NRT Quick Reference Guide: *Bacillus anthracis* (Anthrax)

Report any release of WMD to the National Response Center 1-800-424-8802
For References, Please See: Key References Cited/Used in National Response Team (NRT) Quick Reference Guides (QRGs) for Biological Warfare Agents.

Agent Characteristics

Agent Classification: Biological **Type:** Bacteria *(Bacillus anthracis)*, many strains

Description: B. anthracis is a naturally-occurring, rod-shaped Gram-positive, sporulating bacterium. Anthrax disease is endemic in animal populations in the United States and sporadically occurs in wild and domestic animals (e.g., cattle and antelope). Anthrax disease can also occur in humans when exposed to infected animals or infected animal tissue (cutaneous or gastrointestinal anthrax) or when exposed to aerosolized anthrax spores (inhalation anthrax).

Incubation Period: 1-7 days, up to 60+ days
Duration of Illness: 3-5 Days
Person-to-Person Transmission: No

Infectivity/Lethality: Moderate/High
Infective Dose: LD50: 8000-50,000 spores (estimated)
Persistence/Stability: Spores Highly Persistent/Stable; >40 years in soil

Release Scenarios

Air/Aerosolization: In a bioterrorism event, *B. anthracis* will most likely be aerosolized in the form of a white powder. Powders of *B. anthracis* with characteristics such as high spore concentration, uniform particle size, low electrostatic charge, etc. are considered "weapons-grade." In an aerosol release of *B. anthracis*, re-aerosolization is a consideration depending upon the size, purity, and chemical and physical properties of the manufactured spores. An aerosol release of *B. anthracis* will most likely occur indoors, though an outdoor release is possible. An aerosol release of *B. anthracis* would have the potential to travel many km before dissipating.
Soil/Surfaces: Spores are resistant to adverse environmental conditions and may remain viable for years in soil or in dried or processed hides of animals. Spores of *B. anthracis* may remain viable in soil for 40+ years. For this reason, *B. anthracis* is a threat to the animal population. The spore viability of *B. anthracis* on certain surfaces is not well-known, but it does grow readily on laboratory media.
Water: *B. anthracis* is a probable water threat.
Other: *B. anthracis* is naturally occurring and endemic to the United States, and can cause anthrax disease in humans through contact with infected animals or contaminated animal products; this includes eating contaminated meat products or carcasses.

Health Effects

ONSET	Symptoms may occur within 1-7 days and up to 60 days after an inhalation exposure.
SIGNS/ SYMPTOMS	**Inhalation anthrax:** Fever, malaise, fatigue, cough, chest discomfort, stridor (noisy breathing), respiratory distress, dyspnea (shortness of breath), and cyanosis (bluish discoloration of the skin). **Cutaneous anthrax:** Raised itchy bump to vesicle which progresses to painless ulcer (1-3cm) with black area in the center. Swollen lymph nodes and flu-like symptoms. **Ingested anthrax:** Flu-like symptoms, nausea, loss of appetite, vomiting, fever, abdominal pain, and severe diarrhea.
EXPOSURE ROUTES	**Inhalation anthrax:** Inhalation anthrax occurs when bacterial spores are inhaled, and may be fatal without treatment. **Cutaneous anthrax:** Most common manifestation of naturally occurring anthrax disease; Approximately 95% of skin infections occur when bacteria enters via cut on the skin upon handling contaminated objects; 20% of untreated cases are fatal. **Ingestion:** Caused by consumption of poorly cooked contaminated meat. Intestinal anthrax has two forms: upper & lower GI tract infections, and has a high rate of mortality.

Effect Levels

Lethality reflects the relative ease with which an agent causes death in a susceptible population and can be represented quantitatively by the exposed population mortality rates. Inhalation anthrax is highly lethal.
Infectivity refers to how easily an agent can cause disease in a host. An agent is highly infective when few organisms can cause disease. An infective dose is the number of organisms required to cause disease in an exposed person. Given the uncertainties regarding published infective doses for bioagents, it is important to examine what the infectivity numbers represent, including the routes of exposure and the animal species used for the lab studies. **Responders should not assume that an infective dose estimate represents a safe level.** In the absence of data on infectious dose, the LD50 (the dose of spores in animal experiments which results in the death of 50% of the animals) can be used in its place. Clearly, a dose that could potentially kill 50% of the people exposed is not to be considered safe.

