Comprehensive Procedures for Collecting Environmental Samples for Culturing *Bacillus anthracis* Revised April 2002

Preface

Environmental sampling to determine the presence of *Bacillus anthracis* spores in indoor environments is an important tool for assessing risk for exposure. Environmental sampling can also be used to determine the extent and degree of contamination, to support decisions regarding the need for medical treatment or cleanup, and to provide guidance regarding when cleanup is adequate to permit re-entry into an area.

The decision to collect environmental samples for culturing *B. anthracis* should be made by industrial hygienists and other experts familiar with the organism and the sampling methodologies described in this document. Representatives from laboratories, as well as local, state, and federal agencies, should be consulted during the decision-making process. The decision to sample should be based on the extent and location of any suspected contamination, the potential for the contaminant to migrate, and the activity for which the facility is used.

Currently, no occupational or environmental exposure standards exist for *B. anthracis* spores. In addition, there are presently no validated sampling and analytical methods specifically for *B. anthracis* in environmental samples. Data are lacking on collection efficiency of the sample collection media (swabs, wipes, filters, etc.) for typical porous and non-porous surfaces encountered in indoor environments (e.g., furniture, carpet, letters, clothing, ventilation system filters). The effect of varying concentrations of *B. anthracis*-containing particles and dust loading on sampling efficiency has not been studied. Further, the recovery efficiency of the analytical methods (efficiency of removal of *B. anthracis* spores from the sample collection media) has not been adequately evaluated and limits of detection have not been established.

Culture with positive identification of *B. anthracis* (CDC culture method) is the confirmatory test for environmental samples.[1] The methods described in this document are believed to be more sensitive than the available hand-held rapid-assay devices for the detection of *B. anthracis*. At the present time, PCR- or immune-based assays for *B. anthracis* should not be used alone but should be confirmed with samples analyzed by culture methods to make public health decisions.

This guidance document is based on the experience of CDC field investigators and laboratorians during the recent anthrax response investigations and experience with environmental monitoring for other contaminants in indoor environments. This document will be revised and updated as new information becomes available. Further research is needed to clarify the sensitivity of the sampling and analytical methods for known or suspected *B. anthracis*.

1 Notice to Readers: Use of Onsite Technologies for Rapidly Assessing Environmental *Bacillus anthracis* Contamination on Surfaces in Buildings. MMWR 2001 Dec 7;50(48):1087.

Plan for Investigating *B. anthracis* Environmental Contamination

Several components are essential for the implementation of a successful sampling strategy during a *B. anthracis* investigation. Key components include properly trained personnel, suitable sample media and supplies, appropriate safety policies, and thorough record keeping/documentation procedures. Potentially contaminated areas should be secured to prevent cross-contamination and re-aerosolization of *B. anthracis* spores.

Training

The use of experienced investigators to conduct environmental sampling will provide the greatest probability of locating and identifying *B. anthracis* spores, if present. Personnel should be properly trained in the appropriate disciplines necessary for sample collection, including sampling methods, knowledge of building systems, dissemination pathways, aerosol-generating procedures/equipment, use of personal protective equipment (PPE), safety, and decontamination methods.

Safety

All personnel who enter the contaminated area must follow the safety and infection control plan developed for that particular site. Information regarding PPE for investigators can be found in the CDC Advisory document "Protecting Investigators Performing Environmental Sampling for *Bacillus anthracis*: Personal Protective Equipment," which can be found at http://www.bt.cdc.gov/DocumentsApp/Anthrax/Protective/Protective.asp.

Safety considerations are imperative not only for investigators but for the general public. Depending on the size of the area involved, the types of surfaces potentially contaminated, and the extent of contamination, it may be necessary to isolate and control access to the contaminated area to prevent the spread of contamination through the movement of people or equipment. When selecting sampling equipment, consideration should be given to whether the equipment can be effectively decontaminated or whether it must be properly disposed of after use.

