

YERSINIA PESTIS LEVEL A LABORATORY GUIDELINES

Safety

1. Biosafety level 2 practices for specimen processing.
2. Biosafety level 3 practices for activities involving manipulations of cultures which have potential for aerosol production.

Colony Characteristics

1. Grows well on most standard laboratory media (e.g. sheep blood, chocolate and trypticase soy agars). Pinpoint grey-white, non-hemolytic at 24 hours, by 48 hours, colonies resemble typical enteric gram-negative colonies. After 48-72 hours, grey-white to slightly yellow opaque raised, irregular “fried egg” morphology; alternatively colonies may have a “hammered copper” shiny surface.



Y. pestis on sheep blood agar (72 hours)

2. Although grows more slowly than other *Enterobacteriaceae* at 35-37 C, grows faster than most other enteric bacteria at room temperature (optimal growth temperature for all *Yersinia* species).
3. Grows on MacConkey agar (lactose negative) and cefsulodin-irgasan-novobiocin (CIN) agar (colorless, developing pink centers).
4. In broth, growth is flocculent, producing structures resembling stalactites and clumps at the side and bottom of tubes.

Microscopic Characteristics

1. Small (0.5 x 1.0 uM) gram-negative bacillus.
2. Bipolar staining using Wayson, Wright, Giemsa, or methylene blue stain and may occasionally be seen in Gram-stained preparations.
Note: Although characteristic of *Y. pestis*, bipolar staining is not always observable and is not unique for *Y. pestis*.

Key Characteristics

1. Growth: Grows at 35-37 C and at room temperature.
2. Gram stain: Gram-negative bacillus.

3. Catalase: Positive.
4. Motility: Non-motile. (37 C and room temperature).
Note: *Y. pestis* is the only species of *Yersinia* which is non-motile at room temperature).
5. Oxidase: Negative
6. Biochemical characteristics: Included in most enteric identification systems. However, identification of *Y. pestis* must be considered presumptive until reference laboratory confirmation by phage lysis and direct fluorescent antibody stain.

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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