Designation: E2458 – 17

Standard Practices for Bulk Sample Collection and Swab Sample Collection of Visible Powders Suspected of Being Biological Agents and Toxins from Nonporous Surfaces[1]

This standard is issued under the fixed designation E2458; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 These practices address collection of visible powders that are suspected biological agents and toxins from solid nonporous surfaces using a bulk collection method, using 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 a reference laboratory within the Centers for Disease Control and Prevention (CDC) national Laboratory Response Network (LRN)[2] for confirmatory identification and safe storage. If the source of the powder is a letter or small package, that item is also packaged in a manner that permits it to be safely transported to an LRN reference laboratory. A sterile moistened swab may be used to collect residual powder from the nonporous surface and may be used to conduct on-site biological assessments for the purpose of testing for biological agents and toxins.

1.2 These practices are performed in coordination with the Federal Bureau of Investigation (FBI) as part of a risk assessment including hazard assessment and threat credibility evaluation as recommended and clarified in Guide E2770. The decision to implement these practices and collect a public safety sample will be made by members of the response community of the jurisdiction assuming responsibility through coordination with the FBI and the receiving LRN reference laboratory.

1.3 Sample Collection Method A covers the bulk collection and packaging of suspicious visible powders that are suspected biological agents and toxins from solid nonporous surfaces. All samples suspected to be biological agents and toxins on nonporous surfaces should be collected according to Sample Collection Method A and sent to an LRN reference laboratory for confirmatory testing.

1.4 Sample Collection Method B covers swab sampling of residual suspicious powders that are suspected biological agents and toxins from solid nonporous surfaces. Swab samples can be used for on-site biological assessment; however results from on-site biological assessments are not definitive; confirmatory testing by the LRN reference laboratory is necessary to make public health decisions.

1.5 These practices incorporate reference guidance for packaging and transport of suspicious visible powders to comply with all appropriate federal regulations regarding biosafety and biosecurity.

1.6 These practices should only be used to collect visible samples that are suspected biological agents and toxins and have been field screened according to reference guidance for explosive hazard, radiological hazard, and other acute chemical hazards.

1.7 The values stated in SI units are to be regarded as standard. The values given in parentheses are for information only.

1.8 *This standard does not purport to address all of the safety concerns associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

1.9 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

2. Referenced Documents

2.1 *ASTM Standards:*[3]

[1] These practices are under the jurisdiction of ASTM Committee E54 on Homeland Security Applications and are the direct responsibility of Subcommittee E54.01 on CBRNE Sensors and Detectors.

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[2] The CDC Laboratory Response Network is the network responsible for handling clinical specimens and environmental samples containing suspected biothreat agents.

[3] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

E2770 Guide for Operational Guidelines for Initial Response to Suspected Biological Agents and Toxins
F2412 Test Methods for Foot Protection
F2413 Specification for Performance Requirements for Protective (Safety) Toe Cap Footwear

2.2 *Federal Government Regulations:*[4]
18 USC 178 Prohibitions with respect to biological weapons
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 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

2.3 *Federal Guidance:*
OSHA - CPL 02-02-073 Inspection Procedures for 29 CFR 1910.120 and 1926.65, Paragraph (q): Emergency Response to Hazardous Substance Releases
NIOSH Publication No. 2009-132 Recommendations for the Selection and Use of Respirators and Protective Clothing for Protection Against Biological Agents
FBI Laboratory Publication Handbook of Forensic Services 2013
FBI-DHS-HHS/CDC Coordinated Document Guidance on Initial Response to a Suspicious Letter/Container with a Potential Biological threat, November 2, 2004
CDC/NIOSH Surface Sampling Procedures for *Bacillus anthracis* Spores from Smooth, Non-porous Surfaces, April 26, 2012[5]
DHS Framework for a Biothreat Field Response Mission Capability, April 2011[6]
Sandia National Laboratories SAND2005-3237 (LBNL-54973 (II)) Guidelines to Improve Airport Preparedness Against Chemical and Biological Terrorism[7]

2.4 *NFPA Standards:*[8]
NFPA 472 Standard for Competence of Responders to Hazardous Materials/Weapons of Mass Destruction Incidents, 2013 Edition
NFPA 1994 Standard on Protective Ensembles for Chemical/Biological Terrorism Incidents

2.5 *IATA Standards:*[9]
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

2.6 *ANSI Standards:*[10]
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
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

2.7 *IAFC Guidance:*[11]
Model Procedures for Responding to a Package with Suspicion of a Biological Threat, October 2008

3. Terminology

3.1 *Definitions:*

3.1.1 *aseptic technique, n*—operation or performance of a procedure or method under carefully controlled conditions to reduce the risk of exposure and prevent the introduction of unwanted material/matter (contamination) into a sample.

3.1.2 *biological agent, n*—any microorganism (including but not limited to, bacteria, viruses, fungi, rickettsiae, or protozoa), or infectious substance, or any naturally occuring, bioengineered or synthesized component of any such microorganism or infectious substance, capable of causing: *(1)* death, disease or other biological malfunction in a human, an animal, a plant, or another living organism; *(2)* deterioration of food, water, equipment, supplies, or material of any kind; or *(3)* deleterious alteration of the environment. **18 USC 178**

3.1.3 *bulk powder, n*—a visible powder, at least approximately 5 mL (1 teaspoon) in volume amassed or dispersed over

[4] 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).
[5] Available from http://www.cdc.gov/niosh/topics/emres/surface-sampling-bacillus-anthracis.html.
[6] Available from http://www.hsdl.org/?view&did=767721.
[7] Available from http://share-ng.sandia.gov/news/resources/releases/2005/images/unlsand-2005-3237.pdf.
[8] Available from National Fire Protection Association (NFPA), 1 Batterymarch Park, Quincy, MA 02269-9101.
[9] Available from the International Air Transport Association, 800 Place Victoria, PO Box 113, Montreal-H4Z 1M1, Quebec, Canada.
[10] Available from American National Standards Institute (ANSI), 25 W. 43rd St., 4th Floor, New York, NY 10036, http://www.ansi.org.
[11] Available from International Association of Fire Chiefs (IAFC), 4025 Fair Ridge Drive, Suite 300 Fairfax, VA 22033, http://www.iafc.org.

a limited area (optimally, area should be less than 20 by 20 cm (approximately 8 by 8 in.).

