

## ***Bacillus anthracis* SPORES (ETIOLOGIC AGENT OF ANTHRAX) IN AIR**

Richard Lawrence, OSHA Salt Lake Technical Center

[richard.Lawrence@osha.gov](mailto:richard.Lawrence@osha.gov)

### **ABSTRACT**

*Bacillus anthracis* spores in air are collected utilizing a Dry Filter Unit (DFU) developed by the Joint Program Office for Biological Defense (JPO-BD). The unit consists of a high flow air sampling pump that collects airborne spores on 47-mm polyester filters (PEF-1 filters). After collection, each filter may be transferred into a 50-mL conical tube or may be left in the sample filter holder and transferred into a 50-mL conical tube in the laboratory. In the laboratory, the sample is prepared in a manner that will yield a suspension. Aliquots of the suspension may be assayed by different microbiological techniques depending on the sensitivity needed. Traditional culture techniques provide the most sensitivity. Detection limits are 200 colony forming units (CFU) per sample by Polymerase Chain Reaction (PCR) assay,<sup>1</sup> or 2 CFU per sample by standard culture technique. The DFU sampler has been evaluated by the Battelle Memorial Institute and the testing results were reviewed by OSHA SLTC personnel. This sampling and assay technique is now an approved OSHA method, OSA7.

### **GENERAL DISCUSSION**

#### **Background**

A dry filter unit (DFU) has been developed through funding by the Joint Program Office for Biological Defense (JPO-BD) to determine the presence of biological materials in air. The U.S. military has operated 200 DFU samplers continuously for four months without any sampler failures. Over 20,000 anthrax samples have been collected. Air samples are collected on 47-mm polyester felt filters (PEF-1 filters). After sampling, the filters are removed from the DFU, extracted, and analyzed by various means to determine the presence or absence of biological materials in air. The Battelle Memorial Institute conducted tests to evaluate and optimize the performance of the DFU.<sup>1,2</sup> Among other tests, sampling was done from "clouds" of *Bacillus globigii* (Bg) spores generated at Battelle's Ambient Breeze Tunnel (ABT) test facility.

Both OSHA and NIOSH agree that the Anderson Cascade sampler may be more efficient in collecting anthrax spores than the High Volume Dry Filter Unit. There are several situations where using the Anderson sampler would be the preferred sampling technique (e.g., near surfaces that are being agitated or in small volume rooms or enclosures). Unfortunately the

Anderson sampler is restricted to sampling a small volume of air.<sup>3</sup> The increased efficiency advantage cannot be applied to large volumes of air unless multiple Anderson samples are taken. The Anderson samplers have a flow rate limited to 30 liters per minute. Sampling can proceed for only a few minutes and must stop before the agar begins to desiccate. The DFU units can sample for hours and even days at 300 liters per minute. Similar confidence in sampling results may take tens or hundreds of Anderson samples for equivalent large air volumes. Also, collection on filter media allows for a sample preparation step (heat shock, for example) that is selective for the spores and will eliminate many competitive bacteria if they are present. The direct impact onto agar media, when using the Anderson sampler, does not allow for a similar selectivity. Since OSHA will normally be sampling large volume areas (e.g., large rooms and production areas), high volume samplers will typically be used.

An evaluation of air sampling methods was conducted at the Post Offices' Trenton Processing and Distribution Center. Containment was built around a contaminated Delivery Bar Code Sorter (DBCS). Air sampling was conducted inside of the containment when the DBCS was not operating and also when the DBCS was operating with a test deck of envelopes. An assumption was made that the contaminant concentration in the air was lower when the DBCS was not operating and that the concentration was greater when the DBCS was operating, but the actual contaminant concentration was unknown. Three DFU samples and 65 Anderson samples were collected when the DBCS was not operating. All three of the DFU samples were positive (100%), of the Anderson samples, 19% were positive. Three DFU samples and 65 Anderson samples were collected when the DBCS was operating. All three of the DFU samples were positive (100%), of the Anderson samples, 91% were positive.<sup>4</sup>

### **Description of *Bacillus anthracis***

*B. anthracis* is the etiologic agent of the disease anthrax. It is a large, gram-positive, non-motile, hemolytic, spore-forming bacillus, 1-1.3  $\mu\text{m}$   $\times$  3-10  $\mu\text{m}$ . It grows on ordinary laboratory media at 37 °C, producing round, grayish-white, convex colonies having comma-shaped outshootings with a ground glass appearance that measure 2-5 mm in diameter. The spore form can persist in nature for prolonged periods, possibly years or even decades.<sup>4</sup>

### **Battelle Memorial Institute Evaluation Tests**

*Detection Limit.* The limit of detection, using PCR, was reported to be less than about 2 Agent Containing Particles per Liter of Air (ACPLA) minutes. This was equivalent to 200 CFU per sample.

*Extraction Efficiency.* The average extraction efficiency of *B. anthracis* from liquid-spiked filters was reported to be 68%. The concentration range studied was 9.0E+04 to 9.9E+07 CFU/filter.

## **SAMPLING PROCEDURE**

(All safety practices that apply to the work area being sampled must be followed.) Refer to the OSHA Anthrax e-tool for detailed descriptions of appropriate PPE.<sup>6</sup>

## Sampling Apparatus

*Sampling Pump.* Air sampling is accomplished by using a Dry Filter Unit (DFU) which was developed by the JPO-BD. It is a portable device that operates on AC or DC power and draws air through 1-micron polyester felt filters. It weighs approximately 32 pounds and is 13 inches wide x 13 inches deep x 15 inches high. Single filter unit (SFU) and 3-filter units are available. This procedure is prepared for the (SFU). A single filter DFU is shown in Figures 2.1.1 and 2.1.2.



Figure 2.1.1 DFU with lid up to expose the internal pump and filter assembly. The muffer is stored in the lid.



Figure 2.1.2 DFU with muffer and air inlet snorkel in place.

*Filters.* 47-mm (1-7/8 in.) diameter polyester felt filter disk, 1.0 micron rating. The filter has a 0.1 micron particle collection efficiency of 75%. American Felt and Filter Company, New Windsor, New York.

