**Brucella species**

**Colony Morphology**
- Growth seen on Blood Agar (BA) and Chocolate Agar (CA)
- Non-hemolytic, non-pigmented small grey colonies; may require 48-72 h for discrete colonies to become evident
- CO₂ enhances growth
- Slow grower; punctate colonies (0.6 μm - 1.5 μm) in 48 h
- Clear or white colonies on MacConkey (MAC) agar at 48 h

**Gram Stain**
- Tiny, often faintly stained gram-negative coccobacilli (0.4 μm x 1.0 μm)
- May be mistaken for cocci
- May retain crystal violet stain in blood culture smears

**Biochemical/Test Reactions**
- Urea: Positive
- Oxidase: Positive
- Motility: Non-Motile

**Additional Information**
- Biosafety Level 2/3 agent (once *Brucella* is suspected, work should only be done in a certified Class II Biosafety Cabinet)
- Transmission: Inhalation, consumption of unpasteurized dairy products, direct contact with skin

**Acceptable Specimen Types**
- Whole blood: 5-10 ml blood in EDTA, and/or inoculated blood culture bottle
- Liver or spleen biopsy, or abscess
- Lymph node or bone marrow aspirate (1ml)
- Synovial fluid, CSF (≥ 1ml)

Brucellosis is a commonly acquired laboratory infection; all work on suspect *Brucella* spp. cultures should be performed at a minimum under BSL2 conditions with BSL3 practices.
Sentinel Laboratory Rule-Out of *Brucella* spp.

**Fastidious organism; growth on BA and CA**
Punctate, grey, shiny, circular, convex colonies at 48 - 72 h at 35/37°C
Non-hemolytic; non-pigmented

**Tiny, gram-negative coccobacilli**

Perform all additional work in a certified Class II Biosafety Cabinet

*Oxidase: Positive*
*Urease: Positive* (can be within minutes)
*Motility: Non-Motile*
(Use semi-solid media rather than wet mount; 2,3,5-triphenyltetrazolium chloride indicator)
X and V Factors (optional): No Requirement
Satellite growth (optional): Negative

*X: Oxidase, Urease, and Motility: Appearances of test results are not agent-specific. Photos represent typical reactions*

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**Urease**

- Negative
- Positive

**Motility**

- Non-Motile
- Motile

**Oxidase**

- Positive
- Weak Positive
- Negative

No

Continue laboratory identification procedures

Yes

Immediately notify Wadsworth Center Biodefense/Bacteriology Laboratories if within the 5 boroughs of NYC, please call (212) 447-1091