SENTINEL LABORATORY GUIDELINES FOR SUSPECTED AGENTS OF BIOTERRORISM

Staphylococcal Enterotoxin B

American Society for Microbiology
Credits: Staphylococcal Enterotoxin B

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Table of Contents: Staphylococcal Enterotoxin B

I. General Information
   A. Description of Organism
   B. History
   C. Geographic Distribution
   D. Clinical Presentation
   E. Treatment and Protection

II. Procedure
   A. General
   B. Precautions
   C. Specimen
   D. Materials
   E. Test
   F. Shipping
   G. Interpretation and Reporting
   H. Limitations

III. References
I. GENERAL INFORMATION

A. Description of organism
Staphylococcal enterotoxin B (SEB) is one of several exotoxins produced by *Staphylococcus aureus*. *S. aureus* is a ubiquitous, nonmotile, gram-positive coccus found on the skin and mucous membranes of humans and animals. It is identified by its ability to produce catalase and coagulase. *S. aureus* produces a variety of extracellular proteins and enzymes, which act as virulence factors for the organism. These include toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins (ETA and ETB), leukocidin, and staphylococcal enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SHE, and SEI). The eight staphylococcal exotoxins are characterized as enterotoxins, because they exert their effect on the intestinal tract when ingested. SEB has a molecular mass of 28 kDa and is heat stable and soluble in water. SEB has a broad spectrum of biological activity, and depending on the portal of entry (e.g., gastrointestinal, respiratory, or mucosal), the toxin will elicit a different clinical syndrome.

B. History
SEB is the enterotoxin that most commonly causes classic food poisoning. Usually multiple people are affected after ingesting the same foods at a picnic or a restaurant. SEB also causes nonmenstrual toxic shock syndrome. In addition, it has been studied as a biological weapon, because of its stability and the fact that it can be easily aerosolized. It was part of the U.S. stockpile of bioweapons prior to its destruction in 1972. This enterotoxin produces a debilitating syndrome after inhalation that resolves after 2 weeks. However, inhalation of high concentrations of the enterotoxin can result in multiorgan system failure and death. The effective dose capable of incapacitating 50% of the exposed population is 0.0004 µg/kg of body weight, and the 50% lethal dose (LD₅₀) is 0.02 µg/kg. The effects of SEB when delivered by aerosol exposure are mediated by the stimulation of T lymphocytes in the host’s immune system. The toxin binds to the major histocompatibility class II molecules, which stimulates proliferation of T lymphocytes and release of various cytokines. Therefore, SEB is classified as a “superantigen,” similar to the streptococcal pyrogenic exotoxins and toxic shock syndrome toxin 1 (TSST-1).

C. Geographic distribution
*S. aureus* bacteria and the enterotoxins they excrete are found worldwide. The actual incidence is unknown, because patients with mild forms of the illness do not seek treatment. For symptomatic patients with food poisoning, treatment is empiric, and other causes of gastroenteritis may mimic SEB-induced illness.

D. Clinical presentation
1. Ingestion exposure. The patient may exhibit a sudden onset of symptoms 1 to 8 h after ingestion of the enterotoxin. These patients present with nausea, vomiting, abdominal cramping, and diarrhea. The patient may appear dehydrated, depending on the severity of the illness. Physical examination may reveal hypotension (low blood pressure), tachycardia (rapid heart beat), and hyperperistalsis (active bowel motility). No blood is detectable in the stool. No fever or respiratory symptoms are seen with foodborne intoxication. Infants and debilitated persons can be more severely affected.
In an outbreak, a careful patient history would reveal a common location where all of the patients shared food or drink that had been improperly refrigerated, stored, or handled. For reimbursement purposes, staphylococcal food poisoning is classified as an ICD-9-CM code of 005 or ICD-10 code of A05.0.

2. **Inhalation exposure.** From 3 to 12 h after aerosol exposure to the inhaled form of the enterotoxin, there will be an abrupt onset of high fever (103 to 106°F) that lasts for 2 to 5 days, chills, headache, myalgia, and nonproductive cough persisting for up to 4 weeks. The lungs are clear, with no consolidation or effusion. Some patients complain of shortness of breath and retrosternal chest pain. In heavier exposures, there may be pulmonary edema or signs of adult respiratory distress syndrome (ARDS) with cough and frothy sputum.

