Bacillus anthracis
LEVEL A LABORATORY GUIDELINES

Safety
1. Biosafety level 2 practices for processing clinical materials.
2. Biosafety level 3 practices for activities which have potential for aerosol production.

Colony Characteristics
1. 2-5 mm overnight at 35°C
2. Non-hemolytic, non-pigmented, dry ground glass surface, edge irregular with comma projections (Medusa Head)

![B. anthracis Non-hemolytic on sheep blood agar](image)

3. Consistency sticky (tenacious). When teased with loop, colony will stand up like beaten egg white.

![B. anthracis tenacity positive on sheep blood agar (24 hours)](image)

Microscopic Characteristics
1. Large gram-positive bacillus, singly and in chains. May be gram-variable after 72 hours. May be encapsulated in clinical material and positive blood cultures.
2. Terminal/Subterminal spores do not swell vegetative cells. Spores may be seen on Gram stain, malachite green stain or phase contrast.
3. Spores are Not present in clinical material unless exposed to low CO₂ levels. For example, spores may be seen in material from wound eschars, but would not be seen in body fluids.

Key Characteristics
1. **Growth**: Rapid growth aerobically at 35-37°C on most standard laboratory media. Does not require CO₂
2. **Gram stain**: Large gram-positive bacillus
3. **Hemolysis**: Non-hemolytic
4. **Motility**: Non-motile
5. **Catalase**: Positive
6. **Spores**: Present when cultured aerobically without CO₂
7. **Penicillin Sensitivity**: Inhibited by 10-U PCN disk (zone of inhibition 15-20 mm)

Isolates with the above characteristics should be reported to the patient's physician and forwarded to the South Dakota State Public Health Laboratory for additional testing.
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