

BRUCELLA SPECIES LEVEL A LABORATORY GUIDELINES

Safety

Brucella species are highly infectious! Once this organism is suspected on the basis of clinical and/or laboratory information, do not perform additional testing except within an approved biosafety cabinet and wearing appropriate personal protective equipment!

1. Biosafety level 2 practices for specimen processing.
2. Biosafety level 3 practices for all activities involving manipulations of cultures.

Colony Characteristics

1. Fastidious, usually not visible at 24 hours.
2. Grows slowly on most standard laboratory media (e.g. sheep blood, chocolate and trypticase soy agars). Pinpoint, smooth, entire translucent, non-hemolytic at 48h.



Brucella species on sheep blood agar (48 hours)

3. Some strains grow on MacConkey agar.
4. Grows on Martin-Lewis and Thayer-Martin media.

Microscopic Characteristics

1. Poorly staining, **small gram-negative coccobacillus** (0.5-0.7 x 0.6-1.5 um), seen mostly as single cells and appearing like “fine sand”. Bipolar staining is not a distinctive feature. Although a small gram-negative coccobacillus, cells are typically larger than those of *Francisella tularensis*.

Key Characteristics

1. **Growth:** Growth will occur between 20 and 40 C. Grows slowly, but optimally at 35-37 C. CO₂ enhances growth of some biovars of *B. abortus*.
2. **Gram stain:** Gram-negative coccobacillus.
3. **Catalase:** Positive.
4. **Motility:** Non-motile.
5. **Oxidase:** Positive (*B. canis* is variable).
6. **Urease:** Positive. Strong, typically <2h, some within 5 minutes.



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7. X and V factors: Neither is required.

If a biosafety cabinet is available for testing, oxidase and urease testing may be very useful in differentiating *Brucella* species from the phenotypically similar *F. tularensis*, *Yersinia pestis*, *Haemophilus*, *Bordetella*, *Acinetobacter*, *Pasteurella*, *Moraxella*, and *Oligella* (*Moraxella urethralis*).

Isolates with the above characteristics should be reported to the patient's physician forwarded to the South Dakota State Public Health Laboratory for additional testing.
South Dakota State Public Health Laboratory 615 E 4th St. Pierre, SD 57501.

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