*Sterile conical tubes, with caps and labels.* The sample filters are transferred to individual 50-mL conical tubes after sampling. An example conical tube is shown in Figure 2.1.3.



Figure 2.1.3 Conical 50-mL tube

*Sterile filter forceps.* One for each filter to be removed and one to place the filters into the unit.

*Sterile gauze.* For applying disinfectant to parts of the unit that contact the filter and may contribute to cross contamination from previous samples.

*Sterile latex gloves.*

*Indelible ink pen.*

*Zipper plastic bags.*

### **Sampling Reagents**

*Disinfecting solution.* 0.5% sodium hypochlorite, pH adjusted to  $\approx 6.8$  with hydrochloric acid.

### **Sampling Technique**

Measurement of the DFU flow rate should be performed before and after sampling. In some instances it may be necessary to periodically check the flow rate of the DFU while sampling. An Aalborg mass flow meter with a capacity to measure from 1 to 1000 liters per minute was used.

*Option 1.* (Using the SFU supplied by the manufacturer and using a disinfectant to avoid cross contamination from previous sampling.)

Remove the air inlet and exhaust covers and open the SFU (single filter unit) lid.

Remove the snorkel and attach it to the air inlet port on top of the lid. (The unit is operable without the snorkel attached.)

Remove the muffler and attach it to the exhaust port on the front of the unit. (Note: The unit is operable without the muffler in place. If quiet operation is not needed, do not attach the muffler.) When the muffler is in place, the flow rate through the filter is significantly reduced.

Release the locking nut on the power cord and pull the cord through the front of the unit case. Secure the locking nut.

Open the filter retainer. One hand may be used to hold the clear plastic back portion while the other hand turns the screw on cover.

Wearing clean, powder-free gloves, remove the black filter stay. Soak the sterile gauze with the disinfectant solution. Apply the disinfectant solution to the black filter stay, the screen which the filter is placed against, and the black ring surrounding the filter position. Allow the disinfectant to have a residence time of 10 minutes.

Remove any excess disinfectant from the sampling port with dry sterile gauze.

Replace the filter stay and the filter retainer to their original positions. Turn the device on and allow it to run for 10 minutes. This is done to ensure that all of the disinfectant has been removed or has been dried. It is important that no disinfectant is allowed to wick into the filter prior to sampling.

After the disinfecting and drying steps, again open the filter retainer. Don a new pair of clean, powder-free gloves over the gloves that are a part of the PPE ensemble. Remove the black filter stay. Do not allow it to touch any potentially contaminated surfaces.

Using disinfected forceps, remove a filter from the filter package and insert it into the filter port. Replace the filter stay. Replace the filter retainer to its position in the sampler.

Close and lock down the SFU lid. Verify that the air inlet and outlet are not blocked.

Plug the power cord into a 110 V AC power source.

To begin sampling, turn the power switch to "ON". Time Weighted Average (TWA) sampling can be done up to 24 hours.

After completing the sampling period, turn the power switch to "OFF". Open the SFU lid. Open the filter retainer. One hand may be used to hold the back portion while the other hand twists off the cover. Remove the black filter stay.

Don a new pair of clean powder-free gloves, again over the gloves that are a part of the PPE ensemble. Use disinfected forceps to remove the filter. Transfer the filter to a 50-mL conical vial.

Use an indelible ink pen to record date, identification, on and off times and any other pertinent information on the vial label.

To collect another sample, follow the disinfecting and drying steps previously described, load another filter as described above and proceed.

Once out of the contaminated area, the individual vials are disinfected. Keep the sample away from UV light, including sunlight. After personnel disinfection place tape, Parafilm, and for OSHA purposes, an OSHA-21) across the top of the sealed vial cap and sides of the vial to indicate sample integrity. Place each vial containing a filter sample into an individual zipper bag that was left for this purpose. Enter the sample information on the Log Sheet, complete the chain of custody form and any other required documentation. The chain of custody record and log sheets must accompany samples. Check with the lab for any further sample packaging and submission requirements. Submit samples to the lab for analysis.

*Option 2.* (The sample filter holder in the SFU is modified to minimize cross contamination from previous sampling and to facilitate changing out sample filters.)

An optional filter holder, constructed of PVC and plastic fittings, is shown in Figure 2.3.2. The 6-inch long nipple is 1.5-inch pipe. The 47-mm felt filter is assembled into the 1.5-inch IPS PVC union using hand-tight pressure without the use of PTFE thread sealant tape. All other threads are wrapped with PTFE tape prior to assembling the holders. A number of these units can be pre-assembled and stored in plastic zipper bags.

After sampling, the ends of the holder can be capped and plugged, and the assembly containing the sample filter is placed in a plastic zipper bag. The units can then be shipped to the laboratory where the filters are removed and analyzed.

The SFU is modified to accept the optional holder by removing the nozzle and muffler (not used) and screwing the assembled unit into the reducer attached to the SFU lid. To modify the SFU, remove the filter retainer. Remove the metal locking ring from inside the clear plastic cylinder. Remove the clear plastic cylinder. The reducer is fitted with a 2.25-inch O-ring to form a leak-tight seal and assembled into the lid hole. Replace the metal locking ring (smooth side toward the sampler case) onto the reducer (do not include the clear plastic cylinder). Tighten the metal locking ring until the O-ring seals.

Close the SFU lid. The threads of the 6-inch nipple of the filter holder must be wrapped with PTFE tape. Screw the pre-assembled optional filter holder into the reducer on the outside of the case using hand-tight pressure.

Subsequent samples are taken by removing the entire pre-assembled holder and replacing it with a clean one.



Figure 2.3.2 Optional sample filter holder for DFU. The assembled unit is shown in the bottom of the photograph.

The optional holders are pre-assembled using the following steps and are placed in clean plastic zipper bags. The 1.5-inch PVC unions, which serve as sampling cassettes, are opened. The parts are immersed in disinfecting solution for 20 minutes, rinsed, and allowed to thoroughly dry before assembly. No disinfecting solution should remain in the sampling cassette. The cassettes are comprised of three parts- the bottom (contains an O-ring), the middle (a threaded ring) and the top (similar to the bottom except that it has no O-ring or exterior threads).