Record Keeping/Documentation

Comprehensive documentation of sampling procedures is required. Detailed notes should be kept to document the methodology used to create sampling strategies and sample collection. At a minimum, the following information should be recorded: the discrete sample number or identifier, sample location, type of sample, time and date of sample collection, name of person collecting sample, room description, ventilation factors (e.g., heating, ventilating, and air-conditioning [HVAC] system on or off), and other pertinent information. Taking photographs and/or obtaining a floor plan to document sample locations and results are also helpful. Comprehensive sample records often prove useful to help interpret analytical sample results and to fully evaluate potential risk. Chain-of-custody procedures should be followed and documented as designated by local or state health laboratory reporting requirements. A written report of sample results should be obtained from the laboratory and should include a detailed description of the analytical procedures and any deviations from these procedures that may have occurred.

Sampling Strategy

To design a credible sampling strategy, the investigator must decide what question the data are intended to answer. Defining the goal of a sampling survey is essential to capturing data that are scientifically

meaningful and therefore useful in the decision-making process of an investigation. Once the goal of the sampling is adequately defined, an appropriate sampling strategy can be developed and implemented.

Before sampling is begun, the building's engineer/HVAC facility manager should be consulted on the design and operation of the HVAC system(s) to assess airflow patterns and determine which components (fans, filters, ductwork, etc.) serve a given area. Since most buildings recirculate air through ducted returns or ceiling plenums to other locations in the building, shutting down the ventilation system serving the contaminated area may be necessary to avoid dispersing *B. anthracis* spores. This issue should be discussed with the HVAC engineer with specific attention to some areas, such as computer network areas, which require constant ventilation (cooling) to prevent heat damage to critical systems.

The sampling method and number of samples collected will be influenced by the circumstances of the potential contamination. A sufficient number of samples must be taken to increase the probability that the sampling is representative of the extent of contamination. Obtaining samples from additional locations at varying heights within the area of interest may provide more specific information on the source and dispersion of the contamination. In an initial investigation where there has been a known or suspected release of potentially contaminated material, the first priority should be to collect samples in locations that are near the suspected release source(s). If the aerosol containing *B. anthracis* spores has an aerodynamic size of less than 10 micrometers (μm), the particles will remain suspended in the air for extended periods of time (hours to days). In such cases, the spores can spread throughout an air space and into adjacent areas by following both localized (people walking by) and generalized (airflow from HVAC systems) airflow currents. In determining the extent of contamination, investigators should include coverage of areas along an anticipated contaminant pathway, i.e., those associated with air movement or dust collection, as well as activities that result in re-aerosolization or cross-contamination. In this case, the decision logic typically used in indoor environmental quality investigations of bioaerosols can be applied in identifying other important sampling locations. Spores can also be carried if they attach to clothing, shoes, or other objects; thus, more distant sampling may be needed.

The types of sampling methods utilized in a sampling strategy may include the collection of bulk, surface, and/or air samples. Each sampling method has specific advantages in particular applications. Consultation with laboratory personnel is essential to determine the capabilities and analytical process of the laboratories involved. It may be necessary to utilize a combination of sampling methods to adequately characterize an environment. Those performing the sampling need to be cognizant of how their own activities or the sampling method itself could disturb the existing environment, and therefore alter the results. Additionally, field and media blank samples should be sent to the laboratory to determine if cross-contamination has occurred during sample collection. Field blanks should comprise at least ten percent of the total number of samples.

Bulk Sampling

Bulk samples can help investigators characterize the presence of contamination on building materials such as carpeting, dust cakes on air filters, settled dust (e.g., rafter dusts), and office equipment. However, because extracting spores from bulk samples can pose exposure concerns for laboratory personnel, appropriate precautions (such as double-bagging of samples) should be taken to prevent secondary spreading of spores from contaminated bulk samples. If collected, these samples should be sent to at least a B level laboratory with biosafety level 3 (BSL-3) facilities and should be removed within a biological safety cabinet or glove box. It should be mentioned that some bulk samples pose additional challenges due to unpredictable recovery of spores. This limitation should be recognized when interpreting sampling results.

