

NIST Technical Note 1776

# Best Practices for Sample Collection and Transport During an Initial Response to Potential Biothreat Materials

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## **Abstract**

In the event of a suspected or confirmed biological threat, public safety, law enforcement and public and environmental health authorities may collect samples to support their initial response operational objectives. Responding representatives may include local hazardous materials response teams, law enforcement, and public health authorities, and may also include assistance from the Centers for Disease Control and Prevention (CDC), the Laboratory Response Network (LRN), the National Institute for Occupational Safety and Health and the Federal Bureau of Investigation (FBI). Communication and coordination among members of the different responding agencies on current best practices for the collection and transport of samples for laboratory analysis are critical to the success of these public health and public safety responses. The goals of this document are to provide guidance based on current state of biological surface sample collection, transport and processing science and current best practices to respond when the situation does not conform to available standardized approaches.

## Table of Contents

Acknowledgements.....	1
Abstract.....	2
1. Introduction .....	4
1.1 Background .....	4
2. SCENARIOS FOR WHICH VALIDATED SAMPLING METHODS ARE AVAILABLE.....	6
2.1 Visible powders suspected of being biothreat agents on nonporous surfaces (ASTM E2458) .....	6
2.2 Nonvisible <i>Bacillus anthracis</i> contaminants on smooth, nonporous surfaces (CDC methods) .....	6
3. ALTERNATIVE SCENARIOS FOR WHICH NO VALIDATED SAMPLING METHODS ARE AVAILABLE	7
3.1 Liquid contaminants on nonporous surfaces .....	7
3.2 Unknown nonvisible contaminants on nonporous surfaces .....	7
3.3 Contaminants on hard porous surfaces (e.g. concrete, brick, wood) .....	8
3.4 Contaminants on absorbent surfaces (carpet, upholstery and ventilation filters) ..	8
4. Decision Tool for Selection of Sampling Method .....	9
5. Sample Collection kits.....	2
6. Coordination of Sample Transport and Storage .....	2
6.1 Documentation needs for sample coordination.....	2
7. References .....	4
Appendix A. Existing Standards, Regulations and Guidance Resources.....	6
NFPA Standards: .....	6
IATA Standards:.....	6
Federal Government Regulations: .....	6
Federal Guidance: .....	7
International Standards and Guidance:.....	7
ANSI Standards:.....	7
ASTM Standards:.....	8

## 1. Introduction

### 1.1 Background

Since the fall of 2001, there have been over 30,000 responses to incidents involving suspicious powders and other materials that were considered potential biological threats requiring federal law enforcement and public health expertise [1]. Suspicious powder calls still pervade the public safety mission of our society, diverting resources by requiring law enforcement, public health and safety personnel to respond. These incidents cause fear and anxiety in the communities that are impacted by them. Targets are often high profile community members and locations of high visibility within the community, including court houses and elementary schools.

An essential element of any operational response to a suspected biothreat is active communication and coordination between public safety, public health and law enforcement response personnel. In order to effectively and rapidly address these calls, jurisdictions have developed best practices and protocols. Recently, ASTM International published two standards, E2458-10 – “Standard Practice for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of being Biological Agents from Nonporous Surfaces” and E2770–10 “Standard Guide for Operational Guidelines for Initial Response to a Suspected Biothreat Agent.” The standards were developed to assist jurisdictions in providing an initial response capability and to communicate the resources available to them to date. E2770 is based on current best practices developed by jurisdictions since 2001 and is designed to communicate the essential elements of a response regardless of jurisdiction capability (rural to highly populated urban areas) [2]. The International Association of Fire Chiefs (IAFC) guidelines for responding to suspicious packages provide additional guidance on response considerations and facility operation procedures for the initial response personnel [3].