Health and Safety

CONCERNS	Under ICS, the appointed Health and Safety Officer will determine PPE, Medical Surveillance, and Safety Plans. The PPE levels listed below are general suggestions only and are appropriate only for *B. anthracis*; they may not provide protection for other possible contaminants that may be present onsite. For more info on PPE and health and safety decision-making, please see *http://www.ert.org/products/Anthrax.pdf,* the OSHA/NIOSH interim CBRN guidance document, the EPA's Respiratory Protection Program Draft January 2005 or the EPA's Medical Surveillance Program Implementation Plan Draft January 2005. EPA documents are available at www.epaosc.org. Responders should be trained to Hazardous Waste and Emergency Operations (HAZWOPER) standards and attend an on-site training briefing. Responder training shall meet the requirements specified in 29 CFR 1910.120 (q) (6).
MEDICAL	**Pre-Exposure:** Annual exams to ensure proper respiratory function; ideally, responders should be fully vaccinated against anthrax prior to exposure. **During Exposure:** Wear PPE as designated by the Health and Safety plan. Treat any accidental exposures with the antibiotics Ciprofloxacin and/or Doxycycline. Set up a medical monitoring plan, document PPE levels that were used, exposure incidents, outcome, and if/what types antibiotics used, etc. **Post Exposure:** Monitor responders for signs/symptoms and treat accordingly.
FIRST AID/ DECON	Decon outer PPE with a 10% solution of household bleach (0.5% sodium hypochlorite). Decontaminate skin with warm soapy water (10% bleach solution may irritate skin) for 10-15 minutes. Antibiotics (Ciprofloxacin, Doxycycline) and vaccination can help prevent infection with exposure to spores. With respect to specifics (e.g., value of mechanical scrubbing, contact time of decon solution, and need to rinse completely) OSCs should check with the EPA National Decon Team Subject Matter Experts at 513-487-2420 (after hours call 24-hour pager at 1-800-329-1841).
PPE	**Response to an occurring and ongoing release of *B. anthracis:*** dermal – hooded, protective coverall for hazardous particulate (Tyvek® or equivalent), inner and outer disposable gloves, and boots; respiratory protection – NIOSH-approved, CBRN, positive-pressure self contained breathing apparatus (SCBA). **Response to a controlled incident when release has stopped:** dermal – same protection; respiratory protection – NIOSH has approved Powered Air-Purifying Respirators (PAPRs) and assigned them a protection factor of 50 (e.g., tight-fitting full-faced PAPR); OSHA recently proposed an assigned protection factor of 1,000 for tight-fitting full-faced and certain hood/helmet-style PAPRs that are CBRN-approved, when available, and fitted with P100 filters. **Medical Responders decontaminating incoming victims at the site or hospital:** Same as response to a controlled incident.

Field Detection

The BioWatch Program helps detect aerosol releases of bioagents. Certain buildings, such as postal facilities may have autonomous detection systems (ADS) that continually test for B. anthracis using air samples and PCR.

Immunoassay Tests (smart tickets): These assays are intended for rapid detection of *B. anthracis* and for screening environmental samples. Each ticket employs patented immunochemical tests for specific biological agents. They feature rapid identification, minimal operator training and sample preparation, response time is 5 -15 minutes. **Note:** Immunoassay tests should not be used alone, but should be confirmed with samples analyzed by culturing at LRN lab.

Bacillus anthracis (Anthrax) (side 2)

Sampling

Sampling Location Plans: If release was limited to a letter or container, start with an area thought to be free of contamination and work in concentric Circles either towards or away from the initial point of contamination. Be concerned about other contaminated areas due to foot traffic/ventilation systems (elevator buttons, mail, corners of hallways, baseboards, light switches, door knobs, etc). If point of release or aerosolization is unconfirmed, then use a statistically based sampling method. **Note:** These are general guidelines and do not replace need for a site-specific sampling plan that should be reviewed and approved by appropriate Subject Matter Experts and/or through ICS channels. More specific EPA/NRT sampling procedures/guidance for biowarfare agents can be found in EPA and TetraTech *"Biological Sampling Procedures Booklet for Regional Counterterrorism Response Plans"* TDD: S05-0302-004

Concerns: Different detection/analytical equipment and sampling techniques will be highly site-specific and depend on: **1)** the characteristics of the terrorist agents; **2)** the type of contaminated surfaces (e.g., porous v. nonporous); **3)** the phases/purposes of sampling (initial identification v. post-decon sampling); and **4)** the sampling procedures of the analytical laboratory. **Note:** Before obtaining samples, clearly identify and coordinate with laboratory to be used, as not all laboratories can handle all types of media. Basic Ordering Agreements (BOAs) for laboratory sampling analysis have been established with contract labs. Contact EPA ERT for details; coordinate with investigative units (EPA CID/FBI); ensure plan for appropriate chain-of-custody.