Surface Sampling with Wipes or Swabs

Surface samples are collected by wiping or swabbing a moistened, absorptive medium across a non-porous surface. The absorptive media, wetting agent, and bags used to transport samples should be selected with input from the laboratory personnel who will be analyzing the samples so that collection procedures will be compatible with the laboratory's analytical procedures. There are several absorptive media available, but non-cotton (rayon, polyester, etc.) wipes or swabs are preferred because of applied laboratory procedures. The collection media must be sterile and used with a sterile wetting agent such as sterile water, a sterile saline solution, or a sterile phosphate-buffered solution. Because of the small surface area of swabs, they are best utilized for smooth surfaces that do not have a large accumulation of dust. A larger surface area (>100 square centimeters [cm^2]) can generally be sampled with wipe materials.

Surface Samples Collected by High-Efficiency Particulate Air (HEPA) Vacuuming

Collecting samples by vacuuming offers the advantages of covering large or dusty, non-porous surfaces and porous surfaces such as carpeting, ceiling tiles, ventilation systems filters, and cloth seats. Vacuum samples must be collected using only high-efficiency particulate air (HEPA) vacuum cleaners. Conventional home or industrial vacuum cleaners should not be used for sample collection because these vacuum cleaners can further disperse spores if filtration is insufficient. However, HEPA vacuum samples are not appropriate in sample locations where insufficient dust mass is collected. There are several methods for collecting vacuum samples. One option is to connect a Dust Collection Filter Sock (manufactured by Midwest Filtration Company, Fairfield, Ohio, or equivalent)[2] to the inlet nozzle of a HEPA vacuum cleaner. A second option is to use micro-vacuuming techniques to collect a sample using personal sampling pumps or carbon vane pumps operating at a high flow rate and utilizing a suitable filter substrate contained in a closed-face, conductive sampling cassette to which a short section of plastic tubing cut at a 45° angle is added to the inlet.

Air Samples

Air sampling is conducted in limited situations where a clear need exists to characterize the air concentration of *B. anthracis* spores. The concentration of spores in the air will probably decrease over time as the spores settle out or disperse. Air sampling may be of limited value in areas that are undisturbed or in which ventilation systems have continued to function, for long periods after a known or suspected release. Air sampling can be used to evaluate activities that may result in re-aerosolization of settled spores.

Culturable air sampling can be done by a variety of methods, including the use of cascade impactors. Since *B. anthracis* spores are quite hardy, samples can also be collected using sampling pumps and filters (MCE, polytetrafluoroethylene, gelatin, etc.) placed inside sampling cassettes. To minimize sample loss, it is recommended that conductive sampling cassettes be used. (Additionally, the inside surfaces of the cassette can be rinsed to remove spores that may have adhered to the surfaces and the rinse considered as part of the sample).

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2 A number of suggested commercial sources of materials are given in this document in an attempt to meet the practical needs of the readers. These are sources known to the authors. Their inclusion does not imply endorsement of the products by the U.S. Department of Health and Human Services (HHS), Agency for Toxic Substances and Disease Registry (ATSDR), Centers for Disease Control and Prevention (CDC), or National Institute for Occupational Safety and Health (NIOSH). It is recognized that equivalent products from other sources may be equally satisfactory.

Further information on air sampling for microorganisms can be found in the methods described in the *American Industrial Hygiene Association (AIHA) Field Guide for the Determination of Biological Contaminants in Environmental Samples* (Fairfax, VA, 1996) and the *American Conference of Governmental Industrial Hygienists (ACGIH®) Bioaerosols: Assessment and Control* (Cincinnati, OH, 1999).

Sample Packaging and Shipment

Environmental samples collected for the purpose of determining if *B. anthracis* spores are present should be considered "Infectious Substances." As such, these samples must be packaged, labeled, marked, and shipped according to applicable federal and international regulations (Public Health Service, Department of Transportation, the United States Postal Service, and the International Civil Aviation Organization [as published by the International Air Transport Association, Dangerous Goods Regulation]). General information on sample packaging and shipping can be obtained at http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf.