Patients presenting with fever, myalgias, nonproductive cough, and headache may resemble those infected with influenza, adenovirus, parainfluenza, or mycoplasma. Early clinical manifestations of inhaled SEB may be confused with inhalation anthrax or pneumonia caused by tularemia, plague, or Q fever. The progression of respiratory symptoms stabilizes with SEB intoxication, while the illness continues to become more severe in the other infections if left untreated.

In inhalation exposure to SEB, numerous patients of all ages would display symptoms within a short period of time. There would be a common geographic history among them, such as everyone being at the same athletic event or office building.

3. **Mucosal exposure.** Toxic shock syndrome was initially associated with tampon use, especially super-absorbent tampons. Since their removal from the commercial market, there is less risk. Wound infections with *S. aureus* that produce SEB may also lead to toxic shock syndrome. These isolated cases should not be construed as a bioterrorism event.

E. **Treatment and protection**

Treatment is supportive, and the disease is usually self-limiting. If the patient is severely dehydrated, intravenous fluids should be administered. For patients with pulmonary symptoms, humidified oxygen and pain medication are appropriate. Intubation may be required following significant inhalation exposure.

Antibiotics have not been demonstrated to have any efficacy in SEB intoxication, and steroids have not been shown to be effective in treating the pulmonary edema. There is no vaccine or antitoxin available to treat SEB before or after exposure; however, passive immunotherapy can decrease mortality of inhalation exposure if given within 4 to 8 h of exposure. Experiments with animal models show a favorable response to agents that down-regulate the expression of cytokines and other mediators involved in the development of toxic shock.
The enterotoxin affects only the person who ingested or inhaled the toxin. The *S. aureus* bacterium does not infect the person, and there is no risk of acquiring the toxin from person to person. No isolation precautions are needed.

A military chemical protective mask is effective against inhalation of the toxin.

II. PROCEDURE

A. General
   1. The diagnosis of SEB intoxication is primarily clinical, with confirmation by epidemiologic assays of tissue or body fluids.
   2. Laboratory findings are not very helpful in the diagnosis of SEB intoxication.
   3. Testing is currently performed in selected Laboratory Response Network (LRN) Reference laboratories.
   4. LRN Sentinel (formerly Level A) laboratory guidelines are designed to ensure the proper collection and distribution of appropriate specimens to designated LRN Reference (formerly Level B/C) laboratories.

B. Precautions
   1. Sentinel laboratories should not accept environmental (including food samples) or animal specimens for testing; such specimens should be forwarded directly to the next level LRN Reference laboratory.
   2. These procedures should be performed in LRN Reference laboratories with Biological Safety Level 2 (BSL-2) facilities that follow BSL-3 safety guidelines.
   3. Sentinel laboratories should not attempt to perform toxin analysis.
   4. Health care workers should exercise standard precautions. Contaminated articles can be disinfected with 0.05% hypochlorite solution (1 tablespoon of bleach per gallon of water) for 10 to 15 min.

C. Specimen
   1. Acceptable specimens (for testing at an LRN Reference laboratory). NOTE: Sentinel laboratories should not accept environmental (including food samples) or animal specimens for testing; such specimens should be forwarded directly to the next level LRN Reference laboratory. Exposure to SEB as a result of a bioterrorist event may include exposure to both the organism *S. aureus* and the enterotoxin or exposure to the enterotoxin only. Specimens may be tested for both the presence of enterotoxin and the bacterium.
      a. Serum. Serum is the preferred specimen for testing for inhalation SEB intoxication by detecting antibodies to SEB. Use a red-top or serum separator-type (SST) tube to obtain serum. The tube must be free of anticoagulants. Samples should be obtained as soon as possible after the onset of symptoms to detect the toxin. Approximately 10 ml of blood should be drawn to provide 5 ml of serum. Serum should also be collected 7 to 14 days after onset of illness to compare acute- and convalescent-
phase antibody titers. Do not send whole blood, since hemolysis during transit will compromise the quality of the specimen. Label completely.

b. **Culture isolate.** If an isolate of *S. aureus* is recovered from a specimen, it may be sent for toxin testing on an appropriate agar slant that supports its growth or a transport swab. Label completely.

