

SENTINEL LEVEL CLINICAL LABORATORY GUIDELINES
FOR
SUSPECTED AGENTS OF BIOTERRORISM
AND
EMERGING INFECTIOUS DISEASES

Staphylococcal Enterotoxin B

American Society for Microbiology (ASM)

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For latest revision, see web site below:

<http://www.asm.org/index.php/guidelines/sentinel-guidelines>

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PREANALYTICAL CONSIDERATIONS

I. PRINCIPLE

A. Introduction

Staphylococcal enterotoxin B (SEB) is one of several exotoxins that may be produced by *Staphylococcus aureus*. Depending on the strain, *S. aureus* can produce a variety of extracellular proteins which act as virulence factors. Among these are toxic shock syndrome toxin 1 (TSST-1), exfoliative toxins A and B, and a family of structurally-related proteins classified as enterotoxins.

At least 20 distinct staphylococcal enterotoxins have been described including the classical types (SEA-SEE) and those more recently discovered (SEG-SEU). SEB is a compact polypeptide containing 239 amino acids and has a molecular weight of approximately 28 kD. Because of its compact structure, it is moderately heat-stable and is resistant to proteases, including those found in the gastrointestinal tract. SEB is classified as a superantigen because it interacts directly with MHC class II molecules and T-cell receptors without prior antigen processing and presentation. This direct interaction leads to the activation of an unwarranted number of T-cells and a massive release of inflammatory cytokines ultimately resulting in damage to host tissues.

This procedure describes the processes used by Sentinel Clinical Laboratories to ensure the proper collection and distribution of clinical specimens and food items suspected of containing staphylococcal enterotoxins. Such laboratories are defined as those certified to perform high complexity testing under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) by the Centers for Medicare and Medicaid Services for the applicable Microbiology specialty. Laboratory in-house testing includes Gram stains, and at least one of the following: lower respiratory tract, wound, or blood cultures.

Sentinel Clinical Laboratories are not required to register with the Select Agent Program to conduct diagnostic testing for Select Agents, both Tier I and non-Tier 1. Testing for Select Agents may be performed by laboratories as long as the laboratory follows the policies listed in the reporting section of this document when a Select Agent cannot be ruled out. Staphylococcal enterotoxin types A-E are categorized as non-Tier 1 Select Toxins. Consult with your designated LRN Reference Laboratory or refer to the CDC Division of Select Agents and Toxins website at <http://www.selectagents.gov> for questions.

SEB has been studied as an agent of biowarfare since it is readily prepared, is easily aerosolized, and is able to produce biological effects at much lower

quantities as compared to synthetic chemicals. SEB was weaponized by several countries and was part of the U.S. stockpile prior to its destruction in 1972. The toxin is considered an incapacitating agent since aerosol exposure usually results in a temporary, highly incapacitating illness rather than death. It has been estimated that the 50% effective dose (the quantity of toxin capable of incapacitating half of those exposed) for aerosol exposure is 0.0004 µg/kg while the 50% lethal dose has been estimated at 0.02 µg/kg. Although inhalational exposure to SEB is not likely to produce significant mortality, it could incapacitate 80% or more of exposed individuals leaving them unable to perform routine activities for up to 2 weeks. Pre-existing antibody from natural exposure to SEB does not adequately protect against aerosol challenge.

B. Geographic Distribution

Enterotoxigenic strains of *S. aureus* are found worldwide and food intoxication is both widespread and relatively common.

C. Diseases and Clinical Presentation

Whether ingested or inhaled, the initial manifestations of SEB intoxication are flu-like including myalgias, headache, chills, and fever while subsequent symptoms depend upon the route of exposure. Ingestion of food tainted with SEB leads to nausea, vomiting, abdominal cramping, and diarrhea with symptoms typically resolving within 24 hours. Ingestion of large quantities of toxin can lead to profound dehydration, shock, respiratory failure, and cardiovascular collapse. Inhalation of SEB manifests as non-productive cough, breathing difficulty, and chest pain while more significant exposure may result in acute pulmonary edema and respiratory failure. Fever may persist for up to 5 days and the cough for up to 4 weeks. Gastrointestinal symptoms may also be present due to swallowing toxin after mucociliary clearance or as a systemic manifestation of toxin exposure. Conjunctivitis and periorbital edema along with gastrointestinal symptoms have been described following ocular exposure.

At this time, treatment is limited to symptomatic relief and supportive care; antibiotics have not been demonstrated to have any efficacy in SEB intoxication. Most patients would be expected to completely recover but fluid management and mechanical ventilation may be needed for severe cases. The role of passive immunotherapy or steroid use is unclear and there are no specific therapies or vaccines approved for use in humans.

D. Presumptive Diagnosis

The diagnosis of SEB ingestion or inhalation is largely based on clinical and epidemiologic grounds. The astute clinician would recognize a cluster of acutely ill patients presenting within a short period of time, demonstrating signs and symptoms consistent with toxin ingestion or inhalation. The intentional release of SEB would have to be distinguished from naturally

occurring food poisoning, community-acquired pneumonia, and respiratory virus infections.