Samples that test for re-aerosolization: 1) Wipe sampling of the air duct system (filters, areas of particulate deposition) if exposure occurred indoors. **2)** Sheep blood agar plates determine the presence of bacterial growth. **3)** Andersen Air Sampler & Single Stage Impactors with settle plates capture airborne particulates on a series of agar plates based on their aerodynamic properties. **4)** Dry filter units (DFUs) are the most direct indicator of airborne *B. anthracis* spores. Check for their presence/install DFUs.

Samples that can test Decon efficacy: 1) Wipe Samples: Synthetic, non-cotton (Dacron/rayon) wipes pre-moistened with a nutrient solution, buffer solution, or sterile water. Good for small sample areas of nonporous surfaces. Coordinate methods and buffer solutions with designated Laboratory Response Network (LRN) laboratory. **2)** Swab Samples: Synthetic, moistened, cotton sterile or macrofoam swab moistened with buffer solution (PBST) or sterile water. Most useful for hard-to-reach, nonporous surfaces. CDC study shows that rayon & polyester swabs are not as efficient as cotton/macrofoam swabs in spore recovery. Do not use dry swabs. **3)** HEPA Vacuum Sampling: collect samples in a HEPA sock designed to fit into an inlet nozzle of a vacuum cleaner. Good for screening and determining the extent and location of contamination in large areas. Used on both porous and nonporous surfaces. For sampling method please see: *http://www.bt.cdc.gov/agent/anthrax/environmental-sampling-apr2002.asp*

Sample packaging and shipping: Packaging and transporting samples containing or possibly containing *B. anthracis* spores are subject to various regulations established by DOT, CDC, USPS, OSHA, and IATA. Consult and coordinate with the analytical laboratory receiving the samples to determine packaging or shipping requirements. Details can be found at *www.cdc.gov/od/ohs/biosfty/shipregs.htm or http://www.cdc.gov/od/sap/* OR *http://www.asm.org/ASM/files/LEFTMARGINHEADERLIST/downloadfilename/0000001202/ProtocolPackShip.pdf*

Laboratory Analysis: BSL-3

Laboratory Information: For Biological and Chemical Agent Analyses Contract Vehicles for EPA emergency lab support, contact Battelle Security 24-hour control center at: 614-424-5909. US EPA has IAGs with Aberdeen and Dugway for Analytical Lab Support During a WMD response; for access, contact the EPA ERT 24-hour number: 732-321-6660.
CDC Laboratory Response Network Labs: CDC Bioterrorism Preparedness and Response Program: 404-639-0385
http://www.bt.cdc.gov/agent/anthrax/lab-testing/approvedlrntests.asp

Polymerase Chain Reaction (PCR): Amplifies DNA sequences and compares them to standard gene sequences for *B. anthracis*. PCR is a screening method that should not be used alone. It is useful for analyzing initial samples at sites with suspected contamination. Positive results should be confirmed with bacterial culturing. Field PCR systems are very selective, but do not work well with heterogeneous environmental samples (e.g., dust, soil). PCR is also used as a final confirmation of positive samples taken from plated colonies.
Culturing: The sample is prepared for elution & plating, & inoculated onto sheep blood agar plates at LRN labs. The plates are incubated at 37°C for up to three days & examined for growth of *B. anthracis* colonies

