

Yersinia pestis

(Plague)

***Yersinia pestis* is a large, Gram-negative, enteric rod. It has been attributed to epidemics that have caused high mortality rates. The bacterium can be cultivated in a variety of nutrient media. Plague can cause 2 primary forms of infection in the human body; Bubonic (buboes), and Pneumonic (inhalation), which have specific clinical symptoms based on transmission mode. Plague infections and subsequent specimens are contagious.**

Specimens

- Collect specimens before antibiotic treatment of patient has begun.
- **Bubonic plague**
 - Collect **lymph node aspirate** from the affected bubo.
 - This is the specimen of choice.
 - It may be necessary to inject small amount of sterile saline into a node before aspirating material because nodes in plague-infected patients are usually not purulent.
 - Series of **blood** specimens collected 10–30 min apart may yield *Y. pestis*.
- **Pneumonic plague**
 - Collect **bronchial** or **tracheal washings**.
 - **Sputum** and **throat** specimens are not ideal; contain too many other organisms that may mask *Yersinia*.
 - If it appears unlikely that live organisms will be recoverable (as from autopsy specimens), collect lymphoid tissue, lung tissue and bone marrow and prepare impression smears for DFA staining.

Processing of Specimens

- **Blood**
 - Use routine blood culture methods.
 - When evidence of growth appears, subculture onto 2 sheep blood agar (SBA) plates.
 - If SBA plates are not available, other nutrient-rich media, such as heart infusion agar or trypticase soy agar, may be used.

- **Bubo material, bronchial and tracheal washings**

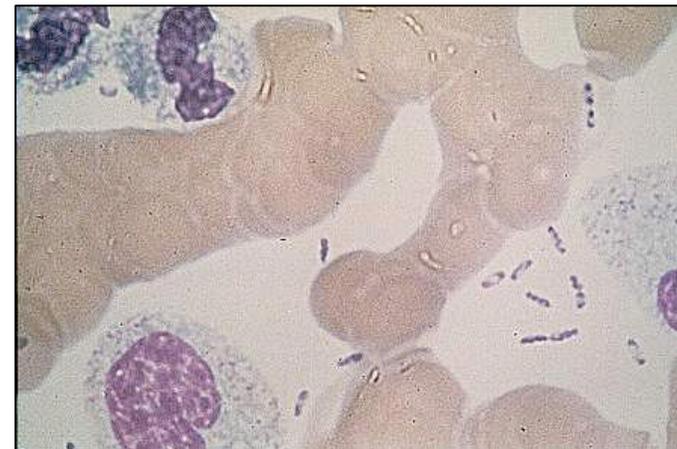
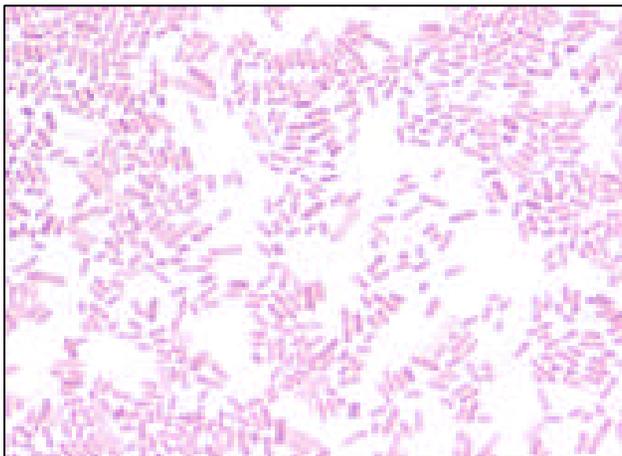
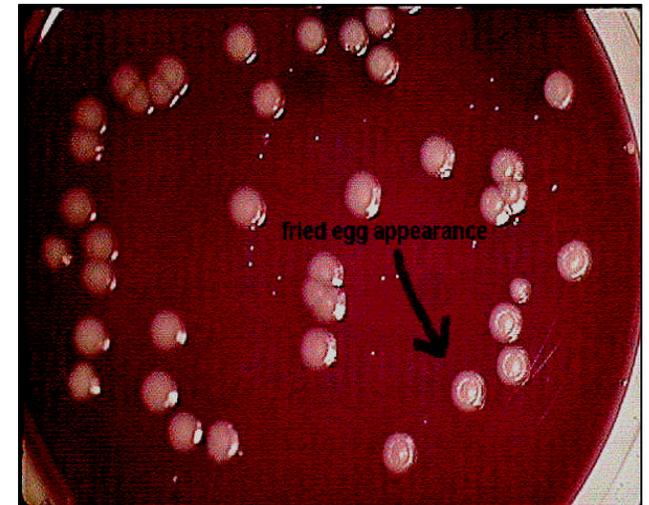
- Inoculate onto 2 SBA plates. If these are not available, use another nutrient-rich medium, such as heart infusion agar or trypticase soy agar.
- Also inoculate an enrichment broth (trypticase soy broth or brain heart infusion broth).
- Prepare direct smears for Gram staining.

Incubation of Cultures

- Incubate one set of plates and broth at 35–37 C in air.
- Incubate the second set at 28 C in air.
- Examine daily.
- Incubate 7 days before reporting culture negative.

Examination of Cultures and Gram Stain

- Colony characteristics on SBA
- 24 h – colonies pinpoint
- 48 h – colonies 1-2 mm in diameter, gray-white to slight yellow, opaque
- 48–72 h – colonies have raised, irregular “fried egg” or “hammered copper” appearance more pronounced when examined under 4X magnification.
- Growth more rapid at 28 C than at 37 C.
- Gram stain: Gram-negative, fat bacilli; 0.5um x 1.0um single cells or short chains may exhibit bipolar staining
- Giemsa or Wayson stain morphology from direct specimen material: Dark blue rod-shaped organisms may show “safety-pin” morphology. (“Safety-pin” feature is neither specific nor sensitive)



Identification Flow Chart

Gram-negative rods from blood, lymph node aspirate, or respiratory specimens.

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Colonies resemble enterics, but grow better at 28°C than at 35°C.

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Non-lactose fermenter on MAC

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Catalase positive

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Oxidase negative

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Indole negative

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Urease negative

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Nonmotile

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Refer to State Laboratory

610-280-3464