

Bacillus anthracis (Anthrax)

Bacillus anthracis is very large, Gram-positive, sporeforming rod, 1 - 1.2µm in width x 3 - 5µm in length. The bacterium can be cultivated in a variety of nutrient media under aerobic or anaerobic conditions. The spores formed are oval and located centrally to subterminally in a nonswollen sporangium. Anthrax can infect the human body through the intestines (ingestion), lungs (inhalation), or skin (cutaneous) and causes clinical symptoms based on transmission mode. Anthrax infection does not usually spread from person to person. Clinical and postmortem samples can remain contagious until sterilized.

Specimens

- Cutaneous anthrax
- **Vesicular stage**
 - Soak 2 dry sterile swabs in **vesicular fluid** of a previously unopened vesicle.
- **Eschar stage**
 - Rotate 2 dry sterile swabs beneath edge of eschar without removing eschar to absorb **serous exudate**
 - If there is no serous exudate, gently massage edge of the lesion with sterile swabs moistened with saline, broth media or sterile water.
- Gastrointestinal anthrax
 - Obtain **stool** specimen if possible.
 - In later stages of illness, collect **blood** specimen.
- Inhalation anthrax
 - If respiratory symptoms present, obtain **sputum**.
 - In later stages of illness (2–8 days post exposure), obtain **blood** specimen.
 - In addition, if symptoms suggest **meningitis**, obtain **cerebrospinal fluid**.



Processing of Specimens

• **Sputum**

- Inoculate 3 media routinely used for sputum specimens [e.g., 5 % sheep blood agar (SBA) plate, MacConkey agar plate, trypticase soy broth (enrichment)].

• **Blood**

- Prepare smear directly from blood for Gram staining.
- Culture specimen using routine blood culture methods.
- If blood culture is positive, prepare a smear for Gram staining.
- Subculture blood cultures to SBA and MacConkey agar plates.

• **Swab specimens**

- Use one swab to inoculate 3 standard media for surface wounds (e.g., SBA plate, MacConkey agar plate, broth enrichment).
- Use second swab to prepare smear for Gram staining.

• **Stool**

- Inoculate specimen onto SBA, and MacConkey agar or phenylethyl alcohol (PEA) agar plates. Do not use hektoen agar.

• **Cerebrospinal fluid**

- Centrifuge cerebrospinal fluid specimens at 1,500 x g for 15 min.
- Collect sediment. Use it to:
 - Inoculate SBA plate and an enrichment broth (trypticase soy broth).
 - Prepare smear for Gram staining.

Incubation of Cultures

Incubate at 35–37 C in air (without increased CO₂).

Examine at 18–24 h incubation.

Examination of Cultures and Gram Stain

Colony Morphology

- Flat to slightly convex, white to grey colonies on SBA at 24 hrs
- Edges may have irregular or wavy comma-shaped protrusions (“Medusa head” colony)
- 2–5 mm in diameter



- Growth has tenacious consistency: when teased with loop, it stands up like beaten egg whites
- Nonhemolytic (*B. cereus* and *B. thuringiensis* are hemolytic); however, weak hemolysis may occur in older cultures under areas of heavy growth.
- MacConkey agar – no growth
- Phenylethyl alcohol (PEA) agar plates – no growth

- **Gram Stain**

(Gram stain of blood or impression smears)

- Large Gram-positive rod (1.0-1.5 μm x 3.0-5.0 μm) (May appear Gram-variable if over-decolorized.) long chains, encapsulated oval, nonswelling spores
- Under appropriate conditions, forms spores (oval, central-to-subterminal, do not significantly swell cell) and capsule.
- Sporulation inhibited by level of CO₂ in body; sporulation occurs when CO₂ levels are low, as in atmosphere.



Identification Flow Chart

Ground glass, nonhemolytic, grey-white colonies on SBA at 24 hrs

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Large Gram-positive rods; long chains, encapsulated oval, nonswelling spores

–
Catalase positive

–
Nonmotile

–
**Refer to State Laboratory
610-280-3464**