Don sterile gloves. Using disinfected forceps, the filter is placed on the bottom portion, positioned inside of the O-ring. The top portion is carefully placed so that the filter remains centered. The middle portion slides over the top portion and is turned until hand tight.

A 6-inch nipple, with PTFE tape on each threaded end, is screwed into the bottom part of the cassette. Loosely screw a cap on the other end of the nipple and loosely put a plug (with PTFE taped threads) into the top of the cassette. Place assembled filter holders into zipper bags.

Carry the modified SFU and the bagged optional pre-assembled sampling devices into the sampling area. Open the SFU. Release the locking nut on the power cord and pull the cord through the front of the unit case. Secure the locking nut. Close the unit. Remove a pre-assembled filter holder from the bag. Remove the plug and cap and place them in the plastic bag so they can be replaced after sampling. Screw the filter holder into the reducer in the SFU lid until hand tight.

Plug the power cord into a 110 V AC power source. To begin sampling, turn the power switch to "ON". TWA sampling can be done up to 24 hours. After completing the sampling period, turn the power switch to "OFF".

Remove the filter holder from the SFU, replace the plug and cap. Use an indelible ink pen to record date, identification, on and off times and any other pertinent information on the sampling assembly label. Place it into the plastic bag. The entire assembly contained in the plastic bag can be shipped to the laboratory.

Once out of the contaminated area, the outside of each bag containing the samples is disinfected. Refer to the OSHA Anthrax e-tool for detailed descriptions of appropriate decontamination.<sup>7</sup> Keep the sample away from UV light, including sunlight. After personnel disinfection, place a seal (tape, or for OSHA purposes, an OSHA-21) across the top of each bag to indicate sample integrity. Enter the sample information on the Log Sheet, complete the chain of custody form and any other required documentation. The chain of custody record and log sheets must accompany samples. Check with the laboratory for any further sample packaging and submission requirements. Submit samples to the laboratory for analysis.

## **SHIPPING PROCEDURES**

Follow all current federal and state regulations with regards to shipping infectious waste. Transport samples at ambient temperature. Attachment 1.0 is a detailed description of the International Air Transport Association (IATA) shipping protocol that was followed for shipping the samples that were used in this evaluation. The IATA updates shipping instructions every two

years to reflect changes in Federal Regulations. The current IATA shipping protocol may differ from those described in Attachment 1.0.

## **ASSAY PROCEDURE**

The sterility of the equipment used in each assay must be assured.

When comparing assay techniques, analyses of aliquots from the same sample suspension provide a better comparison than the results from side-by-side samples. Multiple aliquots from the same sample suspension can also provide quality control and verification of the precision of a single technique. Any collected *B. anthracis* spores will be determined using a standard microbiological culture technique. Discretionary field or laboratory Polymerase Chain Reaction (PCR) assays may also be conducted.

The samples are prepared and an assay is conducted using standard biological techniques. Biosafety level (BSL) 2 practices are required for activities using clinical materials and diagnostic quantities of infectious cultures.<sup>8</sup> BSL 2 facilities and BSL 3 practices were used at the laboratory that conducted the processing and assay of the DFU polyester filters. Sample assays were performed at the New York State Department of Health, Wadsworth Center, Bioterrorism Response Laboratory (NYSDH/WCBRL) for this evaluation. The following is an outline of the sample preparation and assay.

Ensure that the specimens have been kept sealed and dry, before receiving them into the lab and proceeding with testing.

Follow standardized Association of Public Health Laboratories, CDC Laboratory Response Network (APHL/LRN), Level B Laboratory testing protocols,<sup>9</sup> with the following modifications for elution and testing as outlined below.

### **Sample Processing**

All appropriate steps below are conducted inside of a Biological Safety Cabinet.

If the sample is received in a sample filter holder, disassemble the 1.5-inch PVC union. Use forceps to remove the filter and place it in a 50-mL conical vial. If the sample is received in a 50-mL conical vial, open the vial containing the sample. Add 30-40 mL of Sample Processing Solution [0.3% Tween 20 in Phosphate Buffered Saline (PBS)] into the tube. Close the tube.

Place the tube on a shaker/rotator 30 minutes on a medium setting.

Pour the supernatant into a new, sterile 50-mL conical tube. Discard the DFU polyester filter.

Clarify the contents of the tube by allowing it to sit for 5-10 minutes, then pipette the supernatant into a new, sterile 50-mL conical screw-top tube. Discard settled material.

Centrifuge the supernatant in aerosol-tight biosafety carriers at either 3000 rpm for 30 minutes, or 4500 rpm for 15 minutes (preferably at 10 °C). A pellet will form in the bottom of the tube.

The volume of the pellet may vary depending on the amount of dust collected. If the volume of the pellet is less than 0.5 mL, carefully pipette off and discard the supernatant, leaving 1-ml volume in the tube. If the volume of the pellet is greater than 0.5 mL, leave twice that volume of supernatant in the bottom of the tube. Record this volume for dilution factor and detection limit purposes.

Re-suspend the pellet in the bottom of the tube.

This suspension may be assayed by culture. PCR may be conducted on aliquots of this suspension, if desired. The following is an outline of the standard microbiological culture technique and an outline of PCR sample preparation.

### **Culture Technique**

Deliver the entire suspension into a small, sterile 1.5-mL microcentrifuge vial, for heat-shock followed by culture.

Heat-shock the specimen in a 65 °C water bath for 30 minutes.

Vortex briefly. For qualitative positive or negative confirmation, inoculate the sample onto a sheep blood agar (SBA) plate using the entire sample. For quantitative purposes, inoculate the SBA plate using 90% of the sample volume. Freeze the remaining suspension at -70 °C.

A sterile inoculating loop can be used to spread the inoculum over the plate, and then allow it to be absorbed.

Incubate plates at 37 °C in ambient air. Observe for colony growth. Observe plates after 12-18 hours (overnight) and then daily until 48 hours after plating. For quantitative purposes, if overgrowth occurs, thaw and dilute the remaining suspension into 1.0 mL and repeat the inoculation and incubation steps.

Colonies with morphology consistent with *B. anthracis* that are non-hemolytic and have tenacity are tested for confirmation by the APHL/LRN Level A-B protocols for gamma phage lysis and direct fluorescent antibody (DFA) of cell wall and capsule.