It is the responsibility of the shipper to ensure correct identification, classification, packaging, labeling, marking, and documentation for all shipments of infectious substances. Investigators who will be handling and transporting infectious substances must receive training on these regulations prior to collecting samples for submission to an analytical laboratory. Chain-of-custody procedures should be followed and documented.

Sample Analysis

Due to the degree of complexity and safety required during *B. anthracis* analysis, samples should be analyzed at a facility that is part of the Laboratory Response Network for Bioterrorism (LRN), with adequate safety procedures in place. Additional information may be obtained at http://www.phppo.cdc.gov/nltn/pdf/LRN99.pdf.

For example, swab samples collected for rule-out testing can be analyzed at an LRN Level A laboratory (generally a CLIA-certified clinical laboratory) using BSL-2 facilities and BSL-3 safety practices. All other samples including bulks, wipes, air samples, or vacuum samples should be analyzed for *B. anthracis* at an appropriate LRN Level B or C laboratory using BSL-3 facilities. In addition, all culture isolates that cannot be ruled out and are therefore presumptively positive should be referred to an LRN Level B or C laboratory for confirmatory testing. LRN personnel should be consulted when a sampling plan is designed and at subsequent stages of the investigation.

Safety considerations for laboratory personnel involved with analytical processing of samples are paramount. Laboratory personnel must be adequately trained to handle infectious agents and must utilize proper procedures to reduce exposure during analysis. Additional information regarding safe laboratory practices can be obtained at http://www.phppo.cdc.gov/nltn/default.asp and the Biosafety in Microbiological and Biomedical Laboratories (BMBL) 4th Edition located at http://www.cdc.gov/od/ohs/biosfty/bmbl4/bmbl4toc.htm.

Sample Interpretation

A multidisciplinary team including field investigators, laboratory personnel, medical professionals, as well as local, state, and federal agency officials should interpret analytical results. Inclusion of field investigators and laboratory personnel in the interpretation process will provide the best insight into sample collection and recovery. Since analytical methods are not fully validated for *B. anthracis*,

investigators who review and interpret the results of environmental sampling must consider these limitations and use professional judgment in interpreting any positive or negative findings as well as quantitative or semi-quantitative results.

Collecting Bulk Samples

Some laboratories, including all Level A laboratories, cannot accept bulk samples due to safety concerns that arise during sample processing. Therefore, the receiving laboratory should be contacted before bulk samples are collected to determine whether such samples will be accepted. Bulk samples may include items such as sections of carpet, office equipment, supplies, vials of dust, or ventilation filters.

1. Maintain appropriate chain-of-custody documentation and procedures.
2. Don sterile, non-powdered nitrile or vinyl examination gloves over the gloves that are part of standard PPE and clothing.
3. Collect and bag the item; seal the bag.
4. Label the bag and place in another unused, self-sealing bag (such as a Ziploc® bag or Whirlpak®).
5. Document the following items:
   - ♦ Discrete sample number or identifier
   - ♦ Sample location
   - ♦ Type of sample
   - ♦ Time and date of sample
   - ♦ Name of person collecting sample
   - ♦ Map of sample area
6. Clean the outside of the sealed bag with a 0.5 to 0.6% sodium hypochlorite solution just prior to leaving the contaminated area. Typical household bleach sold in the United States contains approximately 5 to 6% sodium hypochlorite. The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution). Final solutions should be in a pH range of 6 to 8. Clorox® bleach diluted 1:10 meets these requirements. When using other brands, one should confirm the buffering capacity and sodium hypochlorite concentrations.
7. Place the cleaned sealed bag in another unused self-sealing bag, and prepare for shipping according to applicable guidelines and regulations (http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf).

To collect another sample, change gloves to prevent cross-contamination and repeat steps 1-6.

8. Submit the samples to the laboratory for culture and/or other analyses (e.g., microscopy).
9. Transport samples to the Level B or C laboratory at ambient temperature.