Sample collection to support initial response to suspected biothreats can be accomplished using a number of different techniques and methods available. When the materials to be collected are powders on a nonporous surface, existing standardized procedures should be used [2, 4-6]. However, responders encounter a variety of suspicious materials (liquids, packages, etc.) on an even broader array of surfaces types (carpet, concrete, upholstery, wallboard etc.). Since the nature of most initial response calls are for suspicious powders, ASTM E2458 is designed to address the collection of unknown particulate suspected of being a biothreat from a nonporous surface and submission of that sample to the receiving LRN reference laboratory. The LRN reference laboratories are prepared to analyze unknown, potential biothreat materials with a range of assays to identify and confirm the presence of the organisms.

In many cases bulk powder may not be present or contamination may be present on porous surfaces and sampling may be necessary. Responders will have to rely on sampling procedures other than those described above in order to assess whether contamination is present. The Centers for Disease Control and Prevention (CDC) has developed surface sampling procedures for the collection of *Bacillus anthracis*, the organism causing anthrax, from nonporous surfaces using sponge sticks and/or swabs.

Sample collection procedures for other agents are limited; therefore general collection procedures are also described to assist local responders.

Biological surface sample collection should fit into a logical approach for collection and should be directed toward the action that the sample will support. Part of the collection process is documenting the approach that will be taken for sample collection in a sampling and analysis plan (SAP). When appropriate through standard evidence collection procedures, SAP development should be coordinated through the incident command structure [7] and shall reference any current federal guidance on sample plan generation. Any evidence collection should be coordinated and overseen by law enforcement.

Due to past real world experiences, current research and available methods are heavily focused on the collection of *Bacillus anthracis*. Validated methods for collection of *B. anthracis* from porous and carpeted surfaces as well as agents other than *B. anthracis* are limited. Therefore, available knowledge on collection method performance and application gathered from a comprehensive literature review is presented in this summary report. This guidance document was designed as a resource for use by member laboratories of the Laboratory Response Network when coordinating with field responders including HazMat teams to determine how best to respond to and collect suspected of biological threat agents. During initial response to a potential bioterror event, first responders should coordinate collection of suspicious materials with their receiving LRN laboratory. This document summarizes applications for existing, standardized methods, innovative approaches and recent scientific reports on surface sample collection methods and emerging operational best practices. Additionally, this document can serve as a guide to collection procedures when sample collection standards and/or validated methods are unavailable.



## 2. Scenarios For Which Validated Sampling Methods Are Available

### 2.1 Visible powders suspected of being bioterror agents on nonporous surfaces (ASTM E2458)

When visible powder materials suspected of being bioterror agents are found deposited on nonporous surfaces, sample collections should be conducted in accordance with ASTM E2458 [4]. The method uses a dry swab and laminated card, followed by a swab sampling method using a sterile moistened swab. Bulk powder samples are collected and packaged in a manner that permits the maximum amount of the sample to be safely transported to an approved LRN reference laboratory for confirmatory identification and safe storage. If the source of the powder is a letter or small package, the source is packaged separately from the powder in a manner that permits it to be safely transported to the laboratory. Once the bulk powder has been collected and preserved, swab samples of remaining powder can be collected using a sterile moistened swab (according to Method B of ASTM E2458-10) for on-site biological assessment; or the swab samples can be sent directly to the receiving LRN reference laboratory. This summary can by no means replace the review and application of the standard and all users are encouraged to access the standard directly and free of charge by becoming a registered user of the Responder Knowledge Base ([www.RKB.us](http://www.RKB.us)) and searching the standards section [8].



