

ENVIRONMENTAL COLLECTION AND SAMPLING PROTOCOLS FOR BIOTHREAT ANALYSIS

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General Instructions:

These procedures are intended for collection, packaging, and transport of bithreat samples, such as white powders suspected to being anthrax or ricin toxin, for laboratory analysis. If a chemical agent or toxin is suspected, please avoid area and contact HazMat immediately (NYS Fire: 518-474-6746).

For a suspected act of bioterrorism, call the NYS Department of Health at 518-474-4177 between 8:00 AM and 5:00 PM work days or the NYSDOH Duty Officer at 1-866-881-2809 after hours and weekends/holidays.

Suspected bithreat samples should only be collected following appropriate risk and credibility assessments (for list of criteria, refer to a *Code Red: Biological Agent Sampling Protocol* quick card). Do NOT collect highly suspect samples following a confirmed bioterrorism event without consultation with HazMat and NYSDOH.

Credible bithreat samples (i.e., potential criminal acts) should be analyzed at one of four facilities in the federal Laboratory Response Network (LRN). These facilities include the New York City Department of Health and Mental Hygiene (for samples originating in the 5 boroughs of New York City; 212-447-1091), Erie County Public Health Laboratory Biodefense Laboratory (716-898-6100), Westchester County Division of Laboratories and Research Biodefense Laboratory (914-231-1610 or 914-231-1600), or the Wadsworth Center Biodefense Laboratory (518-474-4177). **Contact the appropriate testing laboratory prior to sample transport as special collection or transport arrangements may be necessary. If you are uncertain about which testing facility to contact, please call the Wadsworth Center Biodefense Laboratory at 518-474-4177.**

All environmental bithreat samples, collected appropriately and packaged by a certified individual, must be analyzed by a New York State Environmental Laboratory Approval Program (ELAP) accredited testing facility. For a current list of certified testing laboratories, contact ELAP at (518)485-5570.

Suspect bithreat specimens are considered legal evidence relating to non-accidental exposure to biological agents. The Wadsworth Center will accept approved specimens (physical evidence) from law enforcement and first responder agencies following credible threat assessments. Material submitted as physical evidence must comply with the policies set forth to ensure appropriate chain of custody and specimen handling guidelines as described below.

I. COLLECTION AND ENVIRONMENTAL SAMPLING

Collection and submission of biothreat samples (e.g., white powder in an envelope) should be performed by informed and appropriately trained first responders following credible threat assessment. The decision to collect additional environmental samples for biothreat testing should be made with input from informed and appropriately trained medical, environmental, and industrial hygiene professionals familiar with the environmental sampling methodologies described in this document. This decision should be based on the nature and location of the suspected biothreat, any medical diagnoses or opinions relevant to the biothreat, the potential for the biohazard to migrate, and any potential facilities considerations. Representatives from local and state (and federal when appropriate) agencies should be consulted during the decision-making process. Testing laboratories will not accept unopened packages for biothreat testing unless they have been X-rayed and screened for explosives.

Small items (less than 1 cubic feet in size) can be collected and sent to appropriate testing laboratories directly. For larger samples or diffuse biohazards, environmental sampling should be performed. In addition, environmental sampling can be used to help determine the extent and degree of contamination, to support decisions regarding the need for cleanup, and to provide guidance regarding when cleanup is adequate to permit re-entry into an area. The use of experienced investigators to conduct the environmental sampling will provide the best probability of locating and identifying hazardous materials if present. Currently, no environmental exposure standards exist for biohazard agents such as anthrax. Investigators who review and interpret the results of environmental sampling for biothreats must consider these uncertainties and use professional judgment in interpreting any positive or negative findings.

Environmental sampling of surfaces is performed using a pre-wet sterile gauze pad (non-cotton preferably). This method works best for non-porous surfaces but is also sufficient for porous surfaces and fabrics although the recovery efficiency is lower. Surfaces are wiped with an absorptive medium from which biological organisms and biotoxins can be extracted in the laboratory. A sample biothreat collection kit containing a materials order list can be obtained from NYSDOH (order desk information here). Additional kits should be constructed by your agency from the material order list included in the package. The kit and collection procedures described below are consistent with guidelines issued by the US Centers for Disease Control and Prevention (CDC). The absorptive media, wetting agent, containment bags, decontamination materials, and transport container were 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. These guidelines should be consulted for an overview of environmental sampling.

Alternative environmental sampling procedures, such as HEPA vacuum or removal of a piece of material (i.e., cloth sample from chair) can be considered but only in consultation with environmental health specialists and the testing laboratory.

Collection or Sampling should always be performed by a two-person team: one person as the primary collector and one person as a facilitator. Hot and cold zones should be established to minimize exposure and contamination. All PPE should be donned in a cold zone. The primary collector should be considered 'dirty' and the facilitator is considered 'clean'. The primary collector will be the only responder to contact any suspicious materials while the facilitator will only handle only known clean (e.g., empty ziploc bags, sterile gauze) or contained items (e.g., environmental wipes in a ziploc bag). In the protocols below, actions performed by the primary Collector are denoted by the prefix –**C**, while those of the Facilitator are denoted with an **F**-prefix.