Testing status reports need to be faxed after initial reading and at 48 hours.

If *B. anthracis* is confirmed, record the total number of colonies from the plate on the sample documentation form.

Example dilution factor: If the volume of the re-suspended concentrated sample that is delivered onto a culture plate is 1.0 mL, the dilution factor is exactly 1. If a smaller volume is delivered onto a culture plate, an appropriate dilution factor must be calculated, e.g., if 90% of the 1 mL suspension is delivered onto the culture plate, the dilution factor is 1.11, etc.

Each viable spore has the potential to form a colony. Colonies may form from aggregates of spores. Studies were conducted at the NYSDH/WCBRL using serial dilutions of known concentrations of anthrax spores. These studies suggest that sample processing causes aggregates to disperse, and that each colony represents an individual spore.

### **General Description Of PCR Protocol**

The PCR is performed from the same 1 mL sample volume after processing.

Germination step: 100  $\mu$ L of media are added to 100  $\mu$ L of the sample.

DNA extraction and purification is performed in a spin column. The DNA is adsorbed onto silica gel membrane. Impurities are washed away.

The pure DNA is eluted from the silica gel with 150  $\mu$ L of buffer. Ten microliters of this are added to each of 3 PCR capillary tubes that contain primers, probes, fluorescent dyes and other reactive chemicals.

The targets of these three different PCR assays are two dissimilar plasmids and a chromosomal region of the *B. anthracis* genome. These assays will provide a positive identification when the DNA from 20 spores or more is delivered into the capillary tube for the Polymerase Chain Reaction.

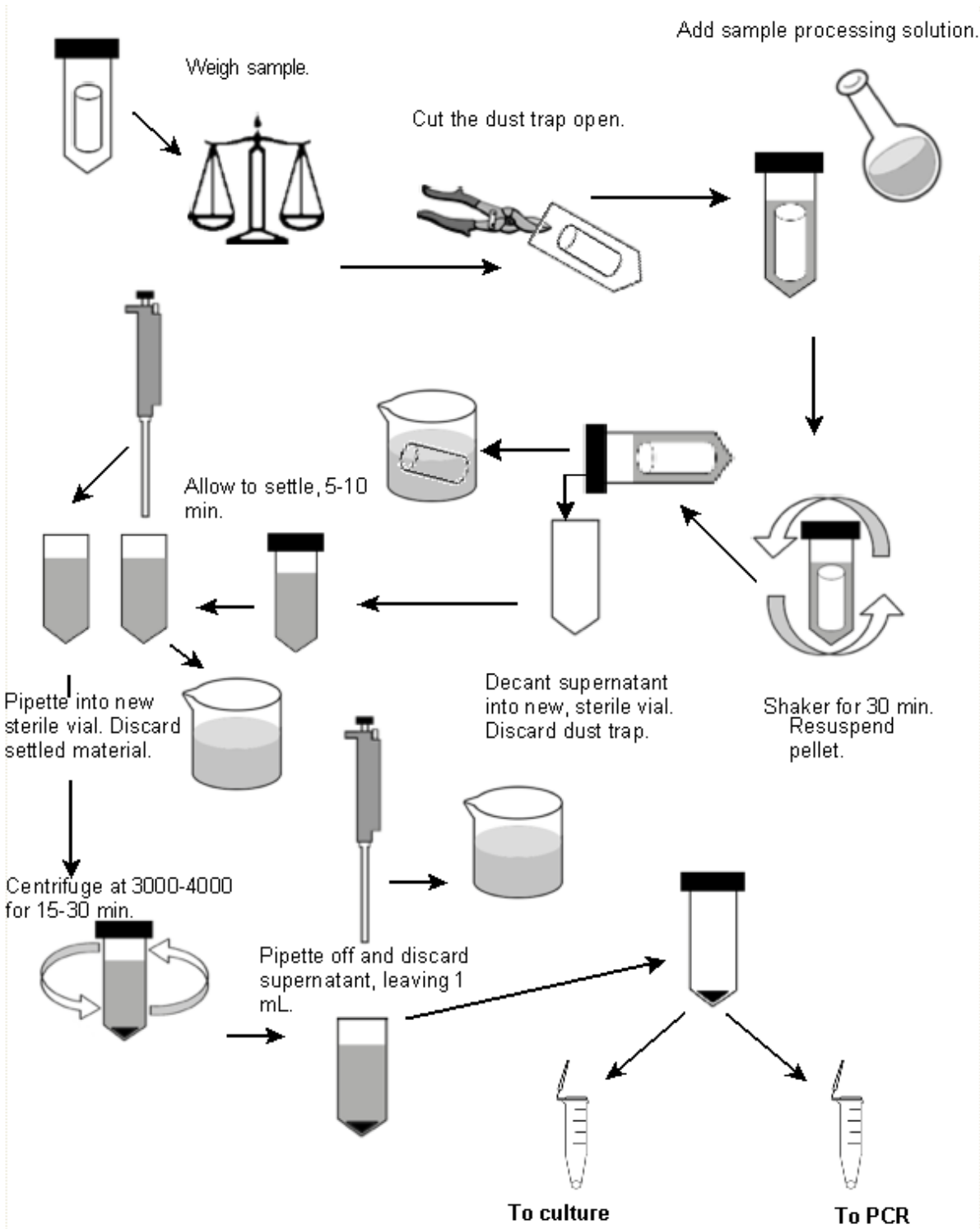
Dilution factor. Concentrated sample is eluted into 1 mL. One-tenth of a milliliter (100  $\mu$ L) of this is extracted with a DNA extraction kit and eluted into 150  $\mu$ L of buffer. A 10- $\mu$ L volume is then added to a PCR reaction. The dilution factor is 0.0067.

If there are 10,000 spores on a DFU filter that are concentrated to this 1-mL volume, 1000 spores will be DNA extracted. After dilution, the DNA of 67 spores will be added to each PCR reaction for detection.

