

Sacred Heart Medical Center  
Microbiology Laboratory Protocol for Anthrax  
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A. Recommended Precautions

1. Biosafety level 2 practices, containment equipment, and facilities are recommended for activities involving clinical specimens.
2. Biosafety level 3 practices, containment equipment, and facilities are recommended for activities with a high potential for aerosol production (refer to “Exposure to Infectious Agent Risk Assessment”, June 2001).

B. Acceptable Specimens for Culture

1. Inhalation Anthrax
  - a. Blood
  - b. Sputum, IF and only IF the patient has a productive cough; chest x-ray is more useful in diagnosis than sputum culture
  - c. CSF, if meningeal symptoms are present
2. Cutaneous Anthrax
  - a. Wound or vesicular fluid
  - b. Blood
3. Gastrointestinal Anthrax
  - a. Stool
  - b. Blood

C. Unacceptable Specimens for Culture

1. Nasal or Throat Swabs
  - a. Nasal swabs are used as part of the epidemiological investigation once there is a known or suspected anthrax exposure. In order for it to be a credible exposure, HAZMAT and the FBI would need to be involved.
  - b. Nasal or throat swabs can NOT rule out an exposure to anthrax. If for some reason a culture has been processed by the lab and is negative for anthrax, the following comment will be attached to the result: **“A negative nasal swab culture result does not rule out exposure or infection with anthrax.”**
2. Environmental Cultures
  - a. Level A labs have been specifically told NOT to culture environmental items. This is a public laboratory function and requires biosafety level 3 practices and facilities.
3. Animal Cultures
  - a. The public health labs will handle animal cultures for anthrax.

D. Specimen Processing

1. Process the specimens under a biosafety hood, according to the existing specimen processing procedure manual. The only modification needed is the addition of a BAP and CNA to a stool culture for anthrax.

- E. Presumptive Identification of *Bacillus anthracis*
1. The role of the level A lab is to rule out *B. anthracis*, not to definitively identify it.
  2. Direct smears from clinical specimens:
    - a. *B. anthracis* appears as broad gram-positive bacilli in short chains, 2-4 cells long. A clear zone around the cells is due to the capsule.
  3. Smears from plated media
    - a. *B. anthracis* appears as non-encapsulated broad gram-positive bacilli in long chains.
    - b. The organism measures 1-1.5 X 3-5  $\mu$ .
    - c. Spores are usually NOT present in clinical specimens unless exposed to atmospheric oxygen. If present, the spores are oval and located centrally to subterminally in the cell and measure 1 X 1.5  $\mu$ , with no significant swelling of the cell.
  4. Colonial Characteristics of *Bacillus anthracis*
    - a. After 15-24 hr incubation on a BAP, well-isolated colonies are 2-5 mm in diameter; heavily inoculated areas may show growth in 6-8 hr.
    - b. The colonies are gray-white, flat or slightly convex, irregularly round, with edges that slightly undulate, and have a "ground glass" appearance.
    - c. The colonies often have comma-shaped protrusions that go out from the edge of the colony = "medusa head" colonies.
    - d. The colonies have a tenacious consistency, such that when pushed up with a loop, the growth will stand up like a beaten egg white.
    - e. The colonies are non-hemolytic. Weak hemolysis may be seen under areas of confluent growth in aging cultures and should NOT be confused with true  $\beta$ -hemolysis.
    - f. The organism will not grow on a MAC.
    - g. The organism is non-motile.
  5. Minimum Criteria for Presumptive Identification of *Bacillus anthracis*
    - a. Non-hemolytic colonies
    - b. Non-motile organism
    - c. Broad, gram-positive rod
  6. If *Bacillus anthracis* is suspected:
    - a. Bring up on Rounds.
    - b. Notify the County Health District first and then the State Health Department for approval to ship
      - i. Spokane County Health District 24 hour emergency pager, 477-4209 Ext 2140
      - ii. State DOH, Seattle, phone: (877)539-4344
      - iii. When the isolate is ready to ship, call the following number at the State DOH to let them know what courier is being used and when it will be shipped/arrive: ( ) - .
    - c. Contact hospital epidemiology, if the patient is hospitalized.
    - d. Notify the patient's physician.

- e. Package the organism, according to 602 regulations, and send to the health department as instructed.

F. References

1. CDC guidelines for state health departments. October 14, 2000. 9 pg.
2. Laboratory protocols for clinical laboratories for the identification of *Bacillus anthracis*. CDC BT public web site: [www.bt.cdc.gov](http://www.bt.cdc.gov)

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Updates and Revisions: