

# *Brucella Spp.* (Brucellosis)

Brucellosis is also referred to as undulant fever or Maltese fever, It is a highly contagious zoonosis caused by ingestion of unpasteurized milk or preparing contaminated meat from infected animals including; (*B. abortus* (cattle), *B. melitensis*, (sheep, and goats), *B. suis* (pigs), and rarely *B. canis* (dogs), or close contact with their secretions. *Brucella* spp. are small, Gram-negative, non-motile, pleomorphic rods. *Brucella* Spp. are facultative intracellular parasites that cause chronic disease. In the United States, the most common cause of infection is ingestion of unpasteurized milk or dairy products. It is highly infectious in the laboratory especially with procedures that produce aerosols. Cultures should be manipulated under BSL-2 conditions using BSL-3 practices.

## Specimens

Collect specimens before antibiotic treatment of patient has begun.

For serologic diagnosis:

- Serum
  - Collect acute phase specimen as soon as possible after onset of illness.
  - Collect convalescent phase serum 2 to 3 weeks after onset of illness.
  - Ship and store frozen.
  - If freezing is not possible, preserve specimens by adding 0.1 ml of a 1% Merthiolate solution per 1 ml of serum.

For culture:

- Blood – multiple specimens recommended
- Bone marrow aspirate – collect 0.5 to 1.0 ml from iliac crest
  - Blood and bone marrow are the specimens of choice.
  - Infected tissues – spleen and liver biopsies
- Abscesses

## Processing of Specimens

Specimens should be cultured within 2 h of collection.

If this is not possible, refrigerate specimen until time of inoculation.

- **Blood**

- Commercial blood culture bottles will support growth of *Brucella* spp.
- Use media with added CO<sub>2</sub>.
- Vent blood culture bottle.
- Make blind subcultures every 4–5 days onto 5% sheep blood agar (SBA) and heart infusion agar or Brucella agar plates.
- Keep blood cultures 30 days before discarding as negative.

- **Bone marrow aspirate and other fluid specimens**

- Inoculate directly onto SBA or Brucella agar plates.
- Incubate at 35–37 C in 5–10% CO<sub>2</sub>.
- Incubate for 10 days before discarding as negative.

- **Tissue specimens**

- Aseptically grind tissue with sterile alundum (aluminum oxide) or sand in a mortar.
- Inoculate ground tissue onto SBA or Brucella agar plates.
- Incubate at 35–37 C in 5–10% CO<sub>2</sub>.
- Incubate for 10 days before discarding as negative.
- If a specimen is likely to be contaminated, inoculate it also onto modified Thayer-Martin medium.



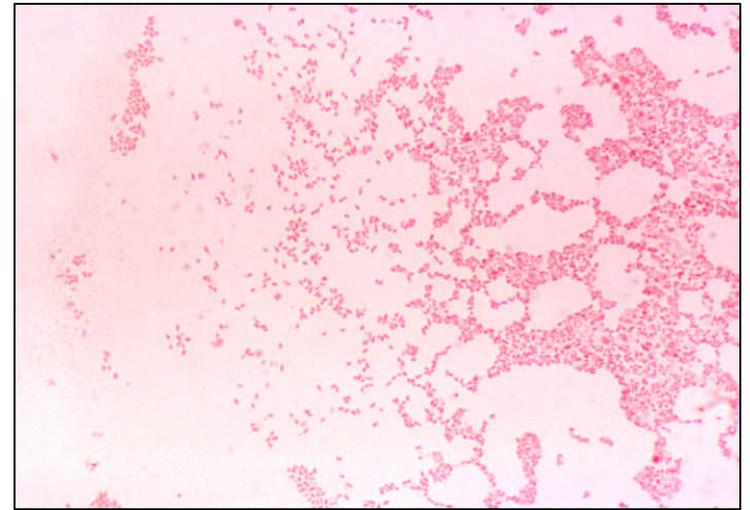
## Examination of Cultures and Gram Stain

- **Colony Morphology**

- SBA
- Chocolate Agar
- Brucella Agar
- Thayer-Martin
- 48 h – punctate, nonpigmented, nonhemolytic
- 4–5 days - 2-7 mm in diameter, spheroidal, moist, slightly opalescent; translucent, bluish-white in reflected light
- MAC-NLF (delayed growth)



- Urea –rapid positive
- Nonmotile
- **Gram Stain**
  - Gram-negative, tiny, faintly-staining coccobacilli.
  - Larger than *Francisella tularensis* individual cells are discrete.



### **Identification Flow Chart**

**Small Gram-negative coccobacilli from blood, lymphoid tissue, or bone marrow,**

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**Isolates growing slowly on Chocolate agar, Blood agar, Thayer-Martin and Brucella agar at 72 hours**

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**Nonmotile**

-

**Catalase positive**

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**Oxidase positive**

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**XV or Satellite negative**

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**Urease positive (rapid)**

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**Refer to State Laboratory**

**610-280-3464**