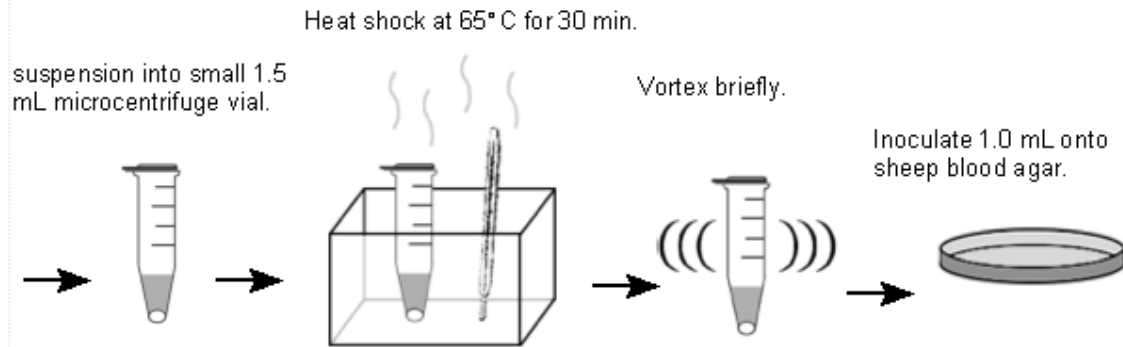
### **DETECTION LIMIT**

As few as 2 colony forming units (CFU) have been reported using the standard microbiological culture technique. Because the dilution factor is 1, the lowest reported amount per sample is 2 CFU. Studies suggest that each colony represents an individual spore.

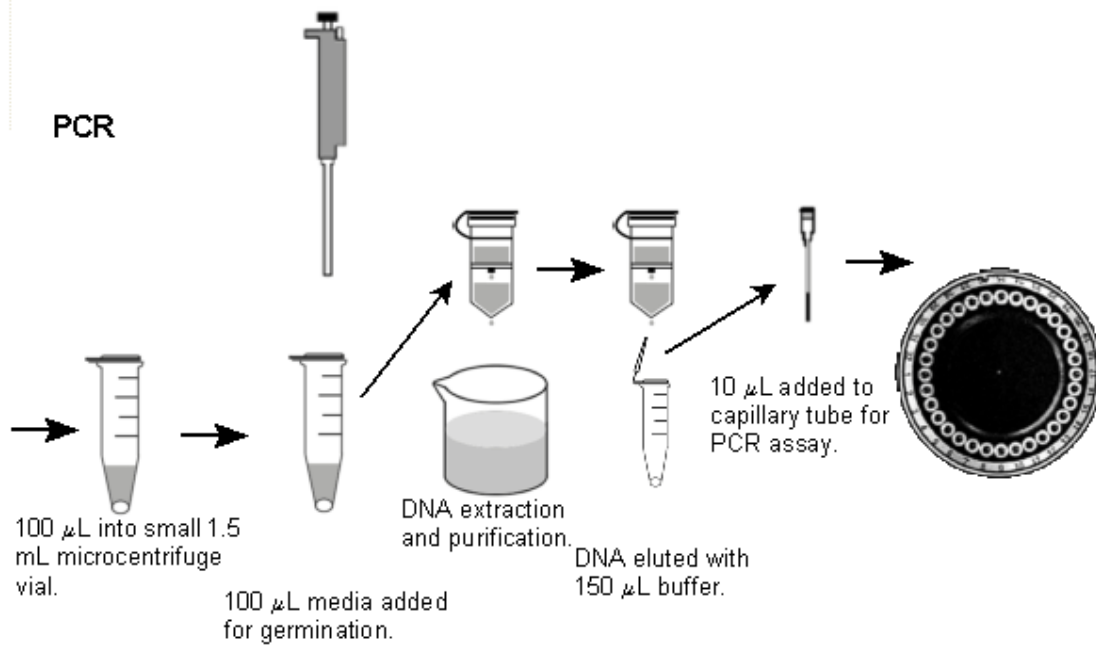
Studies using a Taqman PCR assay have reported a sensitivity to detect the equivalent of 200 spores per sample.<sup>1</sup>



## CULTURE



## PCR



## REFERENCES

1. Shaw, M.; McCandlish, E. et al. Test Report: Ambient Breeze Tunnel Tests of Dry Filter Units and Wet Collectors, PO No. 20902-0123; Battelle Memorial Institute: Columbus, OH, Nov 2001.
2. Black, R.; Shaw, M. et al. Test Report December 2001 Testing of a Variety of Bioaerosol Collection Devices in the Ambient Breeze Tunnel, PO No. 20902-0123; Battelle Memorial Institute: Columbus, OH, Nov 2001.
3. Lonon, M., Bioaerosol Sampling (Indoor Air), Method 0800; U.S. Department of Health and Human Services, National Institute for Occupational Safety and Health, Jan 1998.
4. McCleery, R.; Burr, G.; Martinez, K.; Mattorano, D. Evaluation of Air Sampling Methods for *Bacillus anthracis* Prior and Subsequent to the Operation of a Delivery Bar Code Sorter in the Trenton Processing and Distribution Center. Presented at the Anthrax Incident Management Workshop, Medicine Hat, Canada, 2002.
5. Brachman, P., Evans, A., Eds. Bacterial Infections of Humans, Epidemiology and Control, 2nd ad.; Plenum Publishing Corporation, New York, NY, 1991, pp 75-76.
6. Anthrax e-Tool, Personal Protective Equipment, U.S. Department of Labor, Occupational Safety and Health Administration. <http://www.osha.gov/SLTC/etools/anthrax/ppe.html> (accessed March 2003).
7. Anthrax e-Tool, Decontamination, U.S. Department of Labor, Occupational Safety and Health Administration. <http://www.osha.gov/SLTC/etools/anthrax/decon.html> (accessed March 2003).
8. Biosafety in Microbiological and Biomedical Laboratories, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health, Fourth Edition, U.S.G.P.O., 1999. <http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm> (accessed Jan 2002).
9. Level A Procedures for Identification of *Bacillus anthracis*, Laboratory Response Network, Center for Disease Control and Prevention. <http://www.bt.cdc.gov/Agent/Anthrax/LevelAProtocol/ban-la-cp-031802.pdf> (accessed Feb 2002).