Collecting Sterile Swab Samples (Qualitative or Quantitative)

The following steps are used to collect samples for laboratory culture from small, non-porous surfaces or objects (keyboards, hard-to-reach areas within machinery, mail sorters, ventilation grilles, etc.).

1. Maintain appropriate chain-of-custody documentation and procedures.
2. Don sterile, non-powdered nitrile or vinyl examination gloves over the gloves that are part of standard PPE and clothing.
3. Remove a sterile, non-cotton swab from the package.
4. Moisten the swab with sterile water, sterile saline, or sterile phosphate-buffered saline (PBS) solution using aseptic technique to prevent cross-contamination. Note: check with the laboratory that will do the analysis to determine which type of swab and solution is preferred.
5. Wipe the surface. Recommended wipe area is <100 cm^2. Avoid letting the swab dry completely. Suggested sampling technique: make enough vertical S-strokes to cover the entire sample area.
6. Place the sampled swab in a sterile conical vial, and cap the vial.
7. Label the vial, and place it in a self-sealing bag (such as a Ziploc® bag or Whirlpak®, or similar).
8. Document the following items:
   - ♦ Discrete sample number or identifier
   - ♦ Sample location
   - ♦ Type of sample
   - ♦ Time and date of sample
   - ♦ Name of person collecting sample
   - ♦ Measured size of the area sampled
   - ♦ Map of sample area
9. Clean the outside of the sealed bag with a 0.5 to 0.6% sodium hypochlorite solution just prior to leaving the contaminated area. Typical household bleach sold in the United States contains about 5 to 6% sodium hypochlorite. The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution). Final solutions should be in a pH range of 6 to 8. Clorox® bleach diluted 1:10 meets these requirements. When using other brands, one should confirm the buffering capacity and sodium hypochlorite concentrations.
10. Place the cleaned sealed bag in another unused similar self-sealing bag.

To collect another sample, change gloves to prevent cross-contamination and repeat steps 1-10.

11. Prepare samples for shipping according to applicable guidelines and regulations (http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf) and submit the samples to the laboratory for analysis.
12. Transport samples to the Level A or higher laboratory at ambient temperature.

Collecting Sterile Surface Wipe Samples (Quantitative or

For use on large, non-porous surfaces such as table tops, counters, desks, file cabinets, and non-carpeted floors.

1. Maintain appropriate chain-of-custody documentation and procedures.
2. Don sterile, non-powdered examination gloves over the gloves that are part of standard PPE.
3. Remove a sterile 3" X 3" (or smaller) synthetic (non-cotton) gauze pad (gauze, Handi-Wipe®, sterile sponges) from package.
4. Moisten the gauze with sterile water, sterile saline, or sterile PBS solution using aseptic technique to prevent cross-contamination. Note: check with the laboratory that will do the analysis to determine which gauze and solution is preferred.
5. Wipe the surface. Recommended wipe area is approximately 1 square foot. Avoid letting the gauze pad dry completely. Suggested sampling technique: make enough vertical S-strokes to cover the entire sample area; fold the exposed side of the pad; make horizontal S-strokes over the same area.
6. Place the sampled gauze in a sterile conical vial, and cap the vial.
7. Label the vial, and place it in a self-sealing bag (Ziploc® bag, Whirlpak®, or similar).
8. Document the following items:
   - ♦ Discrete sample number or identifier
   - ♦ Sample location
   - ♦ Type of sample
   - ♦ Time and date of sample
   - ♦ Name of person collecting sample
   - ♦ Measured size of the area sampled
   - ♦ Map of sample area
9. Clean the outside of the sealed bag with a 0.5 to 0.6% sodium hypochlorite solution just prior to leaving the contaminated area. Typical household bleach sold in the United States contains about 5 to 6% sodium hypochlorite. The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution). Final solutions should be in a pH range of 6 to 8. Clorox® bleach diluted 1:10 meets these requirements. When using other brands, one should confirm the buffering capacity and sodium hypochlorite concentrations.
10. Place the cleaned sealed bag in another unused self-sealing bag.

