*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.

- Colonies resemble enterics, but grow better at 28°C than at 35°C.
- Non-lactose fermenter on MAC
  - Catalase positive
  - Oxidase negative
    - Indole negative
    - Urease negative
    - Nonmotile

Refer to State Laboratory
610-280-3464