## Attachment 1.0

Detailed description of the International Air Transport Association (IATA) shipping protocol that was used in this evaluation. The IATA updates shipping instructions every two years to reflect changes in Federal Regulations. The current IATA shipping protocol may differ from those described.

### Table of Contents

1. Applicability
2. Training
3. Certified Packaging
4. Marking and Labeling
5. Shipper's Declaration for Dangerous Goods
6. Air Waybill

Appendix A Suppliers for Certified Infectious Substances Shipping Containers and/or Shipping Labels.

Appendix B: Class 6, Division 6.2 "Infectious Substance" label and Cargo Aircraft Only label.

Appendix C: Guidelines for Completing Shipper's Declaration for Dangerous Goods; Shipment of Anthrax Bacterium Air and Surface Samples.

Appendix D: Step-by-Step Instructions for Shipping Anthrax Bacterium Samples; Utilizing SAF-T-PAK® STP-110 Infectious Substance Shipper 1.

### 1. Applicability

The following guidelines should be followed when shipping anthrax bacterium samples from the field to the laboratory for analysis. This shipping procedure applies to both air and surface anthrax samples gathered according to OSHA Methods OSA7 (air) and OSS3 (surface). Under this procedure, samples are shipped by air to the laboratory via FedEx Priority Overnight service.

### 2. Training

Both the International Air Transport Association (IATA) and the U.S. Department of Transportation (DOT) require specific training for anyone directly involved in the shipping of dangerous goods (IATA 1.5, 49 CFR 172.700). IATA requires training and re-certification every 2 years, while DOT requires training and re-certification every 3 years. It is the responsibility of the employer to ensure that employees are properly trained. Contact the Salt Lake Technical Center (SLTC) for more information regarding training requirements.

### 3. Certified Packaging

All hazardous materials are classified into 9 classes of Dangerous Goods. Anthrax bacterium is classified as an “Infectious Substance,” which is Class 6, Division 6.2. Accordingly, the packaging must meet the performance criteria specified by IATA and DOT for infectious substances. It is strongly recommended to use certified Class 6.2 shipping containers purchased from commercial suppliers. A list of potential suppliers is provided in [Appendix A](#).

Instructions for packing infectious substances are designated in UN Packing Instruction 602, listed in the IATA Dangerous Goods Regulations and DOT 49 CFR 173.196. Again, Class 6.2 commercial shipping containers, which also comply with Packing Instruction 602, are available and recommended. Many of these commercial containers also include the required labels pre-printed on the container surface. A summary of key requirements that apply to shipping dry anthrax samples is as follows:

- Triple packaging is used, consisting of a primary receptacle, secondary packaging, and durable outer container.
- The primary receptacle(s) must be watertight and constructed of glass, metal or plastic. Positive means of ensuring a leak-proof seal must be provided, such as heat seal, skirted stopper or metal crimp seal. If screw caps are used, these must be reinforced with adhesive tape. (Depending on the packaging used, the sample vials may be considered the primary receptacle. It is important to ensure that the screw caps on the vials are properly sealed as described in the sampling methods).
- Secondary packaging must also be watertight. (Absorbent material between the primary receptacles and the secondary packaging is not required for solid substances).
- Where multiple primary receptacles are contained in a single secondary packaging, they must be separated and supported to ensure that contact between them is prevented. (The zipper bags placed around the sample vials according to the sampling methods are sufficient for this purpose).
- Outer packaging must be of adequate strength for its capacity, mass, and intended use, and must meet the specific UN performance tests. The size of the outer packaging must be at least 100 mm (4”) in the smallest overall dimension, yet must also be sufficient size to accommodate all labels placed on a single surface, and labels must not overlap.
- An itemized list of contents must be enclosed between the secondary packaging and the outer packaging. (The chain of custody record and log sheets utilized with the sampling methods may be used for this purpose).
- When transported by passenger airline, DOT and IATA limit the quantity per package to 50 mL or 50 g. When transported by cargo airline, IATA limits the quantity per package to 4 liters or 4 kg. These limits apply to the total amount of actual sampling material i.e., air filter, dust collector trap (HEPA sock), contained within all sampling vials packaged within the shipping container.

#### 4. Marking and Labeling

Each package of infectious substances must be marked durably and legibly on the outside of the shipping container with the following:

- The proper shipping name, technical name, and corresponding UN Number, as follows:

Infectious substance, affecting humans  
(Bacillus species)  
UN2814

- The Name and Telephone Number of a person responsible for the shipment. This number must be that person who is knowledgeable of the material being shipped and has emergency response and incident mitigation information.
- Class 6, Division 6.2 “Infectious Substance” label (see [Appendix B](#)). This diamond-shaped label should contain the telephone number of the CDC in Atlanta to which damaged or leaking packages should be reported.
- Package orientation (“This Way Up”) labels. Two package orientation labels are affixed on opposite sides of the package. Most of the commercial certified infectious substance packages include these markings pre-printed on the outer container.
- Cargo Aircraft Only label for shipments that exceed the maximum quantity specified for passenger aircraft (50 mL or 50 g). The actual wording on this label is “Danger – Do Not Load in Passenger Aircraft” (see [Appendix B](#)).

#### 5. Shipper’s Declaration for Dangerous Goods

Each package must include a “Shipper’s Declaration for Dangerous Goods” form. This form should be attached to the outside of the outer packaging. The form must be complete, accurate, legible, and must be either typewritten or computer generated. Handwritten forms or faxed copies are not acceptable. Blank forms can be obtained directly from FedEx or commercial suppliers.

An example of a completed declaration form is included in [Appendix C](#). Written guidelines for completing the form are also provided.

#### 6. Air Waybill

A standard FedEx USA Airbill must also be completed and included with the documentation. On the form, for type of express package service select “FedEx Priority Overnight”. In the “Special Handling” section on the form, where it is asked “Does the shipment contain dangerous goods?”, check “Yes – As per attached Shipper’s Declaration”. All other areas of the waybill form are completed normally as with any shipment. The waybill is attached to the outer packaging along with the Shipper’s Declaration.