Figure 1- Demonstration of ASTM E2458-10 Method A for sampling visible powders from nonporous surfaces using a sterile non-cotton swab and laminated card

### 2.2 Nonvisible *Bacillus anthracis* contaminants on smooth, nonporous surfaces (CDC methods)

Sampling methods made available by CDC NIOSH were prepared by the CDC to provide standardized procedures for industrial hygienists, or other trained samplers under the direction of sampling experts, to use when sampling for *B. anthracis* spores. The CDC methods should only be utilized during initial response scenarios when properly coordinated with the receiving LRN reference laboratory and the local FBI WMD Coordinator. These and all sampling methods must be coordinated with the LRN reference laboratory to ensure that the laboratory can accept and process the number and type of samples being referred. The CDC methods are meant to be used for collection of samples from smooth, nonporous surfaces and can be used in both indoor and outdoor environments. Examples of nonporous surfaces encompassed by these methods are stainless steel, painted wall board, floor tile, or wood laminate. Each sampling method has its specific uses and advantages and those applications are discussed in the procedure [5]. Covered in the CDC collection methods are the use of macrofoam swabs and cellulose sponge-sticks. Swabs are appropriate for sampling small surfaces or hard to reach

locations of less than 4 inches square, like crevices, corners, supply air diffusers, and air return grills. Sponge-sticks are appropriate for sampling larger surfaces, no greater than 100 in<sup>2</sup>, including walls, desks, and floors. Both swab and sponge-stick methods are described in the detailed method publication at [www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthraxis.html](http://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthraxis.html).

### **3. Alternative Scenarios for Which No Validated Sampling Methods Are Available**

The standardized procedures currently available to the response community [4, 5] are well vetted. However, current standards are only applicable for collection of suspicious powders and powder residues from nonporous surfaces and in the case of the CDC collection procedures, only validated for *B. anthracis* sampling. Responders are often called to collect samples from large areas and diverse surfaces. When materials suspected to be biothreat agents are found on porous surfaces, or when the suspicious materials are in liquid form, the following summary of current findings based on the peer reviewed literature may serve to guide the optimal collection approach based on the on-scene circumstances encountered. The collection and submission of any sample with non-validated methods will require coordination and communication with local public health, including the receiving LRN reference laboratory, as well as the FBI. Additionally, coordination of the submission of any field blanks of collection materials including buffer solutions utilized in sampling of suspected biothreats is highly recommended. Any field blanks must be clearly labeled and indicated in submission documentation.

#### **3.1 Liquid contaminants on nonporous surfaces**

There is a paucity of scientific data to support recommendations for collection of suspicious liquids on nonporous surfaces; however, use of dry sampling materials may be preferable to those that have been pre-wetted in order to maximize absorption of the liquid. Additionally, the absorptive capacity of wipes and sponges is far greater than that of swabs so except in cases where very small amounts of liquid are present, wipes and sponge sticks should be used to maximize the recovery of the liquid from the surface.

#### **3.2 Unknown nonvisible contaminants on nonporous surfaces**

Early in a response, the question of whether a biothreat is present on a surface has not yet been definitively answered. Although validated only for collection of *Bacillus anthracis*, the CDC collection procedures (swabs for sampling areas less than 4 inches square and sponge sticks for larger areas) could be considered for use when the identity of a suspected biothreat contaminant is unknown. For a small surface area, ASTM method B may also be applied. However, sampling results would have to be interpreted cautiously because the performance characteristics of this method applied to other organisms would not be known.

When unknown suspect biothreat contaminants are present on nonporous surfaces the use of foam swab or cellulose sponge stick collection materials are preferable due to consistently higher recovery

efficiencies reported for foam swabs compared to other swab types [9-11], and better variability data available for the sponge-wipe analysis method.

### **3.3 Contaminants on hard porous surfaces (e.g. concrete, brick, wood)**

The differing absorptive natures of various porous surfaces that may be encountered by first responders during a potential biothreat field response pose challenges for standardization of collection methods.

Swabs can be useful when high contaminant concentrations are expected in relatively small surface areas (less than 4 inches square ) [12] or when locations (e.g., crevices or supply air diffusers) that are difficult to access by wipe or vacuum must be sampled [13]. When low contaminant concentrations are expected (e.g. no powder is visible), if contamination is widespread or if the exact site of potential contaminant deposition is unknown, wipes allow sampling of larger surface areas as compared to swabs, and should be used preferentially in order to improve sensitivity of detection [14].