c. **Food specimens.** Sentinel laboratories should forward these specimens directly to an LRN Reference laboratory. Foods should be left in their original containers if possible or placed in sterile unbreakable containers. Place containers individually in leakproof containers (i.e., sealed plastic bags) to prevent cross-contamination during shipment. Empty containers with remnants of suspected contaminated foods can be examined. Label completely.

d. **Environmental samples.** Sentinel laboratories should forward these specimens directly to an LRN Reference laboratory. Paper, powder, swabs, wipes, water, and soil can be sent for SEB testing. Label completely.

e. **Other patient specimens**

1. **Nasal swab.** Collect a nasal swab within 24 h of exposure by rubbing a dry, sterile swab (Dacron or rayon) on the mucosa of the anterior nares. Place in protective transport tube and label completely.

2. **Induced respiratory secretions.** Sputum induced by instilling 10 to 25 ml of sterile saline into the nasal passages should be collected into a sterile screw-top container. Seal tightly and label completely.

3. **Urine.** A 20- to 30-ml urine sample should be collected from the patient into a sterile screw-top container as soon as possible. Seal the container tightly and label completely.

4. **Stool/gastric aspirate.** A 10- to 50-g sample of stool should be placed in a sterile leakproof container with a screw-top lid. Close securely and label completely.

5. **Postmortem.** Obtain specimens of the intestinal contents from different levels of the small and large bowel. Place 10 g of specimen into a sterile unbreakable container and label completely. Obtain serum as previously described.

2. **Specimen handling**

a. In conjunction with instructions from the State Public Health Laboratory, arrange for immediate shipment at 2 to 8°C to the appropriate LRN Reference laboratory.

b. Follow infectious substance regulations for packing and shipping. [Refer to ASM Guideline on Packing and Shipping Infectious Substances, Diagnostic Specimens, and Biological Agents (http://www.asm.org/index.asp?bid=6342)].

c. Sentinel laboratories should not accept environmental (including food samples) or animal specimens for testing; such specimens should be forwarded directly to the next level LRN Reference laboratory.

3. **Rejection criteria**

a. **Incomplete documentation.** All specimens must include the sender’s name and a telephone number to contact for the preliminary report and additional information.

b. **Improper packaging/shipping**
c. **Lack of prior approval.** Do not ship specimens to LRN Reference laboratories without prior approval.

**D. Materials**
1. **Medium.** Use appropriate agar to grow and ship the *S. aureus* isolate.
2. **Supplies**
   a. **Leakproof containers**
   b. **Serum collection tubes** (red-top or SST)
   c. **Packaging materials.** Approved packaging and labels

**E. Test**
1. Since there are no Food and Drug Administration (FDA)-approved toxin assays for clinical use, specimens must be shipped to appropriate LRN Reference laboratories. Currently 50 laboratories in 37 states have the capacity of performing the assay.
2. The testing method is the time-resolved fluorescence (TRF) immunoassay, a solid-phase, noncompetitive sandwich ELISA of capture antibody, antigen, and detection antibody.
3. The turnaround time is 3 to 3.5 h.
4. Mouse assays are no longer performed to detect SEB.

**F. Shipping**
1. Locate the closest LRN Reference laboratory that performs the assay to detect SEB, and notify them that you would like to ship material for testing.
2. Refer to ASM Guideline on Packing and Shipping Infectious Substances, Diagnostic Specimens, and Biological Agents (http://www.asm.org/index.asp?bid=6342). Send specimen with complete documentation according to the directions from the receiving laboratory.

**G. Reporting**
1. Follow institutional reporting protocols if associated with a possible bioterrorist attack.
2. If a cluster of patients presents with similar symptoms, either gastrointestinal or pulmonary, notify institutional infection control and/or the State Public Health Laboratory in accordance with your facility’s regulations.

**H. Limitations**
1. SEB can be identified in nasal swabs collected 12 to 24 h after exposure to respiratory aerosols. Samples taken more than 24 h after exposure may not contain detectable levels of toxin.
2. Data from rabbit studies showed that SEB is transient in the serum. By the time symptoms are noted in a patient, toxin concentrations are below detectable levels. Exposure to toxin can be detected by comparing acute- and convalescent-phase antibody titers in serum.
3. SEB accumulates in urine and may be detected for several hours after exposure.
III. REFERENCES


   http://www.emedicine.com/