3.1.4 *chain of custody, n*—set of procedures and documents to account for the integrity of sample by tracking its handling and storage from point of sample collection to final disposition of the sample.

3.1.5 *cold zone, n*—the uncontaminated area where workers are unlikely to be exposed to hazardous substances or dangerous conditions; also known as Clean Zone or Support Zone. **CPL 02-02-071 Directive**

3.1.6 *confirmatory analysis, n*—a test or a series of assays that definitively identifies the presence of a suspected substance or agent.

3.1.6.1 *Discussion*—Confirmatory analysis of a biological agent for public health action can be performed only by an LRN national or reference laboratory.

3.1.7 *decontamination, n*—the physical or chemical process, or both, of reducing and preventing the spread of contaminants from people, animals, the environment, or equipment involved at hazardous materials/weapons of mass destruction (WMD) incidents. **NFPA**

3.1.8 *field screening, n*—field measurements utilized early in the response to define and characterize the potential hazards present, including corrosive, flammable, volatile, radioactive, or oxidizer hazards, and to support tactical decision making to address operational safety measures.

3.1.8.1 *Discussion*—Field screening does not include measurements of biological properties, which is termed on-site biological assessments (see 3.1.12).

3.1.9 *hazard, n*—something that is potentially dangerous or harmful, often the root cause of an unwanted outcome; a danger or peril. **NIMS**

3.1.10 *hot zone, n*—the area, located on the site where contamination is either known or expected and where potential for greatest exposure exists; also known as Exclusion Zone or ExZ. **CPL 02-02-071 Directive**

3.1.11 *incident commander (IC), n*—the individual responsible for all incident activities, including the development of strategies and tactics and the ordering and release of resources.

3.1.11.1 *Discussion*—The IC has overall authority and responsibility for conducting incident operations and is responsible for the management of all incident operations at the incident site. **NIMS**

3.1.12 *on-site biological assessment, n*—measurements of properties inherent to biological materials performed in the field using rapid, field-based procedures and assays.

3.1.13 *personal protective equipment (PPE), n*—equipment provided to shield or isolate a person from the chemical, biological, physical, and thermal hazards that can be encountered at hazardous materials/weapons of mass destruction (WMD) incidents. **NFPA**

3.1.14 *presumptive test, n*—non-definitive test used to evaluate a material for the presence of a substance or agent, or the presence of signatures of a substance or agent.

3.1.15 *risk, n*—the probability of suffering a loss or harm or injury; peril.

3.1.16 *toxin, n*—the toxic material or product of plants, animals, microorganisms (including but not limited to, bacteria, viruses, fungi, rickettsiae, or protozoa), or infectious substances, or a recombinant or synthesized molecule, whatever their origin and method of production, and includes: (*1*) any poisonous substance or biological product that may be engineered as a result of biotechnology produced by a living organism; or (*2*) any poisonous isomer or biological product, homolog, or derivative of such a substance (18 USC 178).

3.1.17 *threat, n*—an indication of possible violence, harm, or danger and may include an indication of intent and capability. **NIMS**

3.1.18 *warm zone, n*—the transition area between the Exclusion Zone (ExZ or hot zone) and the Support Zone (SZ or cold zone) used to reduce and limit the amount of contamination on people and equipment, and in the air, water, and soil that may be transferred into nonhazardous areas; the CRZ contains decontamination facilities, and functions as a buffer zone surrounding the ExZ; also known as the contamination reduction zone or CRZ. **CPL 02-02-071 Directive**

3.1.19 *weapon of mass destruction (WMD), n*—any weapon or device that is intended, or has the capability, to cause death or serious bodily injury to a significant number of people through the release, dissemination, or impact of (*1*) toxic or poisonous chemicals or their precursors; (*2*) a disease organism; or (*3*) radiation or radioactivity. **U.S. Code Title 50, Ch. 40, Sect. 2302, War and National Defense Definitions**