Pre-moistening swabs and wipes prior to sampling has been shown to maximize contaminant recovery from surfaces [10, 13, 15, 16]. Currently, neutralizing buffer and phosphate buffered saline (PBS) solution with 0.1 % Tween-20 are recommended wetting agents in the standardized methods [4, 5]. Any other buffer solution provided by the manufacturer of an on-site biological assessment kit may be appropriate if communicated with the receiving LRN laboratory prior to application, to ensure compatibility with the detection assays.

If available, and the receiving LRN reference laboratory is able to process vacuum sample collection devices (e.g. 3M Trace evidence filters, Midwest Filtration dust sampling socks, and 37 mm cassettes) such devices may be an alternative to improve detection sensitivity for sampling large surface areas [13, 17, 18]. This type of sampling requires the use of HEPA filtered vacuum devices downstream of the collection device, to ensure spores are not further dispersed by the action of sampling. These devices also require additional manipulation during processing that may put laboratories at risk for exposure. The LRN laboratory analyzing the vacuum sampling devices should be consulted prior to using a vacuum method to determine if that laboratory is capable of processing and analyzing the sample.

### **3.4 Contaminants on absorbent surfaces (carpet, upholstery and ventilation filters)**

When suspected biothreat contaminants are found on carpet or upholstery, wipes and sponges can be used for sample collection and may be preferable over swabs due to reports of increased recovery efficiency for wipes [11, 14, 17]. Performance for rayon/polyester or cellulose/polyester blends have been reported to be superior to cotton gauze wipes in overall recovery efficiency [11].

Pre-moistening swabs and wipes prior to sampling has been shown to maximize contaminant recovery from surfaces [10, 13, 15, 16]. Currently, neutralizing buffer and phosphate buffered saline (PBS) solution with 0.1 % Tween-20 are recommended wetting agents in the standardized methods [4, 5],

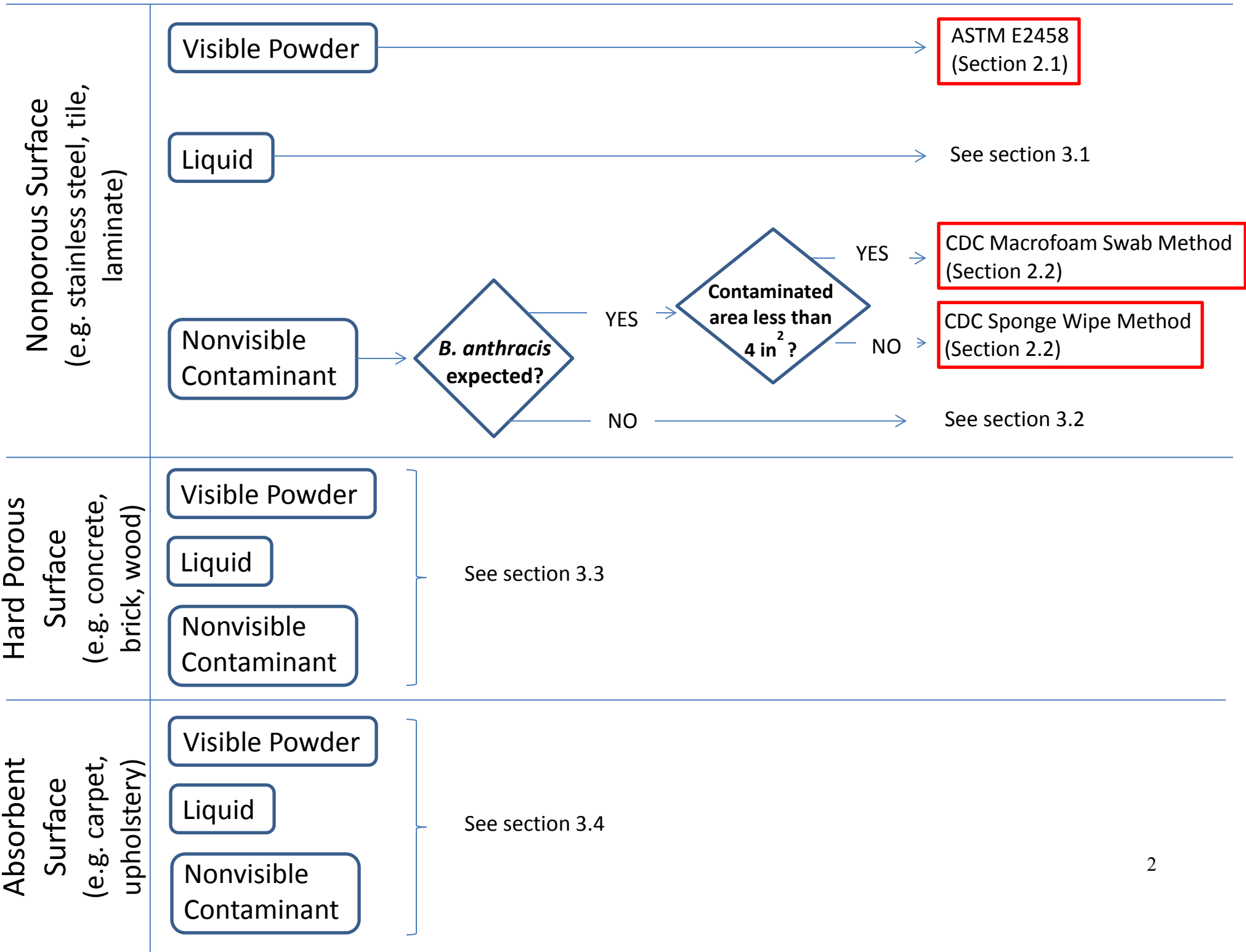
however, a buffer solution provided by the manufacturer of an on-site biological assessment kit may be appropriate if communicated with the receiving LRN reference laboratory prior to application.