Materials

1. Personal protective equipment (2 pair of examination gloves per person and respirators, minimally)
2. 1 Sharpie pen for labeling samples
3. 2 Sterile, 2" x 2" Dacron (or other non-cotton) gauze pads
4. 6 oz. (10 mL) sterile liquid (e.g., water or saline solution)
5. zip-loc plastic bags (3 minimally).
6. 1 large, plastic transport container (e.g., cardboard box or Tupperware)
7. 2 Decontamination wipes (Hype-Wipes®) or 12 oz, (20 mL) fresh 10% bleach.

Procedure for collecting a contaminated item (e.g., an envelope containing powder)

1. **C/F**-Don 2 layers of disposable, sterile, non-powdered examination gloves and respirator (N95 minimally).
2. **C/F**- Enter hot zone.
3. **C**-Using slow, cautious movements, place the item(s) in a sterile plastic bag which is being held open by the Facilitator.
4. **F**- seal the primary bag and label with:
 - Field identification number
 - Collection location
 - Date and time of collection
 - Type and approximate size of sample (e.g., envelope, powder)
 - Name and contact information of person collecting sample

Alternatively, above information can be collected on the chain of custody form.
- 4) **F**- Disinfect the surface of the sealed bag by wiping with fresh, 0.5% hypochlorite solution (i.e., 10% household bleach). The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution).
- 5) **C**- Remove outer layer of gloves and place them into a clean plastic ziploc bag which is being held open by the Facilitator. **F**- Seal the bag.
- 6) **F**- Place the disinfected, primary sample bag(s) in two additional self-sealing bags outside of the contaminated area, disinfecting the surface of each bag by wiping with the hypochlorite bleach solution (multiple samples can be placed in the same secondary bags).
- 7) **F**- Place the triple-contained sample(s) into a hard sided container for transport to an approved testing facility (listed on page 1). Place evidence tape on the final layer of packaging and initial. Transport at ambient temperature is adequate.

- 8) **C-** Disinfect any exposed surfaces that the primary sample may have contaminated by wiping with fresh, 0.5% hypochlorite solution (used in Step 5 above).
- 9) **C-** Once area is decontaminated and cleaned, remove all PPE and discard into trash receptacle.
- 10) **C/F-** Decontamination materials and primary gloves may be sent to testing laboratory for disposal along with the biothreat sample.
- 11) **C/F-** make sure all chain of custody documentation is intact and biothreat sample transporter has contact information for responsible official who will receive analysis results.

Procedure for environmental sampling

1. **C/F-** Don 2 layers of disposable, sterile, non-powdered examination gloves and respirator (N95 minimally).
2. **C-** Remove sterile gauze pad from its package.
3. **C-** Moisten the gauze pad with sterile liquid (wring out any excess fluid). Excess liquid should not wet the surface to be sampled.
4. **C/F-** Enter hot zone.
5. **C-** Using uniform, light pressure and a slow, back-and-forth motion, wipe the contaminated surface with the gauze pad. Try to slightly overlap each stroke. Fold the pad to expose a fresh surface without touching the portion of the pad that has wiped the surface and repeat the wipe effort by drawing the pad over the sample area at a 90° angle to the first wipes. The wiped area should minimally be the size of a standard piece of paper (8.5x11 inches). For remediation purposes, the sampling area should be about 0.25 square meter (50 cm x 50 cm or 20 in x 20 in), but avoid letting the gauze get dry.
6. **C-** Place the gauze pad in a sterile plastic bag which is being held open by the Facilitator.
7. **F-** seal the primary bag and label with:
 - Field identification number
 - Collection location
 - Date and time of collection
 - Type and approximate size of sample (e.g., envelope, powder)
 - Name and contact information of person collecting sample
 - *If Wipe or HEPA sample, include approximate size of area sampled*
 - Name and contact information of person collecting sample

Alternatively, above information can be collected on the chain of custody form.
8. **F-** Disinfect the surface of the sealed bag by wiping with fresh, 0.5% hypochlorite solution (i.e., 10% household bleach). The disinfection solution is made by adding 1 part household bleach to 9 parts water (a 1:10 dilution).
9. **C-** Remove outer layer of gloves and place them into a clean plastic ziploc bag which is being held open by the Facilitator. **F-** Seal the bag.

To collect another sample, change outer gloves (to prevent sample cross-contamination) and repeat steps 2-8.