Appendix A:  
Suppliers for Certified Infectious Substances Shipping Containers and/or Shipping Labels

(Note: Listing of these companies does not imply endorsement by OSHA)

<p><u>Action Pak, Inc.</u> 2550 Pearl Buck Road Bristol, PA 19007 Phone: 800-755-9764 Fax: 215-788-1760</p>	<p>Environmental Packaging Systems, Ltd. 1 Research Drive Dartmouth, N.S., Canada B2Y 4M9 Phone: 800-277-8675 Fax: 902-466-6889</p>	<p><u>Polyfoam Packers Corporation</u> 2320 Foster Avenue Wheeling, IL 60090-6572 Phone: 800-323-7442 Fax: 847-398-0653</p>
<p><u>Air Sea Atlanta</u> 1234 Logan Circle Atlanta, GA 30318 Phone: 404-351-8600 Fax: 404-364-4005</p>	<p><u>EXAKT Technologies, Inc.</u> 7416 North Broadway Extension, Suite E Oklahoma City, OK 73116 Phone: 800-866-7172 Fax: 405-848-7701</p>	<p><u>SAF-T-PAK, Inc.</u> 101, 17872 – 106 Avenue Edmonton, Alberta, Canada T5S 1V4 Phone: 800-841-7484 Fax: 403-486-0235</p>
<p><u>Air Sea Containers, Inc.</u> 2749 NW 82nd Avenue Miami, FL 33122 Phone: 888-272-9883 Fax: 305-599-1668</p>	<p><u>Federal Industries Corp.</u> 2550 Niagara Lane Plymouth, MN 55447 Phone: 800-523-9033 Fax: 612-476-8155</p>	<p><u>Sage Products, Inc.</u> 815 Tek Drive P.O. Box 9693 Crystal Lake, IL 60039-9693 Phone: 815-455-4700 Fax: 815-455-3310</p>
<p><u>All-Pak, Inc.</u> Corporate One West 1195 Washington Pike Bridgeville, PA 15017-2854 Phone: 800-245-2283 Fax: 412-257-3001</p>	<p><u>HAZMATPAC, Inc.</u> 5301 Polk Avenue, Bldg 18 Houston, TX 77023 Phone: 800-923-9123 Fax: 713-923-1111</p>	<p>Shamrock, Inc. 34 Davis Drive Bellwood, IL 60104 Phone: 800-323-0249 Fax: 800-248-1907</p>
<p><u>Casing Corporation</u> P.O. Box 820369 Dallas, TX 75382-0369</p>		<p><u>Source Packaging of New England, Inc.</u></p>

<p>Phone: 800-358-6866 Fax: 214-320-1682</p> <p><u>Cin-Made Corporation</u> 1780 Dreman Ave. Cincinnati, OH 45223 Phone: 513-681-3600</p> <p>The Compliance Center, Inc. 2150 Liberty Dr. Niagara Falls, NY 14304 Phone: 800-767-7231</p> <p><u>Cargo Pak Corporation</u> 306-A White Street South Wake Forest, NC 27587 Phone: 800-266-0652 Fax: 919-554-9055</p> <p><u>DG Supplies, Inc.</u> 28 C Industrial Dr. Hamilton, NJ 08619 Phone: 800-347-7879 Fax: 609-860-0285</p>	<p><u>Inmark, Inc.</u> 220 Fisk Drive, S.W. Atlanta, GA 30336 Phone: 800-646-6275 Fax: 404-349-5249</p> <p><u>Label Master</u> 5724 North Pulaski Chicago, IL 660646 Phone: 800-621-5808 Fax: 800-723-4357</p> <p><u>Nalge Nunc International</u> 75 Panorama Creek Drive P.O. Box 20365 Rochester, NY 14625 Phone: 716-586-8800 Fax: 716-586-8987</p> <p><u>O'Berk International, Inc.</u> 3 Milltown Court P.O. Box 1690 Union, NJ 07083 Phone: 800-577-7624 Fax: 908-687-5157</p>	<p>405 F Kilvert Street Warwick, RI 02866 Phone: 800-200-0366 Fax: 401-738-7762</p> <p>United Ad Label Company, Inc. P.O. Box 2216 Brea, CA 92622-2216 Phone: 800-423-4643 Fax: 800-962-0658</p>
---	--	--

Appendix B:  
Class 6, Division 6.2 "Infectious Substance" label and Cargo Aircraft Only Label



Class 6, Division 6.2 "Infectious Substance" label



Cargo Aircraft Only Label

Appendix C:  
Guidelines for Completing Shipper's Declaration for Dangerous Goods; Shipment of Anthrax  
Bacterium Air and Surface Samples

(see example below)

Form must be legible, typewritten, or computer-generated. All alterations must be signed with the same signature used to sign the declaration.

SHIPPER: Enter name and address of shipper. A telephone number is also preferred.

CONSIGNEE: Enter the name and address of recipient. A telephone number is also preferred.

AIR WAYBILL NO.: Leave blank unless instructed otherwise by the carrier.

TRANSPORT DETAILS: Delete the option that does not apply to the shipment:

"Passenger and Cargo Aircraft" may be used for up to 50 mL or 50 g of material.

"Cargo Aircraft Only" must be used for material quantities over 50 mL/50g. (Maximum quantity per package is limited to 4 liters or 4 kg.)

AIRPORT OF DEPARTURE: Enter "N/A".

AIRPORT OF DESTINATION: Enter "N/A".

SHIPMENT TYPE: Delete the "radioactive" option.

PROPER SHIPPING NAME: Enter "Infectious substance, affecting humans (Bacillus species)".

CLASS OR DIVISION: Enter "6.2".

UN OR ID NO.: Enter "UN2814".

PACKING GROUP: Leave blank.

SUBSIDIARY RISK: Leave blank.

QUANTITY AND TYPE OF PACKAGING: Enter "1 Fiberboard Box X \_\_\_\_\_ g" or "1 Fiberboard box X \_\_\_\_\_ mL" (Enter the total amount of g or mL of material within the package, as applicable. This may be completed in the field by hand, if desired).

PACKING INST.: Enter the packaging instruction number, "602".

AUTHORIZATION: Leave blank.

ADDITIONAL HANDLING INFORMATION: Enter "Prior arrangements as required by the IATA Dangerous Goods Regulations 1.3.3.1 have been made".

