 44, bins 171-174	Wipe - 044	130,000
Column 45, bins 175-178	Wipe - 045	60,000
Column 46, bins 179-182	Wipe - 046	110,000
Column 47, bins 183-186	Wipe - 047	21,000
Column 48, bins 187-190	Wipe - 048	70,000

Appendix A. Wipe Sample Results for *B. anthracis*

After DBCS Machine in Operation		
Sample Location	**Sample #**	**Concentration (CFUs)**
Jogger - table and frame	Wipe - 116	26
Feeder table (open end)	Wipe - 117	12
Feeder assembly (interior)	Wipe - 118	27
Reader (interior) - front section	Wipe - 119	57
Reader (interior) - back section	Wipe - 120	3,500
Column 1, bins 1-3	Wipe - 061	3
Column 2, bins 4-7	Wipe - 062	32
Column 3, bins 8-11	Wipe - 063	45
Column 4, bins 12-15	Wipe - 064	7
Column 5, bins 16-19	Wipe - 065	18
Column 6, bins 20-23	Wipe - 066	68
Column 7, bins 24-27	Wipe - 067	17
Column 8, bins 28-31	Wipe - 068	6
Column 9, bins 32-35	Wipe - 069	22
Column 10, bins 36-39	Wipe - 070	6
Column 11, bins 40-43	Wipe - 071	15
Column 12, bins 44-47	Wipe - 072	1
Column 13, bins 48-51	Wipe - 073	15
Column 14, bins 52-55	Wipe - 074	5
Column 15, bins 56-59	Wipe - 075	22
Column 16, bins 60-63	Wipe - 076	70
Column 17, bins 64-67	Wipe - 077	100
Column 18, bins 68-71	Wipe - 078	49
Column 19, bins 72-75	Wipe - 079	32
Column 20, bins 76-79	Wipe - 080	56
Column 21, bins 80-83	Wipe - 081	17

Appendix A. Wipe Sample Results for *B. anthracis*

After DBCS Machine in Operation		
Sample Location	**Sample #**	**Concentration (CFUs)**
Column 22, bins 84-87	Wipe - 082	26
Column 23, bins 88-91	Wipe - 083	111
Column 24, bins 92-95	Wipe - 084	123
Column 25, bins 96-99	Wipe - 085	97
Column 26, bins 100-103	Wipe - 086	150
Column 27, bins 104-107	Wipe - 087	3,200
Column 28, bins 108-111	Wipe - 089	1,800
Column 29, bins 112-114	Wipe - 088	5,200
Column 30, bins 115-118	Wipe - 090	5,700
Column 31, bins 119-122	Wipe - 091	15,000
Column 32, bins 123-126	Wipe - 092	6,500
Column 33, bins 127-130	Wipe - 093	12,000
Column 34, bins 131-134	Wipe - 094	9,800
Column 35, bins 135-138	Wipe - 095	12,000
Column 36, bins 139-142	Wipe - 096	20,000
Column 37, bins 143-146	Wipe - 097	11,000
Column 38, bins 147-150	Wipe - 098	12,000
Column 39, bins 151-154	Wipe - 099	11,000
Column 40, bins 155-158	Wipe - 100	25,000
Column 41, bins 159-162	Wipe - 101	13,000
Column 42, bins 163-166	Wipe - 102	20,000
Column 43, bins 167-170	Wipe - 103	25,000
Column 44, bins 171-174	Wipe - 104	8,000
Column 45, bins 175-178	Wipe - 105	3,500
Column 46, bins 179-182	Wipe - 106	8,700
Column 47, bins 183-186	Wipe - 107	6,200
Column 48, bins 187-190	Wipe - 108	6,800

