

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

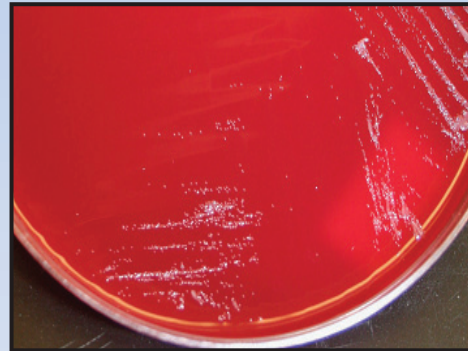
- May be misidentified as: *Moraxella* spp., *Micrococcus* spp., *Corynebacterium* spp., "slow growing" *Staphylococcus* spp., *Oligella ureolytica*, *Bordetella bronchiseptica*, *Haemophilus* spp., *Pasteurella* spp. by automated systems
- Biosafety Level 2/3 agent (once *Brucella* is suspected, work 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

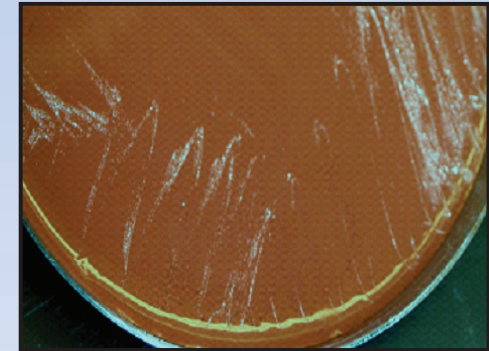
- Whole blood: 5-10 ml blood in EDTA, and/or Inoculated blood culture bottle
- Liver or spleen biopsy, or abscess
- Lymph node or bone marrow aspirate (1 ml)
- Synovial fluid, CSF (≥ 1 ml)

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.

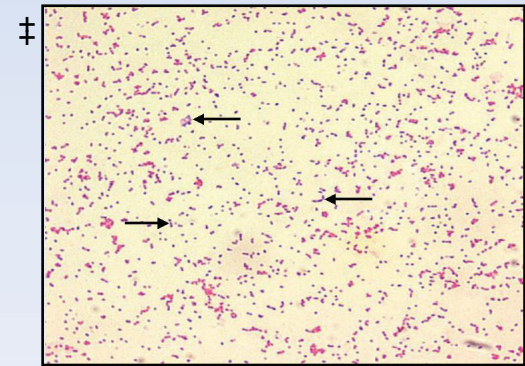
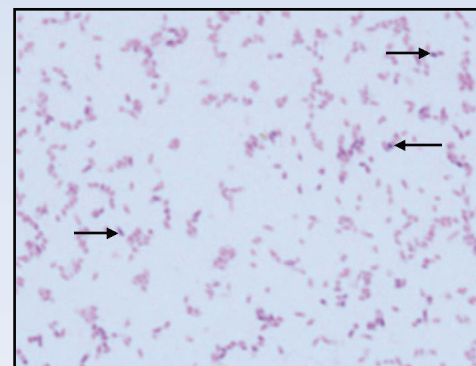
Growth on BA at 48 h



Growth on CA at 48 h



Gram stain



As indicated by the arrows, some gram-negative coccobacilli may retain crystal violet stain

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**

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

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

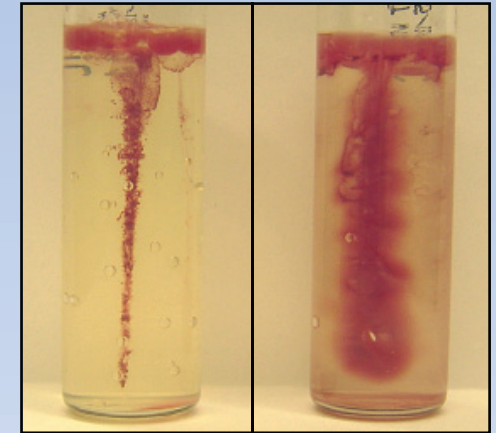
Urease



Negative

Positive

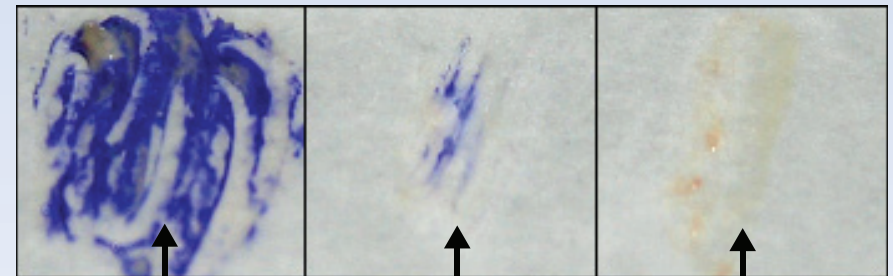
Motility



Non-Motile

Motile

Oxidase



Positive

Weak Positive

Negative