EMERGENCY TELEPHONE NUMBER: Enter the 24-hour emergency contact number. (Usually either the telephone number for the responsible person or contracted response company, if used (i.e., Chemtrec)).

NAME/TITLE OF SIGNATORY: Enter name and job title of shipper. (If the form is typed beforehand, it may be advisable to leave this item blank and have it completed in the field by hand at the time of shipment).

PLACE AND DATE: Enter the city and date of shipment. (If the form is typed beforehand, it may be advisable to leave this item blank and have it completed in the field by hand at the time of shipment).

SIGNATURE: Sign the form with a complete signature.

Shipper's Declaration for Dangerous Goods  
 \*\* EXAMPLE FOR 50 mL / g OR LESS \*\*

**SHIPPER'S DECLARATION FOR DANGEROUS GOODS**

(Provide at least two copies to the airline)

Shipper John Doe (321) 444-5555  
 ABC Company  
 1200 Main St.  
 Big City, CA 55555

Air Waybill No.  
 Page 1 of 1 Pages  
 Shipper's Reference Number  
(optional)

Consignee Mary Smith (456) 789-0123  
 XYZ Laboratory  
 700 Elm St.  
 Midtown, NY 23232



**Two completed and signed copies of this Declaration must be handed to the operator.**

**WARNING**

Failure to comply in all respects with the applicable Dangerous Goods Regulations may be in breach of the applicable law, subject to legal penalties. This Declaration must not, in any circumstances, be completed and/or signed by a consolidator, a forwarder, or an IATA cargo agent.

TRANSPORT DETAILS			
This shipment is within the limitations prescribed for: <small>(delete non-applicable)</small>	Airport of Departure  N/A		
<table border="1"> <tr> <td>PASSENGER AND CARGO AIRCRAFT</td> <td><del>CARGO AIRCRAFT ONLY</del></td> </tr> </table>	PASSENGER AND CARGO AIRCRAFT	<del>CARGO AIRCRAFT ONLY</del>	Airport of Destination:  N/A
PASSENGER AND CARGO AIRCRAFT	<del>CARGO AIRCRAFT ONLY</del>		

Shipment type: (delete non-applicable)

<input type="checkbox"/> NON-RADIOACTIVE	<input checked="" type="checkbox"/> <del>RADIOACTIVE</del>
--	--

**NATURE AND QUANTITY OF DANGEROUS GOODS**

Dangerous Goods Identification					Quantity and type of packaging	Packing Inst.	Authorization
Proper Shipping Name	Class or Division	UN or ID No.	Pack- ing Group	Subsi- dary Risk			
Infectious substance, affecting humans (Bacillus species)	6.2	UN2814			1 Fibreboard box X <u>30</u> g	602	

Additional Handling Information  
 Prior arrangements as required by the IATA Dangerous Goods Regulations 1.3.3.1 have been made.

Emergency Telephone Number: (321) 444-5555

I hereby declare that the contents of this consignment are fully and accurately described above by the proper shipping name, and are classified, packaged, marked and labelled/placarded, and are in all respects in proper condition for transport according to applicable International and National Governmental Regulations.

Name/Title of Signatory  
 John Doe / Ind. Hygienist  
 Place and Date  
 Washington D.C. 01/07/02  
 Signature *John Doe*  
(see warning above)

IF ACCEPTABLE FOR PASSENGER AIRCRAFT, THIS SHIPMENT CONTAINS RADIOACTIVE MATERIAL INTENDED FOR USE IN, OR INCIDENT TO, RESEARCH, MEDICAL DIAGNOSIS, OR TREATMENT.

Appendix D:  
Step-by-Step Instructions for Shipping Anthrax Bacterium Samples;  
Utilizing SAF-T-PAK<sup>®</sup> STP-110 Infectious Substance Shipper

The SAF-T-PAK<sup>®</sup> STP-110 Infectious Substance Shipper is useful for shipping anthrax bacterium samples. Using this kit, the sample vials are considered the primary container. The STP-110 kit includes the following items:

- (2) leak-proof inner bags (STP-711) into which sample vials are placed.
- (2) Tyvek envelopes utilized as a secondary container (STP-710), each designed to hold one inner bag.
- Inner fiberboard box into which up to two of the Tyvek envelopes may be placed.

Outer fiberboard box. This outer shipping container includes most of the necessary markings pre-printed on the sides of the box.



To utilize this shipper, follow these instructions:

1. Make sure the 50-mL sample vials obtained from the sampling method are ready for packaging. The caps should be sealed with tape or sample seals and each vial should be in a zippered bag.
2. Insert up to four sample vials into the STP-711 inner bag. Seal the bag according to instructions on the bag. If there are more than four samples vials, use the second STP-711 bag provided. Note: The maximum number of 50-mL primary containers allowed per bag is four.



3. Place one STP-711 inner bag into one STP-710 Tyvek envelope. If using the additional STP-711 inner bag, place it into the second STP-710 Tyvek envelope provided. Seal the envelope(s) according to the instructions provided.



4. Assemble the inner fiberboard box. Tape may be necessary. Place the STP-710 Tyvek envelope(s) into the inner box. Seal the inner box.



5. Assemble the outer fiberboard box according to the manufacturer's instructions. Place the inner fiberboard box into the outer fiberboard box. Place the itemized list of contents/chain of custody form inside the outer box. Seal the outer box.



6. On the side of the outer box, the proper shipping name and UN number for infectious substances is already provided. However, complete the information by writing "Bacillus Species" in the blank provided. Additional labels are provided to place over the pre-printed markings if a mistake is made.

7. The 6.2 Infectious Substance label is pre-printed on the box; no additional 6.2 labels are necessary.

8. If there is more than 50 mL or 50 g of sample material in the entire container, place a Cargo Aircraft Only label on the side of the box.

9. In the blank section next to "Shipper" printed on the outside of the box, write the name and

phone number of the responsible person. The name and phone number of the consignee may be written in the “Consignee” section, although it is not required.



10. Complete the Shipper’s Declaration for Dangerous Goods form and Air Waybill according to previous instructions. Place the documentation in a clear shipping envelope and attach the envelope to the container. If you are unsure how to attach the documentation, have FedEx provide assistance at the time of shipment.



11. The package is now ready for shipment.