3.2 *Acronyms:*

3.2.1 *AHJ*—Authority Having Jurisdiction

3.2.2 *ANSI*—American National Standards Institute

3.2.3 *ASTM*—American Society for Testing and Materials

3.2.4 *CDC*—Centers for Disease Control and Prevention

3.2.5 *CFR*—Code of Federal Regulations

3.2.6 *CRZ*—Contamination Reduction Zone

3.2.7 *CST*—Civil Support Team

3.2.8 *DHS*—Department of Homeland Security

3.2.9 *DOT*—Department of Transportation

3.2.10 *EOC*—Emergency Operations Center

3.2.11 *EPA*—Environmental Protection Agency

3.2.12 *ExZ*—Exclusion Zone

3.2.13 *FBI*—Federal Bureau of Investigation

3.2.14 *FEMA*—Federal Emergency Management Agency

3.2.15 *HAZMAT*—Hazardous Materials

3.2.16 *HHS*—Health and Human Services

3.2.17 *IAFC*—International Association of Fire Chiefs

3.2.18 *IATA*—International Air Transport Association

3.2.19 *IC*—Incident Commander

3.2.20 *ICS*—Incident Command System

3.2.21 *IEC*—International Electrotechnical Commission

3.2.22 *ISEA*—International Safety Equipment Association

3.2.23 *ISO*—International Organization for Standardization

3.2.24 *LRN*—Laboratory Response Network

3.2.25 *MACS*—Multiagency Coordination System

3.2.26 *NFPA*—National Fire Protection Association

3.2.27 *NIMS*—National Incident Management System

3.2.28 *NIOSH*—National Institute for Occupational Safety and Health

3.2.29 *NRC*—Nuclear Regulatory Commission

3.2.30 *OSHA*—Occupational Safety and Health Administration

3.2.31 *PBS*—Phosphate Buffered Saline

3.2.32 *PPE*—Personal Protective Equipment

3.2.33 *SZ*—Support Zone

3.2.34 *US&R*—Urban Search and Rescue

3.2.35 *WMD*—Weapons of Mass Destruction

4. Significance and Use

4.1 These practices should be used only to collect visible samples that are suspected biological agents and toxins and have been field screened as defined by the FBI-DHS-HHS/CDC Coordinated Document for explosive hazard, radiological hazard, and other acute chemical hazards.

4.2 These practices provide standardized methods for collecting, packaging, and transporting suspicious visible powder samples that are suspected biological agents and toxins. Collection of a bulk powder material from a nonporous surface using a sterile swab and laminated card as the collection devices to move the material into a container will depend on several factors, including (but not limited to): *(1)* amount of visible powder present; *(2)* sample composition; *(3)* choice of collection device; *(4)* size and shape of the collection container; *(5)* ability of the powder to become aerosolized; *(6)* texture and porosity of the surface; *(7)* humidity; *(8)* air movement; and *(9)* electrostatic properties of powders and collection tools/containers.

4.3 Similarly, these practices standardize methods for sampling suspicious visible powders for on-site analysis, although wipe and swab sampling is often employed in the field for subsequent LRN reference laboratory analysis. The ability to collect suitable samples from nonporous surfaces using a sterile moistened swab will depend on the following factors: *(1)* swabbing procedure; *(2)* swab material; *(3)* sample composition; and *(4)* texture of the surface.

4.4 These practices standardize suspicious powder collection and packaging procedures and swab sampling procedures in order to reduce exposure risk, to reduce variability associated with sample handling and sample analysis, and to increase reliability of sampling visible powder samples from nonporous surfaces.

4.5 The bulk sample collection practice and the swab sampling practice are recommended for collecting amassed or dispersed powder samples from all nonporous surfaces on which the suspicious powder sample is clearly visible.

4.6 These practices are not recommended for samples on porous materials such as upholstery, carpeting, air filters, or ceiling tiles.

4.7 These practices are recommended for collecting visible powders where the bulk of the powder sample is amassed or dispersed over a limited area (optimally, area should be less than 20 by 20 cm (approximately 8 by 8 in.) or 400 cm^2 (approximately 64 $in.^2$).

4.8 These practices are to be performed by personnel who are adequately trained to work with hazardous materials in the hot zone (see NFPA 472, or OSHA - 29 CFR 1910.120). Personnel performing collection or screening under these practices shall be adequately trained in the use of sampling equipment, materials, and procedures. This includes personnel performing the prior initial chemical and radiological screening. Personnel should use the appropriate level of personal protective equipment (PPE) to mitigate hazards during collection and screening. Personnel performing collection or screening under these practices shall be aware of evidence preservation and sampling procedures (NFPA 472 section 6.5).

4.9 These standard practices should be used in accordance with Guide E2770 for best practices for planning, training and evaluation of competency.

SAMPLE COLLECTION METHOD A—BULK SAMPLE COLLECTION FOR LABORATORY ANALYSIS

5. Scope of Method A

5.1 This sample collection method applies to the bulk collection and packaging of suspicious visible powders that are suspected biological agents or toxins, or both, from solid nonporous surfaces.

5.2 These practices are performed in coordination with the FBI and receiving LRN reference laboratory after a risk assessment including a hazard assessment and threat credibility evaluation is conducted and the sample is deemed potentially to be a credible threat as recommended and clarified in Guide E2770.

5.3 This sample collection method applies to suspicious visible powders that are amassed or dispersed in a limited area where the bulk of the powder sample is in an area that is less than 20 by 20 cm (approximately 8 by 8 in.) or 400 cm^2 (approximately 64 $in.^2$).

5.4 These practices should be used only to collect samples that are suspected biological agent and toxin hazards that have been field screened as defined by the FBI-DHS-HHS/CDC Coordinated Document for explosive, radiological, and chemical hazards.

6. Summary of Sample Collection Method A

6.1 A visible powder sample that is a suspected biological agent or toxin, or both, and its source should be field screened for non-biological hazards as defined in the FBI-DHS-HHS/CDC Coordinated Document and according to the reference

guidance including appropriate NFPA documents. Non-biological hazards include explosive, radiological, and chemical hazards. The visible powder sample, amassed or dispersed in a limited area, is collected from a nonporous surface using a swab and a laminated card to move the sample into a sterile dry collection container that is held close to the surface.

6.2 The method provides guidance on performing these procedures in a manner that will minimize sample loss and aerosolization of the powder. The bulk powder sample and swab are sent to an LRN reference laboratory for confirmatory analysis.

7. Sampling and Packaging Equipment and Supplies

7.1 *Personal Protective Equipment*—Level A, B, or C personal protective equipment ensembles as indicated (see Section 2 for additional guidance, including OSHA - 29 CFR 1910.120 Appendix B and NFPA 1994).

7.2 Clean drop cloth to create an isolated work area.

7.3 *Sample Transport Container*—Bucket or large heavy duty plastic bag used to transport the samples and materials from the hot zone through to the decontamination line.