To collect another sample, repeat steps 1-9. Change gloves between samples.

11. Prepare samples for shipping according to applicable guidelines and regulations (http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf) and submit the samples to the laboratory for analysis.
12. Transport samples to a Level B or C laboratory at ambient temperature.

Collecting Samples with a HEPA Vacuum Cleaner

The following steps should be used to collect samples for laboratory culture from large, porous or dusty, non-porous, dust/dirty surfaces such as carpeting, upper surface of ceiling tiles, ventilation systems, and papers. If the number of CFUs per gram of dust is desired, then pre-weighed filter socks should be used. Alternatively, the mean filter weight of several socks could be used as a background, representative weight.

1. Maintain appropriate chain-of-custody documentation and procedures.
2. Don sterile, non-powdered nitrile or vinyl examination gloves over the gloves that are part of the standard PPE and clothing.
3. Insert a cone-shaped Dust Collection Filter Sock manufactured by Midwest Filtration Company, Fairfield, Ohio, or equivalent) into the vacuum cleaner nozzle.
4. Fold the plastic sleeve over the outside of the nozzle, and secure it with an elastic band, or hold firmly in place using a gloved hand.
5. HEPA-vacuum the surface. Note: 1-2 tablespoons of vacuumed debris are desired.
   Technique: make one pass of the entire sampling area at a slow rate (12 inches per 5 seconds).
6. After collecting the sample, remove the tape or elastic band and discard these items as contaminated waste.
7. Remove the cone-shaped dust collection filter sock, and place it in a self-sealing bag (such as a Ziploc® bag or Whirlpak®), roll the filter, and place it in a sterile conical vial.
8. Place the sample in a clean self-sealing bag and label it with a discrete identifier.
9. Document the following items:
   - ♦ Discrete sample number or identifier
   - ♦ Sample location
   - ♦ Type of sample
   - ♦ Time and date of sample
   - ♦ Name of person collecting sample
   - ♦ Measured size of the area sampled
   - ♦ Map of sample area
10. Clean the outside of the sealed bag with a 0.5 to 0.6% sodium hypochlorite solution just prior to leaving the contaminated area. Typical household bleach sold in the United States contains about 5 to 6% sodium hypochlorite. The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution). Final solutions should be in a pH range of 6 to 8. Clorox® bleach diluted 1:10 meets these requirements. When using other brands, one should confirm the buffering capacity and sodium hypochlorite concentrations.
11. Place the cleaned sealed bag in another unused self-sealing bag.

To collect another sample, wipe the nozzle with an alcohol wipe, change gloves, and repeat steps 1-10.

12. The use of alcohol wipes will physically remove contamination from the nozzle surface but will not sterilize the surface.
13. Prepare samples for shipping according to applicable guidelines and regulations (http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf) and submit the samples to the laboratory for analysis.
14. Transport samples to the Level B or C laboratory at ambient temperature.

Collecting Air Samples

Air Cassettes

The sampling train consists of an air pump, Tygon® tubing, and a filter cassette (3-piece 37-mm cassette with a mixed cellulose ester membrane, polytetrafluoroethylene, or gelatin filter). The appendix below pertains to a mixed cellulose ester membrane (0.8 µm pore size). If gelatin filters are used, a modified procedure should be developed.