II. SAFETY CONSIDERATIONS

- A. The enterotoxin is not contagious and affects only the person who was exposed. There is no risk of acquiring the toxin from person to person. Therefore, health care workers should exercise standard precautions; no transmission-based precautions are required.
- B. SEB is able to withstand boiling for several minutes, gamma irradiation and dry heat treatments may not completely inactivate the toxin, and freeze-thawing is ineffective. To ensure complete inactivation, contaminated items should be steam autoclaved at $>121^{\circ}\text{C}$ for one hour (2 hours for volumes greater than 1 liter) and then incinerated. Contaminated surfaces should be treated with fresh 0.5% hypochlorite for 15 minutes (<http://www.cdc.gov/biosafety/publications/bmbl5/>).

III. MATERIALS

- A. Media
 - 1. Culture media suitable for the isolation of *S. aureus*
 - 2. Agar slant for isolate shipment
- B. Reagents
 - 1. Appropriate disinfectant such as 10% bleach (0.5% hypochlorite)
- C. Equipment and supplies
 - 1. Biosafety cabinet
 - 2. PPE (gloves, solid front gown)
 - 3. Screw-capped leak-proof containers
 - 4. Sealable plastic bags
 - 5. Sample collection/transport swabs (Dacron or rayon)
 - 6. Packing and shipping materials

IV. SPECIMEN COLLECTION AND TRANSPORT

A. Collection and Transport of Specimens	
Culture isolate	<ul style="list-style-type: none"> • Fresh subculture on agar slant or transport swab • Transport at 2 to 8°C • Store at room temperature
Food	<ul style="list-style-type: none"> • Sentinel laboratories should forward these specimens directly to an LRN Reference or FERN laboratory • When possible, it is preferable to send the entire food item in its original container. Alternatively, representative samples (2 g or 100 mL) may be placed in sterile unbreakable containers • Place food containers individually into separate leak-proof secondary containers (i.e., sealed plastic bags) to prevent cross-contamination during shipment. Empty containers with remnants of suspected contaminated foods can also be examined. Label completely. • Transport at 2 to 8°C • Store at 2 to 8°C
Environmental (non-food)	<ul style="list-style-type: none"> • Sentinel facilities should not attempt to collect these samples • Contact your designated LRN Reference level laboratory for guidance
Clinical	<ul style="list-style-type: none"> • Contact your designated LRN Reference level laboratory for specific guidance prior to specimen collection

B. Follow infectious substance regulations for packing and shipping. [Refer to the current version of the ASM Guideline on Packing and Shipping Infectious Substances, Diagnostic Specimens, and Biological Agents (<http://clinmicro.asm.org/index.php/bench-work-resources>)].

C. Rejection criteria

1. Incomplete documentation. All specimens must include the sender's name and a telephone number to contact for the preliminary report and additional information.
2. Improper packaging/shipping.
3. Lack of prior approval. Do not ship specimens to LRN Reference or FERN laboratories without prior consultation with your state or local epidemiologist.

ANALYTICAL CONSIDERATIONS

V. SPECIMEN PROCESSING

- A. The LRN no longer has specific testing available. Notify your designated LRN Reference Laboratory who will assist you in locating the appropriate testing laboratory. Do not ship specimens to these laboratories without prior consultation with your state or local epidemiologist.
- B. Sentinel laboratories should not accept environmental (including food samples) for testing; such specimens should be forwarded directly to the next level LRN Reference or FERN laboratory.

VI. INCUBATION AND EXAMINATION OF CULTURES

Follow institutional protocols if isolation of *S. aureus* is attempted from clinical specimens.

VIII. IDENTIFICATION

- A. Since there are no Food and Drug Administration (FDA)-approved SEB detection assays for clinical use, specimens must be shipped to appropriate LRN Reference or FERN laboratories. Contact your assigned LRN laboratory for availability.
- B. Exposure to SEB as a result of a bioterrorist event may include exposure to the enterotoxin alone or both the organism *S. aureus* and the enterotoxin. Specimens may be tested for the enterotoxin, SEB gene sequences, or the bacterium.
- C. The diagnosis of SEB intoxication is primarily clinical, with confirmation by epidemiologic assays of environmental or clinical specimens. Routine laboratory findings are nonspecific and therefore not helpful in the diagnosis of SEB intoxication. Specialized testing is currently performed in select laboratories which may include polymerase chain reaction (PCR) to detect SEB gene sequences in environmental, food, and clinical specimens, or enzyme immunoassay (EIA) to detect the toxin in food and toxin production by *S. aureus* isolates. Culture may also be performed on any sample type.

POST ANALYTICAL CONSIDERATIONS

VIII. SUMMARY/SPECIAL CONSIDERATIONS

- A. Follow institutional reporting protocols if associated with a possible bioterrorist

attack.

- B. If a cluster of patients presents with similar symptoms, either gastrointestinal or pulmonary, notify your institutional infection control and your identified LRN Reference Laboratory in accordance with local and state reporting requirements.
- C. Destruction
 1. Once the presence of a Select toxin is confirmed, the Sentinel Laboratory Select Agent regulations require that the residual specimens be destroyed or transferred to an approved Select Agent entity within 7 days of confirmation. Your designated LRN Reference Laboratory must advise you on destruction or transfer of specimens.
 2. Residual specimens should be steam autoclaved at $>121^{\circ}\text{C}$ for one hour (2 hours for volumes greater than 1 liter) and then incinerated (<http://www.cdc.gov/biosafety/publications/bmb15/>).
- E. Packing and shipping
 1. Refer to the ASM Packing and Shipping Sentinel Guidelines.
 2. All materials sent to your designated LRN Reference Laboratory must be shipped in compliance with IATA and DOT regulations.

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