7.4 *Non-powdered Nitrile or Vinyl Examination Gloves.*

7.5 *Two Sterile Polypropylene Wide-mouth Screw-capped Sample Collection Containers (Sample Containers)*—Containers must possess a leak-resistant seal; Diameter of container mouth must be large enough to accomodate the 4 by 6.5 cm plastic cards (Section 7.10); each pre-labeled as "POWDER SAMPLE" with unique sample identifier numbers.

7.6 *Bleach Solution*—Fresh pH-adjusted bleach solution (household bleach diluted 1:9; pH-adjusted to 6.8-8.0) to be prepared outside of the hot zone just prior to use by (step 1) mixing one part household bleach (5.25 to 6.0 % sodium hypochlorite) with 5 parts water (volume/volume); (step 2) adding 1 part white vinegar; (step 3) adding 3 parts of additional water.[12]

7.7 *Labeling or Marking Tape.*

7.8 *Solvent-resistant Indelible Marker.*

7.9 *Sterile Culture Swabs,* (rayon, macrofoam, or polyester) individually packaged and sterile, self-contained in sealed plastic tubes, with absorbent material wrapped around one end of a plastic stick, unopened.

7.10 *Two at least 4 by 6.5 cm (approximately 1.5 by 2.5 in.) Sterile Plastic Laminated Cards.*

7.11 *Six 1-gal Sealable Plastic Bag(s)*—Both bags with sliding lock and Trademark Whirl-Pak[13] bags are not recommended for this purpose since they may not seal completely; the use of colored or opaque bags is discouraged because it makes viewing of the sample more difficult once transported back to the laboratory in the CDC Laboratory Response Network.

7.12 *Bucket,* or other container marked "BIOHAZARD WASTE".

7.13 *1-gal Sealable Plastic Bag(s),* pre-labeled as "BIOHAZARD WASTE".

7.14 *Field Screening Results Form*—See example in Appendix X1.

7.15 *Sample Collection Sheet*—See example in Appendix X1. Note that a single sample collection sheet can be used for all items collected at a single location including primary source, swab, and powder sample(s).

7.16 *Chain-of-Custody Form*—See example in Appendix X1.

7.17 *Two Plastic Transparent Document Pouches,* with adhesive on back.

7.18 *Durable Hard-sided Outer Container,* with lid or screw cap, for sample transport (such as metal can with lid, or plastic container with lid).

7.19 Attach documentation described in the Sample Collection and Submission section of Guide E2770. Documentation should include the contact information for local, state, and federal law enforcement, local health officials and local/national testing facilities.

8. Procedure

8.1 Practices employed herein preserve the integrity of the material in the event that it becomes evidence. Efforts should be made to minimize or reduce air currents that may re-aerosolize or disturb suspected biological agents, including ceasing the operation of heating, ventilation, and air conditioning (HVAC) and exhaust fans and other forms of air movement. The location of the work area should be considered prior to entry into the hot zone. When selecting work location, teams should consider proximity to sample location to reduce possible contamination of the work area, while also minimizing the distance necessary to move materials during sample collection. Additionally these actions should be supported by a written incident action plan and site health and safety plan developed under the direction of the Incident Commander. A coordinated risk assessment including a hazard and threat credibility evaluation should be performed prior to determination of a sampling mission. These practices assume that a sampling site/hot zone has already been defined. These practices are to be performed by personnel who are adequately trained to work with hazardous materials in the hot zone (NFPA 472, and 29 CFR, Part 1910.120).

8.2 Prior to allowing people to enter the hot zone for sampling purposes, proper site safety practices should be implemented, including establishing decontamination areas, and assuring that the appropriate PPE is selected based on a risk-assessment.

8.3 Prior to performing these practices, review the sampling plan and sampling procedures and assemble all necessary equipment before entering the hot zone. At minimum, a two-person team is required to perform these sampling procedures in the hot zone.

[12] Additional information about decontamination with pH-adjusted bleach solution is available at nepis.epa.gov.

[13] Whirl-Pak is a trademark of Nasco International, Inc.

8.4 Prior to entering the hot zone for sampling purposes, label the two unopened, sterile plastic sample collection containers. The sample collection containers are the primary containment for bulk dry powder sample and are labeled as "POWDER SAMPLE" with unique sample identifier numbers on each container. Properly label five 1-gal self-sealing bags in the following manner: *(1)* and *(2)* containment tubes for two dry swabs each labeled as "DRY SWAB" and with unique sample identification numbers; *(3)* if applicable, the primary containment for source of powder (letter or small package) labeled as "PRIMARY SOURCE" and with a unique sample identifier number; and *(4)* and *(5)* containment for two sample collection containers labeled as "POWDER SAMPLE" and with unique sample identifiers.

8.5 After entering the hot zone, ensure that the sample material is protected from wind currents and moisture until all sampling is completed.

8.6 Perform basic field screening on material prior to sample collection to assess explosive hazards, radiological hazards, and acute chemical hazards (NFPA 472). Use screening methods to minimize sample consumption, thereby conserving as much of the sample as possible. Ensure that the results of field screening are documented indicating which tests have been performed and their outcome (see example of field screening results form in Appendix X1). In situations where biological agents or toxins, or both, are suspected, the item(s) should be field screened for explosive devices and substances, radiological materials, corrosive materials and volatile organic compounds as defined in the joint FBI-DHS-HHS/CDC Coordinated Document guidelines for responders to suspicious letters and packages.

8.7 This instruction is provided for a two-person sampling team to conduct the sampling procedure in the hot zone; the procedure should not be performed by a single individual. The first team member (assistant sampler, also known as the facilitator) is responsible for communication, photography (FBI Laboratory Publication, Handbook of Forensic Services 2003), ensuring that the sample collection sheet is filled out, and facilitating collection (for example, opening and handing materials to the sampler as required, including sample collection containers, gloves, swab, laminated card, other sampling materials, and packaging materials). The second team member (sampler) is the person collecting the sample and should be the only person to come in contact with the suspicious material. The sampler is also responsible for signing the final chain-of-custody form outside of the hot zone.