1. Maintain appropriate chain-of-custody documentation and procedures.
2. Calibrate the sampling train at 2 to 4 liters per minute. If a high volume sampler (similar to that used for asbestos sampling) is available, use it at the highest sample rate (which may be up to 16 liters per minute).
3. Remove the cap from the cassette (retain this cap for use later), and collect the sample closed-faced. Sampling time: as long as practical, generally a minimum of 6 to 8 hours at 2 to 4 liters per minute is suggested. Note: if gelatin filters are used, then an alternate sampling time and flow rate may be needed. (Consult manufacturers' specifications).
4. After the sample is collected, turn off pump, replace cap, and remove the filter cassette from the sampling train. Label the cassette sample, and place it in a clean self-sealing bag (Ziploc® bag, Whirlpak®, or similar).
5. Document the following items:
   - ♦ Discrete sample number or identifier
   - ♦ Sample location
   - ♦ Type of sample
   - ♦ Time and date of sample
   - ♦ Name of person collecting sample
   - ♦ Map of sample area
   - ♦ Pump start time
   - ♦ Pump stop time
   - ♦ Pump flow rate
6. Clean the outside of the sealed bag with a 0.5 to 0.6% sodium hypochlorite solution just prior to leaving the contaminated area. Typical household bleach sold in the United States contains about 5 to 6% sodium hypochlorite. The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution). Final solutions should be in a pH range of 6 to 8. Clorox® bleach diluted 1:10 meets these requirements. When using other brands, one should confirm the buffering capacity and sodium hypochlorite concentrations.
7. Place the cleaned sealed bag in another unused self-sealing bag.
8. Submit the samples to the laboratory for analysis.

To collect another sample, repeat steps 1-7.

9. Prepare samples for shipping according to applicable guidelines and regulations (http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf) and submit the samples to the laboratory for analysis.
10. Transport samples to the Level B or C laboratory at ambient temperature.

Collecting Air Samples

Impactors

There are many commercially available impactors. Before selecting a particular device, the investigator must consider the unique properties and specifications of the impactor (i.e., particle size cut points, operating flow rate, collection media) prior to making a determination that it is acceptable for its intended use. Impactors are primarily used in two ways: (1) using nutrient agar directly for culturable sample analysis, or (2) using filters for subsequent culture or spore analysis (counting, morphology, etc.). The appendix below pertains to impactors used with agar plates for culturable samples. If filter samples are used, an alternate procedure should be developed.

Note: prior to initial sample collection, the impactors should be autoclaved.

1. Maintain appropriate chain-of-custody documentation and procedures.
2. Program sampler for the volume of air to be sampled and aseptically place an open tryptic soy agar (TSA) with 5% sheep blood plate in the sampler. (Note: specific milliliters of pre-prepared agar are required for plates used in some impactor samplers (i.e., Andersen N-6), check these requirements when ordering the amount of fill in your plates.
3. Allow to run for the specified period of time.
4. Collect the plates and cover.
5. Seal plates with gas-permeable tape (i.e., masking tape).
6. Document the following items:
   - ♦ Discrete sample number or identifier
   - ♦ Sample location
   - ♦ Type of sample
   - ♦ Time and date of sample
   - ♦ Name of person collecting sample
   - ♦ Map of sample area
   - ♦ Pump start time
   - ♦ Pump stop time
   - ♦ Pump flow rate
7. After the sample is collected, remove the plate and place it in a clean self-sealing bag (Ziploc® bag, Whirlpak®, or similar).
8. Clean the outside of the sealed bag with a 0.5 to 0.6% sodium hypochlorite solution just prior to leaving the contaminated area. Typical household bleach sold in the United States contains about 5 to 6% sodium hypochlorite. The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution). Final solutions should be in a pH range of 6 to 8. Clorox® bleach diluted 1:10 meets these requirements. When using other brands, one should confirm the buffering capacity and sodium hypochlorite concentrations.
9. Place the cleaned sealed bag in another unused self-sealing bag.
10. Submit the samples to the laboratory for analysis.

To collect another sample, wipe the impactor components with an alcohol wipe, change gloves, and repeat steps 1-9. The use of alcohol wipes will physically remove contamination from the nozzle surface but will not sterilize the surface. After sample collection is complete, each impactor should be properly

decontaminated with a disinfection solution. The impactors should be autoclaved before use in an alternate site.

11. Prepare samples for shipping according to applicable guidelines and regulations (http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf) and submit the samples to the laboratory for analysis.
12. Transport samples to the Level A laboratory at ambient temperature.
13. Maintain appropriate chain-of-custody documentation and procedures.