Decontamination

Decon Planning: Site specific decon/cleanup plan should be developed and approved by all necessary organizations/SMEs via ICS channels. Responders should develop a plan that takes into account: **1)** The nature of contamination including purity, spore size, chemical/physical properties, how it entered the facility, etc.; **2)** The extent of contamination, including the amount and possible pathways that have or could have spread *B. anthracis* spores. It is advisable to isolate the contaminated area; and **3)** The objectives of decon, including decon of critical items for re-use and the treatment, removal, or packaging of other items for disposal. **Note:** Crisis exemptions from EPA Office of Pesticides might be necessary depending on decontaminating agents used.
Decon Methods: OSCs should check with the EPA National Decon Team Subject Matter Experts regarding specific decontamination parameters, as well as specifics on the use of readily available commercial items such as standard bleach, at 513-487-2420 (after hours call 24-hour pager at 800-329-1841).
Methods used on surfaces: 1) Source reduction steps, including HEPA vacuuming; **2)** Liquid Antimicrobial products such as pH amended bleach (A mixture of 1 part bleach (5.25% to 6.0%) to 1 part white vinegar to 8 parts water, is recommended). This product affects surfaces differently in terms of corrosiveness, staining, and residue. The product will be most efficient a) At higher temperatures (>70F) b)when plain bleach (e.g., no added fragrance) is used to make the pH-amended bleach solution, c) when pH is equal to 7,d) when presence of other surface contaminants is minimal, and e) when surfaces remain wet with amended bleach solution for 60 minutes. pH-amended bleach can be deployed as a spray or liquid. **Note:** Store-bought bleach does degrade with time – check the expiration date. Alternate antimicrobial products include: chlorine dioxide, hydrogen peroxide, and peroxyacetic acid. Fumigation: Uses gas or vapor to decontaminate facilities in which there is evidence of high levels of contamination, re-aerosolization of spores or if decontamination of limited access areas is required (e.g. HVAC systems). The history of usage of the agents as fumigants, materials compatibility, penetration capacity, method of removal at the end of fumigation, as well as their physical, chemical, & toxicological properties should be taken into account. Fumigants: chlorine dioxide, vaporized hydrogen peroxide, and paraformaldehyde. Each chemical has a specified range for process variables (e.g., temperature, relative humidity, conc. & contact time) that must be followed.
Other Decon: 1) Ethylene oxide sterilization is used to decontaminate items in an off-site sterilization chamber. **2)** Irradiation uses cobalt-60 and electron beam technologies to destroy *B. anthracis* in mail, and other paper goods at off-site locations. This procedure may destroy magnetic media. Irradiation and chemical sterilization may be useful in decontaminating items that are intended to be returned to owners. The Brentwood, Trenton, and Capitol Hill remediation teams used Chloride Dioxide liquid and fumigation to decontaminate the site (ClO2 at 750ppmv for 12 hours at a minimum of 75°F and 75% relative humidity).
Decon Effectiveness: Multi-agency, multi-disciplinary experts should be consulted for advice in developing a post-decon verification sampling strategy and establishing criteria for verifying decon effectiveness. Expert input is especially important if contamination is extensive. Rigorous environmental sampling should be performed after decontamination. The samples obtained should be verified via culture. Targeted or judgemental sampling should be performed in areas which were known to be contaminated prior to decontamination. In addition, statistically relevant sampling in the decontaminated area(s) should also be performed. Air sampling after decon may also be appropriate if spores are likely to re-aerosolize. If fumigation is to be performed, use biological indicators in hard-to-reach areas to provide assurance that the fumigant adequately penetrated all of the contaminated areas.
Clean-up Adequacy Verification: There is currently no scientifically sound basis for determining a "safe" number of residual viable spores in a decontaminated area. In response to bioterrorist events involving *B. anthracis*, EPA follows the recommendation of the National Academy of Sciences that decontamination be continued until there is "no growth" of *B. anthracis* on post-decon samples. Viable spores may remain, but the risk of contracting anthrax in that area would be considered extremely low. In workplace situations, OSHA offers alternative criteria to the "no growth" decon goal, especially where PPE, special work practices, and engineering controls can be used to minimize the risk. OSHA provides guidance RE: alternative controls at: *http://www.osha.gov/SLTC/etools/anthrax/transition_program.html*

Waste/Disposal

B. anthracis is not regulated under Subtitle C of RCRA, but should be handled with caution. In some states and localities, waste management will vary; for instance, B. anthracis waste may be considered municipal waste, medical waste or an infectious substance with special requirements for handling and disposal. Contact the state or local regulatory agency to determine appropriate waste management practices. *B. anthracis* spores are subject to DOT regulations and the CDC's Select Agent program requirements. See *http://hazmat.dot.gov/training/rmgmt/guide_anthrax.htm* or *http://www.asm.org/ASM/files/LEFTMARGINHEADERLIST/downloadfilename/0000001202/ProtocolPackShip.pdf*. Prior to disposal, wastewater from contaminated sites should be pre-treated using a chemical like 5.25 - 6.0 % sodium hypochlorite (household bleach) or another sterilization process. Neutralization of the resulting solution may be required prior to final disposal (e.g. wastewater treated with bleach can be neutralized with sodium thiosulfate)