Appendix B. Andersen Sampler Air Sampling Results for *B. anthracis*

Before DBCS Machine in Operation				
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**Concentration (CFU/m^3)**
SBA - 054	1	0948 - 0958	280	4
SBA - 053	1	1006 - 1016	280	0
SBA - 060	1	1020 - 1030	280	0
SBA - 044	1	1049 - 1102	364	3
SBA - 025	1	1102 - 1112	280	0
SBA - 018	1	1120 - 1130	280	0
SBA - 005	1	1135 - 1145	280	0
SBA - 032	1	1153 - 1203	280	0
SBA - 034	1	1211 - 1221	280	0
SBA - 029	1	1229 - 1239	280	0
SBA - 013	1	1350 - 1400	280	0
SBA - 017	1	1554 - 1604	280	0
SBA - 071	1	1710 - 1720	280	4
SBA - 055	2	0947 - 0958	308	0
SBA - 052	2	1006 - 1016	280	0
SBA - 059	2	1033 - 1043	280	0
SBA - 042	2	1049 - 1102	364	0
SBA - 024	2	1102 - 1112	280	0
SBA - 022	2	1120 - 1130	280	0
SBA - 019	2	1135 - 1145	280	4
SBA - 020	2	1153 - 1203	280	4
SBA - 010	2	1211 - 1221	280	0
SBA - 049	2	1229 - 1239	280	0
SBA - 003	2	1350 - 1400	280	0
SBA - 016	2	1554 - 1604	280	0
SBA - 070	2	1710 - 1720	280	0

Appendix B. Andersen Sampler Air Sampling Results for *B. anthracis*

Before DBCS Machine in Operation				
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**Concentration (CFU/m^3)**
SBA - 056	3	0947 - 0958	308	0
SBA - 051	3	1006 - 1016	280	0
SBA - 041	3	1020 - 1030	280	0
SBA - 043	3	1049 - 1102	364	0
SBA - 038	3	1102 - 1112	280	0
SBA - 004	3	1120 - 1130	280	0
SBA - 006	3	1135 - 1145	280	0
SBA - 033	3	1153 - 1203	280	0
SBA - 011	3	1211 - 1221	280	0
SBA - 001	3	1229 - 1239	280	0
SBA - 002	3	1350 - 1400	280	4
SBA - 050	3	1554 - 1604	280	0
SBA - 073	3	1710 - 1720	280	7
SBA - 057	4	0947 - 0958	308	0
SBA - 048	4	1006 - 1016	280	0
SBA - 046	4	1033 - 1043	280	0
SBA - 036	4	1049 - 1102	364	0
SBA - 037	4	1102 - 1112	280	4
SBA - 021	4	1120 - 1130	280	0
SBA - 031	4	1135 - 1145	280	0
SBA - 039	4	1153 - 1203	280	0
SBA - 009	4	1211 - 1221	280	0
SBA - 014	4	1229 - 1239	280	0
SBA - 015	4	1350 - 1400	280	0
SBA - 079	4	1554 - 1604	280	0
SBA - 072	4	1710 - 1720	280	0

Appendix B. Andersen Sampler Air Sampling Results for *B. anthracis*

Before DBCS Machine in Operation				
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**Concentration (CFU/m^3)**
SBA - 058	5	0947 - 1058	308	3
SBA - 047	5	1006 - 1016	280	4
SBA - 045	5	1033 - 1043	280	0
SBA - 035	5	1049 - 1102	364	0
SBA - 023	5	1102 - 1112	280	0
SBA - 027	5	1120 - 1130	280	0
SBA - 007	5	1135 - 1145	280	0
SBA - 008	5	1153 - 1203	280	0
SBA - 028	5	1211 - 1221	280	7
SBA - 030	5	1229 - 1239	280	0
SBA - 012	5	1350 - 1400	280	0
SBA - 077	5	1554 - 1604	280	0
SBA - 080	5	1710 - 1720	280	4

Appendix B. Andersen Sampler Air Sampling Results for *B. anthracis*

After DBCS Machine in Operation				
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**Concentration (CFU/m^3)**
SBA - 103	1	0931 - 0941	280	159
SBA - 131	1	0947 - 0957	280	297
SBA - 125	1	0958 - 1008	280	244
SBA - 154	1	1011 - 1021	280	no impact holes (blank)
SBA - 152	1	1023 - 1033	280	141
SBA - 118	1	1036 - 1046	280	127
SBA - 123	1	1049 - 1059	280	14
SBA - 107	1	1102 - 1112	280	85
SBA - 093	1	1114 - 1125	308	45
SBA - 082	1	1133 - 1143	280	32
SBA - 134	1	1330 - 1340	280	21
SBA - 150	1	1532 - 1542	280	0
SBA - 146	1	1703 - 1713	280	0
SBA - 109	2	0931 - 0941	280	360
SBA - 129	2	0947 - 0957	280	300
SBA - 127	2	0958 - 1008	280	226
SBA - 159	2	1011 - 1021	280	223
SBA - 115	2	1023 - 1033	280	191
SBA - 114	2	1036 - 1046	280	141
SBA - 111	2	1049 - 1059	280	99
SBA - 143	2	1102 - 1112	280	92
SBA - 158	2	1114 - 1125	308	67
SBA - 089	2	1133 - 1143	280	49
SBA - 094	2	1330 - 1340	280	4
SBA - 148	2	1532 - 1542	280	4
SBA - 145	2	1703 - 1713	280	7