Vacuum sampling, described previously in Section 3.3, is also effective for spore collection from carpet or upholstery and could be used on these surfaces, particularly if high contaminant concentrations are expected [18], however, the LRN laboratory analyzing the vacuum sampling devices should be consulted prior to using a vacuum method to determine if that laboratory is capable of processing and analyzing the sample.

Alternatively, a bulk sample of the surface (e.g., sections of ventilation filters, carpet, or upholstery) can be removed and transported to the LRN reference laboratory for processing and testing [19, 20]. However, prior to collecting a bulk sample, sampling teams must ensure receipt of bulk samples has been communicated and agreed to by the recipient LRN reference laboratory and a risk assessment has been performed to determine suitability of the bulk sample for testing. Additional considerations that must be addressed prior to sample collection include meeting safe packaging and shipping regulations and the size constraints of the biosafety cabinet of the receiving laboratory.

#### **4. Decision Tool for Selection of Sampling Method**

To further summarize efforts to support biological surface sample collection methods selection, the following decision tool is provided. Please note that the decision tool is to direct laboratory support personnel and trained field users to sections in this document that summarize available method performance data.



## **5. Sample Collection Kits**

Sample collection kits should be flexible to accommodate the different types of sample collection scenarios that may be encountered during a response. When designing a kit for sample collection of unknown materials, kit contents should align with receiving LRN laboratory methods for sample collection and processing procedures. Responders may wish to submit samples collected using materials provided by the manufacturer of on-site biological assessment tools. When proper coordination and communication procedures have been followed, those samples can be submitted to the LRN reference laboratory and should be packaged and shipped as described in ASTM E2458 or the CDC method depending on which one was followed. Additionally, a number of sample collection kit manufacturers provide well characterized and quality controlled (sterilization verification, lot numbers and expiration dates) materials that have been assembled into kits to meet various scenarios and applications. Kit manufacturers are listed on the Responder Knowledge Base ([www.rkb.us](http://www.rkb.us)).