8.8 Some jurisdictions may have standard operating procedures requiring the collection of negative controls. Sampling teams should refer to standard operating procedures regarding the collection of any negative controls (also referred to as field and media blanks). Negative controls include unopened sampling tools and any wetting solutions. Blanks must be submitted for each lot number used.

8.9 All team members must put on a new pair of non-powdered nitrile or vinyl examination gloves over the gloves that are part of standard PPE ensemble (that is, team members will have multiple layers of gloves on) for sample collection.

NOTE 1—The use of appropriate aseptic techniques including doffing of gloves between sample collections is done to minimize cross contamination.

8.10 *Assistant Sampler*—Lay down the clean drop cloth to create an isolated work area, and place materials on drop cloth.

8.11 *Assistant Sampler*—Ensure the following items are documented through radio communication between hot and cold zone personnel and finalization of paperwork in the cold zone prior to sending the sample to the laboratory (see example in Appendix X1).

8.11.1 Unique sample number or identifier,
8.11.2 Sample location and address,
8.11.3 Type of sample,
8.11.4 Type and texture of surface sampled,
8.11.5 Time and date of sample,
8.11.6 Names and signatures of persons collecting sample,
8.11.7 Measured size of the area sampled, and
8.11.8 Map of sample area and photograph is possible. Additional efforts to photograph the materials associated with the primary source (for example, envelope, package outer wrap, or letter, or combinations thereof) to discern writings and markings prior to collection are useful to support the law enforcement mission.

8.12 *Assistant Sampler*—If the source of the powder is present on the surface, and the source is a letter or other small package that can fit easily into a 1-gal sealable plastic bag, position the pre-labeled bag labeled as "PRIMARY SOURCE" above the surface next to the source, and hold the bag open.

8.13 *Sampler*—Place the source gently into the plastic bag taking care to make sure all writing and markings are visible through the bag. If there are both documents and packaging, collect them in separate bags to ensure visibility if needed. Limited handling of the primary source is recommended to preserve forensic attributes for additional testing and to minimize resuspension potential.

NOTE 2—Written and other markings on documents associated with the threat are valuable to law enforcement early in the investigation. Special care should be taken to ensure law enforcement entities at the end of the decontamination line can clearly see anything written or labels, or both, on packing associated with the primary source.

8.14 *Assistant Sampler*—Carefully dispel excess air, and seal the bag. Place the sealed bag containing the source into another transparent sealable bag.

8.15 *Assistant Sampler*—Carefully dispel excess air; seal the bag, and place into sample transport container for decontamination.

8.16 *Assistant Sampler*—Give laminated card to sampler. Loosen the cap of the tube containing the swab.

8.17 *Sampler*—Remove the swab from the tube while the assistant sampler holds the tube. Hold the laminated card at an angle on the surface next to the powder. If the surface is smooth, use the card to push the powder into a pile on the surface. Use the sterile swab to gently push the dry powder onto the laminated card. Be sure to use slow, deliberate motions while moving the powder.

8.18 *Assistant Sampler*—Firmly hold the tube for the sampler.

8.19 *Sampler*—Place swab firmly into tube, taking care not to touch the outside of the tube with the swab.

8.20 *Assistant Sampler*—Close the tube. Place the tube containing the swab into a transparent sealable bag labeled as "DRY SWAB". Carefully dispel excess air; seal the bag, and place into sample transport container for decontamination.

8.21 *Assistant Sampler*—Open the sample container labeled as "POWDER SAMPLE" and hand the open container to the sampler.

8.22 *Sampler*—Hold the sample collection container on its side parallel to the surface and place the laminated card (with the powder on top) into the sample collection container. Do this slowly and gently so as to minimize aerosolization of the powder.

8.23 *Sampler*—Take the lid from the assistant sampler and place the lid on the sample collection container containing the laminated card and dry bulk powder.

8.24 *Sampler*—Place the closed, pre-labeled container containing the dry bulk powder into a transparent sealable bag held open by the assistant sampler. Do not touch the outside of the bag.

8.25 *Assistant Sampler*—Carefully dispel excess air; seal the bag, and place into sample transport container for decontamination.

8.26 If the amount of powder is too large to collect it all using a single laminated card and swab, repeat steps described in 8.16 – 8.25 with a new card, swab, and pre-labeled sample collection container. In situations where significant material is present after the initial sample is collected for the laboratory, the sampling team should coordinate and discuss with the receiving laboratory and FBI the disposition of the residual material.

8.27 If there is residual powder and conditions allow for the team to remain in the hot zone, the team may perform Sample Collection Method B now prior to leaving the hot zone.

8.28 Sampling team transports all samples out of the hot zone and into the decontamination line in the warm zone.

8.29 *Assistant Sampler*—Rinse or wipe the outside of the sealed plastic bags containing the primary source, powder sample(s), and swab(s) with decontamination solution—do not dry the outside of the bags afterwards as this will allow appropriate decontamination solution contact time. The outer surface of the sealed plastic bag should be decontaminated using a 10 % bleach solution adjusted to a pH of 7. A contact time of 10 min is needed for treating the outer surface of bagged items. Treated outer surfaces of bagged items should remain wet when placed in additional containment bags to insure adequate contact times are achieved. Collect any bleach runoff in a container marked "BIOHAZARD WASTE".

8.30 *Assistant Sampler*—Place the rinsed, sealed bags containing the primary source, powder sample(s), and swab(s) into separate sealable plastic bags and seal.

8.31 Any unused supplies that were carried into the hot zone (plastic bags, sample containers) should also be placed into "BIOHAZARD WASTE" bag(s).