Appendix B. Andersen Sampler Air Sampling Results for *B. anthracis*

After DBCS Machine in Operation				
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**Concentration (CFU/m^3)**
SBA - 104	3	0931 - 0941	280	219
SBA - 130	3	0947 - 0957	280	251
SBA - 138	3	0958 - 1008	280	230
SBA - 153	3	1011 - 1021	280	170
SBA - 140	3	1023 - 1033	280	159
SBA - 105	3	1036 - 1046	280	95
SBA - 155	3	1049 - 1059	280	78
SBA - 157	3	1102 - 1112	280	74
SBA - 144	3	1114 - 1125	308	58
SBA - 084	3	1133 - 1143	280	25
SBA - 122	3	1330 - 1340	280	14
SBA - 126	3	1532 - 1542	280	0
SBA - 135	3	1703 - 1713	280	7
SBA - 110	4	0931 - 0941	280	300
SBA - 116	4	0947 - 0957	280	233
SBA - 117	4	0958 - 1008	280	223
SBA - 100	4	1011 - 1021	280	205
SBA - 120	4	1023 - 1033	280	194
SBA - 097	4	1036 - 1046	280	194
SBA - 151	4	1049 - 1059	280	95
SBA - 147	4	1102 - 1112	280	46
SBA - 142	4	1114 - 1125	308	77
SBA - 112	4	1133 - 1143	280	21
SBA - 096	4	1330 - 1340	280	14
SBA - 133	4	1532 - 1542	280	0
SBA - 136	4	1703 - 1713	280	4

Appendix B. Andersen Sampler Air Sampling Results for *B. anthracis*

After DBCS Machine in Operation				
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**Concentration (CFU/m^3)**
SBA - 102	5	0931 - 0941	280	353
SBA - 128	5	0947 - 0957	280	346
SBA - 139	5	0958 - 1008	280	247
SBA - 101	5	1011 - 1021	280	240
SBA - 119	5	1023 - 1033	280	205
SBA - 121	5	1036 - 1046	280	134
SBA - 160	5	1049 - 1059	308	71
SBA - 091	5	1102 - 1112	280	102
SBA - 141	5	1114 - 1125	308	64
SBA - 113	5	1133 - 1143	280	49
SBA - 124	5	1330 - 1340	280	11
SBA - 108	5	1532 - 1542	280	4
SBA - 137	5	1703 - 1713	280	0

Appendix C. Mixed-Cellulose Ester Filter Air Sampling Results for *B. anthracis*

Before DBCS Machine in Operation					
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**10%[$] ($CFU/m^3$)**	**Remainder (CFU/m^3)**
MCE - 004	1	0949 - 1145	232	0	0
MCE - 001	1	0949 - 1724	910	0	0
MCE - 007	2	0949 - 1145	232	0	0
MCE - 003	2	0949 - 1724	910	0	0
MCE - 005	3	0949 - 1145	232	0	0
MCE - 011	3	0949 - 1724	910	0	0
MCE - 019	4	0949 - 1145	232	0	0
MCE - 006	4	0949 - 1724	910	0	0
MCE - 009	5	0949 - 1145	232	0	4
MCE - 018	5	0949 - 1724	910	0	0
MCE - 013	6	0949 - 1145	232	0	0
MCE - 016	6	0949 - 1724	910	0	0