## **6. Coordination of Sample Transport and Storage**

After sample collection in the field, samples are packaged and transported to the receiving LRN reference laboratory for processing and analysis. For the CDC *B. anthracis* method, all samples should be sent to the laboratory on wet ice or on cold packs. These samples may be stored at 2°C–8°C prior to processing and should be processed within 48 hours of collection. CDC has developed a protocol for safe packaging of the vacuum socks for shipping. This protocol is described at [www.cdc.gov/niosh/topics/anthrax/pdfs/socksguidance.pdf](http://www.cdc.gov/niosh/topics/anthrax/pdfs/socksguidance.pdf) [6]. Packaging materials should be consistent with the DOT requirements for shipping/transporting hazardous materials as described in both ASTM E2458 and E2770 [2, 4]. Storage of suspected biothreat samples at the receiving LRN reference laboratory must be coordinated by the FBI to meet forensic and evidence storage condition recommendations.

### **6.1 Documentation needs for sample coordination**

In order to maintain a record of the incident and to insure proper communication of information on the sample collected and any field screening done to assess the hazard, documentation should be submitted to the receiving LRN reference laboratory. Specific documentation is recommended and first responders should contact their local LRN reference laboratory prior to an event to find out which forms are required and the protocol for submission in their jurisdiction. As an example of information that may be required, Section 14 of E2770 has a comprehensive list of items to be disclosed in the laboratory submission documentation [2]. Example documents are contained in the appendix of ASTM E2458 and E2770 that include, but are not limited to, the chain of custody forms, field screening results, and sample submission form. Additional

information such as sample collection procedure description (noting surface type, area sampled and collection of field blanks), and a record of exposed individuals with contact information for possible follow-up public health response may all be useful. In order to decrease time to results reporting, responders can fax or email a copy of the chain of custody form in advance of the shipment to allow laboratories to pre-enter data into their management system and prepare supplies for processing.

## 7. References

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## **Appendix A. Existing Standards, Regulations and Guidance Resources**

### **NFPA Standards:<sup>1</sup>**

NFPA 471 Recommended Practice for Responding to Hazardous Materials Incidents, 1997 Edition

NFPA 472 Standard for Competence of Responders to Hazardous Materials/Weapons of Mass Destruction Incidents, 2008 Edition

NFPA 1994 Standard on Protective Ensembles for Chemical/Biological Terrorism Incidents

NFPA 1600 Standard on Disaster/Emergency Management and Business Continuity Programs, 2007 Edition

### **IATA Standards:<sup>2</sup>**

IATA PI 602 Infectious Diseases (Infectious Substances)

IATA PI 650 Shipping of Diagnostic Samples

IATA DGR 46th Edition, 2005

IATA DGR Addendum I, January 2005

IATA DGR Addendum II, March 2005

IATA DGR Addendum III, July 2005

### **Federal Government Regulations:<sup>3</sup>**

DOT - 49 CFR, Parts 171-180, Hazardous Materials Regulations

DOT - 49 CFR 172 Subpart H, Transportation Training

DOT - 49 CFR 173, General Requirements for Shipments and Packagings

DOT - 49 CFR 178, Specifications for Packagings

EPA - 40 CFR 300, National Oil and Hazardous Substances Pollution Contingency Plan (NCP)

EPA - 40 CFR 311, Worker Protection

NRC - 10 CFR 20, Standards for Protection against Radiation

NIOSH - 42 CFR 84, Respiratory Protective Devices

OSHA - 29 CFR 1910 Subpart Z and 29 CFR 1926 Subpart Z, Toxic and Hazardous Substances

OSHA - 29 CFR 1910.1096 and 29 CFR 1926.53, Ionizing Radiation

OSHA - 29 CFR 1910.120, Hazardous Waste Operations and Emergency Response (HAZWOPER) standard

OSHA - 29 CFR 1910 Subpart I (Sections 132 to 139), Personal Protective Equipment

OSHA - 29 CFR 1910.1200, Hazard Communication

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<sup>1</sup> Available from the National Fire Protection Association, 1 Batterymarch Park, Quincy, MA 02269—9101

<sup>2</sup> Available from the International Air Transport Association, 800 Place Victoria, PO Box 113, Montreal-H4Z 1M1, Quebec, Canada.