8.32 *Assistant Sampler and Sampler*—After following all proper personnel decontamination procedures (NFPA 472), move the samples to the cold zone. Place the rinsed, sealed bags into a durable hard-sided outer container. Responding personnel should contact the receiving LRN reference laboratory to ensure that the dimensions of the outer container will fit in the laboratory biosafety cabinet. Place the lid on the container.

8.33 *Assistant Sampler and Sampler*—Initial and date the sample package with the indelible marker.

8.34 *Assistant Sampler and Sampler*—Confirm radio communicated information is consistent or if only a 2 person team responded, transfer all sample and field screening information to a clean sample collection sheet and field screening results form in the cold zone.

8.35 *Assistant Sampler and Sampler*—Transfer or verify, or both, all unique sample numbers or identifiers and other pertinent information from the sample collection sheet onto a chain-of-custody form (see example in Appendix X1).[14]

8.36 *Assistant Sampler and Sampler*—Attach a self-sticking document pouch to the durable outer container. Place the clean sample collection sheet and field screening results form and any additional paperwork or documentation inside the document pouch. Place labeling tape over the opening of the document pouch and initial and date the tape with the indelible marker.

8.37 *Sampler*—Sign a chain-of-custody form at the receiving area. Hand the package to the person who is responsible for transporting the package (transporter).

8.38 *Transporter*—Examine the outer package to determine that it is properly packaged and sealed, and that the chain-of-custody form is completed. Sign the original chain-of-custody form. Give one copy of the form to the submitter (sampler) and retain the original form. Place the original signed chain-of-custody form into a second self-sticking document pouch and adhere to the outside of the package. Notify receiving LRN reference laboratory of package transportation and estimate time of delivery. Transport all samples under secure conditions to the predesignated, approved LRN reference laboratory. Any transfers of the materials from one person to another should be documented on the chain-of-custody form with signatures.

8.39 The primary source, powder sample(s), and swab(s) are transported in a manner that complies with all state and federal regulations (IATA PI 602, IATA PI 650, IATA DGR 46th Edition, IATA DGR Addendum I, IATA DGR Addendum II, IATA DGR Addendum III, and DOT 49 CFR, Parts 171-180). All materials are maintained under chain of custody in a secure and biosafe area at the LRN reference laboratory until testing is completed.

[14] Prior to shipping any specimen suspected to contain a biological agent, contact your state public health laboratory or nearest laboratory in the national response network (currently the CDC Laboratory Response Network) for specific guidance. Any materials that might be used as evidence in any investigation must be controlled by a chain of custody at all times.

SAMPLE COLLECTION METHOD B—SWAB SAMPLE COLLECTION FOR ON-SITE ANALYSIS

9. Scope of Method B

9.1 This sample collection method applies to swab sampling of suspected biological agents and toxins from nonporous surfaces to collect residual powder after Method A has been applied. The swab sampling procedure produces a sample that may be used for optional, on-site biological assessments. While the sample collected with Method B may be used for on-site biological assessments, it is recommended that assessment methods have been validated by nationally recognized consensus standards (for example, AOAC International, Stakeholders' Panel on Agent Detecting Assays (SPADA) performance specifications[15]) and supported as defined in Guide E2770.

9.2 These practices are performed only after collecting the bulk sample in the hot zone as described in Sample Collection Method A-Bulk Sample Collection.

9.3 These practices should be used only to collect samples that are suspected biological hazards and that have been field-screened for explosive hazards, radiological hazards, and acute chemical hazards.

10. Summary of Sample Collection Method B

10.1 A nonporous surface from which a suspicious visible bulk powder, a suspected biological agent, has previously been collected is swabbed using a moistened swab to collect any residual powder. The sample may be utilized in on-site biological assessment to support public safety operations.

11. Sampling Equipment and Supplies for Sample Collection Method B

11.1 *Personal Protective Equipment*—Level A, B, or C as necessary (OSHA - 29 CFR 1910.120 Appendix B and NFPA 1994).

11.2 Sample collection tools are the following: *(1)* sterile culture swab (rayon, macrofoam, or polyester), individually packaged and self-contained in sealed plastic tube, with absorbent material wrapped around one end of a plastic stick, and, if available, *(2)* sample collection device provided by manufacturer of the on-site biological assessment kit.

11.3 Sterile solutions for moistening the swab are as follows: *(1)* sterile vial with lid, pre-labeled as "BUFFER," containing minimally 0.5 mL of Phosphate Buffered Saline (PBS) solution with 0.1 % Tween-20,[16] or if available, *(2)* buffer solution from manufacturer of on-site biological assessment kit if required by manufacturer. Background control samples of collection materials including buffer solutions utilized in sampling of residual powder should be submitted to the receiving LRN reference laboratory. Any control buffers or materials must be clearly labeled and indicated in submission documentation.

11.4 *1-gal Sealable Bag*—Pre-labeled as "WET SWAB" with unique sample identifier number.

11.5 *Sample Collection Sheet.*

12. Procedure

12.1 *Sampler*—The residual powder that remains on the surface following bulk powder sample collection may be used for on-site biological assessments. Follow instructions provided by the manufacturer regarding sample collection for on-site biological assessments using a commercially available kit. Personnel performing on-site biological assessments should be adequately trained in the use of biological assessment technologies (NFPA 472).

12.2 *Assistant Sampler*—Record the methods and results of on-site biological assessments. If the residual powder sample is consumed for on-site analysis, a chain-of-custody form is not needed.

12.3 If there is significant amount of residual powder on the surface and no on-site biological assessment is performed, perform the steps described in 12.4 – 12.11 to collect residual powder for packaging and transport to the receiving LRN reference laboratory.

12.4 *Assistant Sampler*—Remove the lid from the vial labeled as "BUFFER" and hand the vial to the sampler. Loosen swab from tube (but do not remove swab) and hand tube with swab to sampler. Sampler holds the tube and "BUFFER" vial in the same hand.