Appendix C. Mixed-Cellulose Ester Filter Air Sampling Results for *B. anthracis*

After DBCS Machine in Operation					
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**10%[$] ($CFU/m^3$)**	**Remainder (CFU/m^3)**
MCE - 038	1	0927 - 1130	246	0	49
MCE - 025	1	0927 - 1734	974	0	28
MCE - 030	2	0927 - 1140	266	0	64
MCE - 036	2	0927 - 1730	966	0	7
MCE - 035	3	0927 - 1134	254	0	31
MCE - 029	3	0927 - 1731	968	0	7
MCE - 032	4	0927 - 1135	256	4	■
MCE - 031	4	0927 - 1732	970	0	11
MCE - 021	5	0927 - 1138	262	4	■
MCE - 022	5	0927 - 1728	962	1	■
MCE - 023	6	0927 - 1132	250	40	■
MCE - 026	6	0927 - 1734	974	0	38

Appendix D. Polytetrafluoroethylene Filter Air Sampling Results for *B. anthracis*

Before DBCS Machine in Operation					
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**10%[$] ($CFU/m^3$)**	**Remainder (CFU/m^3)**
PTFE - 004	1	0949 - 1145	232	0	0
PTFE - 001	1	0949 - 1724	910	0	0
PTFE - 007	2	0949 - 1145	232	0	0
PTFE - 006	2	0949 - 1724	910	0	0
PTFE - 015	3	0949 - 1145	232	0	0
PTFE - 013	3	0949 - 1724	910	0	0
PTFE - 017	4	0949 - 1145	232	0	0
PTFE - 009	4	0949 - 1724	910	0	1
PTFE - 003	5	0949 - 1145	232	0	0
PTFE - 008	5	0949 - 1724	910	0	0
PTFE - 002	6	0949 - 1145	232	0	0
PTFE - 014	6	0949 - 1724	910	0	0

Appendix D. Polytetrafluoroethylene Filter Air Sampling Results for *B. anthracis*

After DBCS Machine in Operation					
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**10%[$] ($CFU/m^3$)**	**Remainder (CFU/m^3)**
PTFE - 038	1	0927 - 1131	248	0	56
PTFE - 022	1	0927 - 1734	974	0	26
PTFE - 030	2	0927 - 1134	254	0	98
PTFE - 031	2	0927 - 1730	966	3	■
PTFE - 029	3	0927 - 1134	254	4	■
PTFE - 039	3	0927 - 1731	968	1	■
PTFE - 025	4	0927 - 1135	256	4	■
PTFE - 040	4	0927 - 1732	970	3	■
PTFE - 027	5	0927 - 1138	262	4	■
PTFE - 026	5	0927 - 1728	962	0	16
PTFE - 036	6	0927 - 1132	250	0	120
PTFE - 037	6	0927 - 1734	974	0	39

Appendix E. Gelatin Filter Air Sampling Results for *B. anthracis*

Before DBCS Machine in Operation					
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**10%[$] ($CFU/m^3$)**	**Remainder (CFU/m^3)**
GEL - 027	1	0949 - 1050	122	0	0
GEL - 001	1	1050 - 1145	110	0	0
GEL - 019	1	0949 - 1145	232	0	0
GEL - 031	1	1145 - 1351	252	0	0
GEL - 025	1	1351 - 1548	234	0	0
GEL - 026	1	1548 - 1724	192	0	0
GEL - 020	2	0949 - 1051	124	0	0
GEL - 009	2	1051 - 1145	108	0	0
GEL - 008	2	0949 - 1148	238	0	0
GEL - 013	2	1148 - 1355	254	0	0
GEL - 036	2	1356 - 1549	226	0	0
GEL - 022	2	1549 - 1724	190	0	0
GEL - 018	3	0949 - 1052	126	0	0
GEL - 002	3	1052 - 1145	106	0	9
GEL - 004	3	0949 - 1149	240	0	0
GEL - 023	3	1149 - 1358	258	0	0
GEL - 030	3	1358 - 1550	224	0	0
GEL - 038	3	1550 - 1724	188	0	0
GEL - 006	4	0949 - 1053	128	0	0
GEL - 010	4	1053 - 1145	104	0	0
GEL - 011	4	0949 - 1130	202	0	0
GEL - 015	4	1150 - 1358	256	0	0
GEL - 021	4	1358 - 1551	226	0	0
GEL - 028	4	1551 - 1724	186	0	0