<sup>3</sup> Available from U.S. Government Printing Office Superintendent of Documents, 732 N. Capitol St., NW, Mail Stop: SDE, Washington, DC 20401, and also available online from Occupational Safety and Health Administration ([www.osha.gov](http://www.osha.gov)).

**Federal Guidance:**

FBI-DHS-HHS/CDC Coordinated Document, Guidance on Initial Response to a Suspicious Letter/Container with a Potential Biological threat, November 2, 2004.

NIMS 2008 National Incident Management System<sup>4</sup>

Planning Guidance for Recovery Following Biological Incidents, Biological Decontamination Standards Working Group, Subcommittee on Decontamination Standards and Technology Committee on Homeland and National Security, National Science and Technology Council, May 2009

NRF 2008 National Response Framework<sup>5</sup>

OSHA - CPL 02-02-073, Inspection Procedures for 29 CFR 1910.120 and 1926.65, Paragraph (q): Emergency Response to Hazardous Substance Releases

EPA - Safety, Health, and Environmental Management (SHEM) Guide No. 44, Personal Protective Equipment, October 2004

EPA - Safety, Health, and Environmental Management (SHEM) Guideline No. 46, Respiratory Protection, dated October 2004

EPA - Order 1460.1, Occupational Medical Surveillance Program, June 18, 1996

NIOSH Publication No. 2009-132: Recommendations for the Selection and Use of Respirators and Protective Clothing for Protection Against Biological Agents

CDC Surface Sampling Procedures for Bacillus anthracis spores from smooth, nonporous surfaces, May 2012

FBI Laboratory Publication: Handbook of Forensic Services 2003

DOT, current version, Emergency Response Guidebook (ERG)<sup>6</sup>

**International Standards and Guidance:**

International Association of Fire Chiefs (IAFC) Guidance, Model Procedures for Responding to a Package with Suspicion of a Biological Threat, October 2008

ISO/IEC Guide 43-1:1997 Proficiency Testing by Interlaboratory Comparison, Part1: Development and Operation of Proficiency Testing Schemes

**ANSI Standards:**

ANSI Z41-1999: American National Standard for Personal Protection - Protective Footwear

ANSI Z87.1-2003: American National Standard for Occupational and Educational Personal Eye and Face Protection Devices

ANSI Z88.2-1992: American National Standard Practices for Respiratory Protection

ANSI Z88.10-2001: American National Standard for Personal Protection - Respirator Fit Testing Methods

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<sup>4</sup> For access to document, go to [www.fema.gov](http://www.fema.gov).

<sup>5</sup> For access to document, go to [www.fema.gov](http://www.fema.gov).

<sup>6</sup> Available from <http://hazmat.dot.gov/pubs/erg/gydebook.htm>.

ANSI/ISEA Z89.1-2003: American National Standard for Personal Protection -  
Protective Headwear for Industrial Workers Requirements

ANSI/Compressed Gas Association, CGA G-7.1-1997, Commodity Specification for Air

**ASTM Standards:**

ASTM E 2601-08: Standard Practice for Radiological Emergency Response

ASTM F 2412-05: Standard Test Methods for Foot Protection

ASTM F 2413-05: Specification for Performance Requirements for Foot Protection

ASTM E 2458-10: Practices for Bulk Sample Collection and Swab Sample Collection of  
Visible Powders Suspected of Being Biological Agents from Nonporous Surfaces

ASTM E 2770-10: Guide for Operational Guidelines for Initial Response to a  
Suspected Biothreat Agent