12.5 *Sampler*—Remove swab from tube and place into "BUFFER" vial to moisten, using aseptic technique to prevent cross contamination. Press the swab against the side of the vial to remove excess liquid. Remove swab from "BUFFER" vial and drop "BUFFER" vial into biohazardous waste container.

12.6 *Sampler*—Wipe the swab over the surface where the powder was originally found, using closely spaced vertical S-strokes or Z-strokes over the entire sampling area.

12.7 *Sampler*—Roll the swab handle (end of the plastic stick furthest from absorbent material) between fingers to rotate the swab, thereby exposing a fresh surface. Wipe the swab over the entire area again, this time using horizontal S-strokes or Z-strokes over the surface. The swab area should preferably not exceed the maximum recommended area of 20 by 20 cm (8 by 8 in.) or 400 cm^2 (approximately 64 $in.^2$).

12.8 *Sampler*—Place swab into tube, and seal tube by pressing firmly.

12.9 *Sampler*—Place the closed tube containing the swab into a transparent sealable bag labeled as "WET SWAB" held open by the assistant sampler. Do not touch the outside of the bag.

12.10 *Assistant Sampler*—Carefully dispel excess air; seal the bag, and place into sample transport container for decontamination.

12.11 Continue with procedure in 8.28 through to the end of Method A as determined appropriate through correspondence with the receiving LRN reference laboratory.

13. Keywords

13.1 packaging; sample collection; suspicious powders; swab

[15] SPADA specifications are available at www.aoac.org.
[16] Tween-20 is a trademark of ICI Americas, Inc.

APPENDIX

(Nonmandatory Information)

These appendices provide example forms for the user. Use of these specific forms is not mandatory.

X1. EXAMPLE OF BEST PRACTICES FORMS

X1.1 See Table X1.1, Fig. X1.1, Table X1.2, Fig. X1.2, Fig. X1.3, and Fig. X1.4.

TABLE X1.1 Field Screening Results Form

Location:

Date:

Screening Team:

Time:

Sample #	Color	Rad (uR/h)	Flammable (% LEL)	PID/VOC (ppm)	O_2 (%)	Oxidizer or energetic material	Observations/Comments/Other Screening Tools Used

Monitoring Instruments Used:

MDPH William A. Hinton State Laboratory Institute
Biological/Chemical Specimen Submission Form / Environmental Threat

Do not write in this box; SLI use only
SLI TRACKING NUMBER (One SLI Tracking # Per Package)

SPECIMEN SCREENING INFORMATION

SPECIMEN WAS SCREENED FOR: (check any applicable boxes and write additional information if the box is checked)

☐ **RADIATION**
Screening Method(s): _____
Background Reading: _____
Sample Reading (units): _____

☐ **EXPLOSIVES**
Screening Method(s): _____
Results: _____

☐ **CHEMICALS**
☐ **WMDs** Screening Method(s): _____
Results: _____
☐ **pH** Results: _____
☐ **OTHER** Screening Method(s): _____
Results: _____

☐ **OTHER**
Screening Method(s): _____
Results: _____

SAMPLE SCREENED BY:
(Fill out this section completely)
Technician Name(s): _____ Organization(s): _____
Address: _____ Telephone(s): _____
COLLECTOR/SUBMITTER INCIDENT IDENTIFIER #: _____

The Hinton State Laboratory Institute does not accept explosive or incendiary material or chemical WMD.
Call the 24/7 Laboratory Emergency Cell phone at 617-590-6390 for guidance.

Questions regarding the submission and analysis of explosive or incendiary material should be referred to the Arson & Explosives Unit at the State Crime Lab
8 am – 5 pm Telephone ~ 508-358-3220 / After Hours Pager ~ 508-899-3770 / After Hours Cell ~ 508-241-2052

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FIG. X1.1 Example Specimen Screening Form

TABLE X1.2 Example of Sample Collection Sheet

Sample Number or Sample Identifier: _____

Date/Time of Sample: _____

Type of Sample: _____

Type/texture of surface sampled: _____

Description of Material Sampled (e.g., color, texture, homogeneity etc.):

Name of Persons Collecting Sample:

Sampler

Printed Name: _____

Signature: _____

Phone Number: _____

Facilitator

Printed Name: _____

Signature: _____

Phone Number: _____

Measured Size of Area Sampled: _____

Sample Location (include agency, address, room number, description of sample location):

_____

_____

_____

_____

Map of Sample Area:

Other Comments:

1. NAME OF SAMPLE COLLECTOR		2. LOCATION OF SAMPLE COLLECTION ADDRESS (CITY, ST, ZIP)			
3. REASON OBTAINED		4. TIME/DATE OBTAINED			
5. ITEM #	6. QUANTITY	7. DESCRIPTION OF SAMPLE (Liquid, Solid, Color, etc)			
8. ITEM #	9. QUANTITY	10. DESCRIPTION OF SAMPLE PACKAGING			
12. ITEM #	13. DATE	14. RELEASED BY	15. RECEIVED BY	16. PURPOSE OF CHANGE OF CUSTODY	17. SHIPMENT DESCRIPTION
		Signature	Signature		
		Print: Name, Grade, Title	Print: Name, Grade, Title		
		Signature	Signature		
		Print: Name, Grade, Title	Print: Name, Grade, Title		
		Signature	Signature		
		Print: Name, Grade, Title	Print: Name, Grade, Title		