Appendix E. Gelatin Filter Air Sampling Results for *B. anthracis*

Before DBCS Machine in Operation					
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**10%[$] ($CFU/m^3$)**	**Remainder (CFU/m^3)**
GEL - 007	5	0949 - 1054	130	0	0
GEL - 005	5	1054 - 1145	102	0	0
GEL - 016	5	0949 - 1151	244	0	0
GEL - 012	5	1151 - 1402	262	0	0
GEL - 037	5	1404 - 1551	214	0	0
GEL - 029	5	1552 - 1724	184	0	0
GEL - 003	6	0949 - 1055	132	0	0
GEL - 014	6	1056 - 1145	98	0	0
GEL - 017	6	0949 - 1152	246	0	0
GEL - 032	6	1152 - 1404	264	0	0
GEL - 035	6	1404 - 1553	218	0	0
GEL - 024	6	1554 - 1724	180	0	0

Appendix E. Gelatin Filter Air Sampling Results for *B. anthracis*

After DBCS Machine in Operation					
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**10%[$] ($CFU/m^3$)**	**Remainder (CFU/m^3)**
GEL - 098	1	0927 - 1027	120	0	75
GEL - 079	1	1027 - 1130	126	0	40
GEL - 096	1	0927 - 1128	242	4	■
GEL - 067	1	1128 - 1321	226	0	4
GEL - 064	1	1322 - 1521	238	0	0
GEL - 063	1	1521 - 1734	266	0	0
GEL - 089	2	0927 - 1029	124	8	■
GEL - 094	2	1029 - 1140	142	0	56
GEL - 090	2	0927 - 1140	272	0	120
GEL - 075	2	1140 - 1322	204	5	■
GEL - 070	2	1323 - 1522	238	0	0
GEL - 074	2	1522 - 1730	256	0	0
GEL - 100	3	0927 - 1027	120	8	■
GEL - 076	3	1027 - 1134	134	7	■
GEL - 085	3	0927 - 1135	256	8	■
GEL - 077	3	1135 - 1323	216	5	■
GEL - 095	3	1324 - 1522	236	0	4
GEL - 065	3	1523 - 1731	256	0	0
GEL - 062	4	0927 - 1027	120	8	■
GEL - 081	4	1027 - 1135	136	0	37
GEL - 080	4	0927 - 1135	256	4	■
GEL - 071	4	1136 - 1324	216	5	■
GEL - 088	4	1324 - 1523	238	0	4
GEL - 099	4	1524 - 1732	256	0	0

Appendix E. Gelatin Filter Air Sampling Results for *B. anthracis*

After DBCS Machine in Operation					
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**10%[$] ($CFU/m^3$)**	**Remainder (CFU/m^3)**
GEL - 072	5	0927 - 1028	122	8	■
GEL - 092	5	1028 - 1137	138	0	87
GEL - 073	5	0927 - 1139	264	0	80
GEL - 066	5	1140 - 1325	210	0	10
GEL - 068	5	1325 - 1524	238	0	4
GEL - 084	5	1525 - 1728	246	0	0
GEL - 086	6	0927 - 1028	122	8	■
GEL - 083	6	1028 - 1132	128	0	8
GEL - 061	6	0927 - 1133	252	4	■
GEL - 091	6	1133 - 1326	226	0	18
GEL - 069	6	1326 - 1526	240	0	0
GEL - 082	6	1526 - 1734	256	0	0

Appendix F. Dry Filter Unit Air Sampling Results for *B. anthracis*

Before DBCS Machine in Operation					
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**10%[$] ($CFU/m^3$)**	**Remainder (CFU/m^3)**
DFU - 001	4	0949 - 1153	49,600	0	< 1
DFU - 015	5	0949 - 1153	49,600	0	< 1
DFU - 005	6	0949 - 1729	184,000	0	< 1

After DBCS Machine in Operation					
Sample #	**Sample Location**	**Sample Time (military)**	**Volume (liters)**	**10%[$] ($CFU/m^3$)**	**Remainder (CFU/m^3)**
DFU - 008	4	0927 - 1134	50,800	6	■
DFU - 010	5	0927 - 1134	50,800	15	■
DFU - 011	6	0927 - 1727	192,000	5	■

Appendix G. Test Kit Sheet Sample Results for *B. anthracis*

Test Kit Sheet	10%[$] (CFUs)	Remainder (CFUs)
1	0	1
2	0	3
3	0	4
4	0	3
5	0	3
6	0	3
7	0	4
8	0	2
9	1	#
10	0	5

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