10. **F-** Place the disinfected, primary sample bag(s) in two additional self-sealing bags outside of the contaminated area, disinfecting the surface of each bag by wiping with the hypochlorite bleach solution (multiple samples can be placed in the same secondary bags).
11. **F-** Place the triple-contained sample(s) into a hard sided container for transport to an approved testing facility (listed on page 1). Place evidence tape on the final layer of packaging and initial. Transport at ambient temperature is adequate.
12. **C-** Disinfect any exposed surfaces that the primary sample may have contaminated by wiping with fresh, 0.5% hypochlorite solution (used in Step 8 above).
13. **C-** Once area is decontaminated and cleaned, remove all PPE and discard into trash receptacle.
14. **C/F-** Decontamination materials and primary gloves may be sent to testing laboratory for disposal along with the biothreat sample.
15. **C/F-** make sure all chain of custody documentation is intact and biothreat sample transporter has contact information for responsible official who will receive analysis results.

II. TRANSPORT OF BIOTHREAT SAMPLES

Transport of Biothreat samples by law enforcement officials

CDC sample packaging and shipping guidelines are available at <http://www.cdc.gov/od/ohs/biosfty/shipregs.htm> or <http://www.bt.cdc.gov/LabIssues/PackagingInfo.pdf> [Appendix A]

Appropriately packaged Biothreat samples should be transported to the nearest approved LRN testing laboratory (listed on page 1) in the most rapid and expeditious manner while in the custody of the appropriate law enforcement agency. Law enforcement agents are exempt from federal Department of Transportation (DOT) and International Air Transport Association (IATA) packaging and shipping regulations and thus need not adhere to these regulations. The packaging and shipping procedures described herein insures safe and secure transport of biothreat samples although they do not meet the federal transportation regulations.

Evidence Receipt

The biothreat sample transporter will provide appropriate contact information to the designated representative via a chain of custody or sample submission form.

The technologist receiving the package will examine it to determine that it is properly packaged, sealed and that there is a chain of custody document completed.

1. The package must be sealed with appropriate evidence tape and the tape must be initialed and dated. The tape must be affixed in a way that tampering would be obvious. In addition, a container is considered to be properly sealed only if it is intact and not compromised.
2. A specimen submission form, as designated by the submitting agency, should be attached to the evidence container. Intact initialed and dated evidence tape must be

placed perpendicular across the submission form to indicate that there is no evidence of tampering. The submission document must contain sufficient information to identify the submitting agency and accurately identify those items enclosed in the container.

3. The submitter and the technologist receiving the evidence will sign a 4 part original specimen chain of custody form at the evidence receiving area. One copy is handed to the submitter and the remaining 3 part form is retained by the technologist and submitted to the individual designated as responsible for the chain of custody procedure.
4. The specimens will be transported under secure conditions to the BSL3 testing suite.

Transport of Biothreat samples by non-law enforcement officials:

Regulations on the transportation of biological agents are aimed at ensuring that the public and the workers in the transportation chain are protected from exposure to any agent that might be in the package. Protection is achieved through (a) the requirements for rigorous packaging that will withstand rough handling and contain all liquid material within the package without leakage to the outside, (b) appropriate labeling of the package with the biohazard symbol and other labels to alert the workers in the transportation chain to the hazardous contents of the package, c) documentation of the hazardous contents of the package should such information be necessary in an emergency situation, and (d) training of workers in the transportation chain to familiarize them with the hazardous contents so as to be able to respond to emergency situations.

Regulations

Public Health Service 42 CFR Part 72. Interstate Transportation of Etiologic Agents. This regulation is in revision to harmonize it with the other U.S. and international regulations. A copy of the current regulation may be obtained from the Internet at: <http://www.cdc.gov/od/ohs>

Department of Transportation. 49 CFR Parts 171-178. Hazardous Materials Regulations. Applies to the shipment of both biological agents and clinical specimens. Information may be obtained from the Internet at: <http://www.dot.gov/rules.html>

United States Postal Service. 39 CFR Part 111. Ability to Mail Etiologic Agents. Codified in the Domestic Mail Manual 124.38: Etiologic Agent Preparations. A copy of the Domestic Mail Manual may be obtained from the Government Printing Office by calling 1-202-512-1800 or from the Internet at: <http://www.access.gpo.gov>

B. General Packaging Requirements for Transport of Biological Agents

All specimens and isolates are to be placed in UN approved containers and over-packing. These IATA certified mailers are available from several commercial vendors. All packages should be labeled in accordance with current CDC Federal Regulation 42 CFR Part 73 and U.S. Postal Regulation 39 CFR Part iii.

A generalized "triple" (water tight primary receptacle, water tight secondary packaging, preferably a third watertight packaging and a durable outer packaging) packaging is required for a biological agent of human disease or materials that are known or suspected of containing them. This packaging requires the "Infectious Substance" label on the outside of the package. This packaging must be certified to meet rigorous performance tests as outlined in the DOT, USPS, PHS, and IATA regulations. Note that the shipper's name, address and telephone number must be on the outer and inner containers. The reader is also advised to refer to additional provisions of the Department of Transportation (49 CFR, Parts 171-180) Hazardous Materials Regulations. See Appendix C, Figures 1 and 2 for reference.