FIG. X1.2 Example of Chain of Custody Form

14. ITEM #	15. DATE	16. RELEASED BY	17. RECEIVED BY	18. PURPOSE OF CHANGE OF CUSTODY	19. SHIPMENT DESCRIPTION
		Signature	Signature		
		Print: Name, Grade, Title	Print: Name, Grade, Title		
		Signature	Signature		
		Print: Name, Grade, Title	Print: Name, Grade, Title		
		Signature	Signature		
		Print: Name, Grade, Title	Print: Name, Grade, Title		
		Signature	Signature		
		Print: Name, Grade, Title	Print: Name, Grade, Title		
		Signature	Signature		
		Print: Name, Grade, Title	Print: Name, Grade, Title		

20. BACKGROUND INFORMATION

a. Wind Speed	b. Wind Direction (from)	c. Temperature	d. Humidity	e. Visibility	f. Terrain	g. Other Remarks

21. FINAL DISPOSAL ACTION

RELEASE TO OWNER OR OTHER (Name/Unit) _____

DESTROY _____

OTHER (Specify) _____

22. FINAL DISPOSAL AUTHORITY

ITEM(S) _____ ON THIS DOCUMENT, PERTAINING TO THE INVESTIGATION INVOLVING

(Grade)

_____ (IS) (ARE) NO LONGER

(Name) (Organization)

REQUIRED AS EVIDENCE AND MAY BE DISPOSED OF AS INDICATED ABOVE. (If article(s) must be retained, do not sign, but explain in separate correspondence.)

(Typed/Printed Name, Grade, Title) (Signature) (Date)

23. WITNESS TO DESTRUCTION OF EVIDENCE

THE ARTICLE(S) LISTED AT ITEM NUMBER(S) _____ (WAS) (WERE) DESTROYED BY THE EVIDENCE CUSTODIAN, IN MY PRESENCE, ON THE DATE INDICATED ABOVE.

(Typed/Printed Name, Organization) (Signature)

FIG. X1.2 Example of Chain of Custody Form *(continued)*

New York State Department of Health
Wadsworth Center
Biodefense Laboratory
120 New Scotland Avenue
Albany, NY 12208 Phone (518) 474-4177

Laboratory Response Network
Biothreat Tracking Form

Specimen Information
Incident ID#_____ **Collection Date/Time**_____
Investigating agency and contact information_____
Collection County_____
Collection Site (address)_____
Targeted Individual's name (if any)_____
Specimen Description_____
_____

Collection Site Information

Building evacuated	no ☐	yes ☐	details _____
Ventilation system shut down	no ☐	yes ☐	details _____
Site/building locked-down	no ☐	yes ☐	details _____
Media on-site	no ☐	yes ☐	details _____
Medical response initiated	no ☐	yes ☐	details _____

Credible Biohazard Assessment Criteria

Stated or implied Threat	no ☐	yes ☐	describe _____
Visible, testable Material	no ☐	yes ☐	describe _____
Uncertain or suspicious Origin	no ☐	yes ☐	describe _____
Exposure or illness			
Targeted individual	no ☐	yes ☐	illness ☐ _____
First responders	no ☐	yes ☐	illness ☐ _____
Sample collectors	no ☐	yes ☐	illness ☐ _____

Credible Biohazard Assessment performed by _____
Field Hazard Screens performed by_____

Explosive Device	negative ☐	Instrument used_____
Chemical Hazard	negative ☐	Instrument used_____
Rad/Nuc Hazard	negative ☐	Instrument used_____

ALL samples must be NEGATIVE by ALL Screens to be accepted at testing laboratory*

Sample Collected by_____
Sample Container Decontaminated by_____
UNYRIC notified (by whom)_____ **date/time**_____
NYSPIN BIO1 submitted (by whom)_____ **date/time**_____

Submitter Information
Name_____
Phone( ) - _____ After hours ( ) - _____ Message OK? yes ☐ no ☐

Report Results To (if different than Submitter)
Name_____
Phone( ) - _____ After hours ( ) - _____ Message OK? yes ☐ no ☐

Incomplete information reporting on this tracking form or a failure to conduct hazard screening procedures as outlined will result in this sample being refused at the laboratory and/or returned to the submitting agency.

White and Yellow copies to lab *Pink copy to law enforcement* *Blue copy to local health department*

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FIG. X1.3 Example of Biological Agent Tracking Form

William A. Hinton State Laboratory Institute
Massachusetts Department of Public Health
305 South Street, Jamaica Plain, MA 02130
(617) 590-6390

Biological/Chemical Specimen Submission Form

Environmental Threat

Do not write in this box; SLI use only	
SLI TRACKING NUMBER (ONE SLI Tracking # Per Package)	BT LAB NUMBER(S):

Received By Print Name: _____
Signature: _____
Date Received: ____/____/____/____/ Time Received: _____ am pm
Priority Sample ☐ Yes ☐ No

1 COLLECTOR/SUBMITTER INCIDENT IDENTIFIER#: _____ **INCIDENT REPORT ATTACHED?** Yes No **EVIDENCE?** Yes No **SPECIMEN SCREENED?** Yes No (if yes, fill out back of form)

SAMPLE DESCRIPTION: _____

DATE COLLECTED: ____/____/____ **TIME COLLECTED:** _____ am pm **COLLECTED BY:** _____ (print name)

2 LOCATION WHERE SAMPLE WAS COLLECTED:

Location Name: _____ Telephone: _____
Address: _____ Fax: _____
_____ Contact Name: _____

3 COLLECTOR INFORMATION:

Contact Name (Lab Report To): _____
Organization: _____
Address: _____
_____
Telephone: _____

4 SUBMITTER INFORMATION: ☐ SAME AS COLLECTOR

Contact Name (Lab Report To): _____
Organization: _____
Address: _____
_____
Telephone: _____

5 DELIVERY TO STATE LABORATORY INFORMATION:

Delivered By (Name): _____ Organization: _____
Delivered By (Title): _____ Badge Number: _____

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FIG. X1.4 Example of Specimen Submission Form