

SENTINEL LEVEL CLINICAL LABORATORY GUIDELINES
FOR
SUSPECTED AGENTS OF BIOTERRORISM
AND
EMERGING INFECTIOUS DISEASES

Brucella species

American Society for Microbiology (ASM)

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For latest revision, see web site below:

<https://www.asm.org/Articles/Policy/Laboratory-Response-Network-LRN-Sentinel-Level-C>

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PREANALYTICAL CONSIDERATIONS

I. PRINCIPLE

A. Introduction

Brucella is a fastidious, aerobic, small, gram-negative coccobacillus that is neither motile nor spore-forming (11, 19). *Brucella* is considered an “Overlap select agent” because it not only has the potential to pose a threat to public health and safety, but it also poses a threat to animal health and animal products.

This procedure describes the steps to rule out, recognize, and presumptively identify this organism in clinical specimens in Sentinel Clinical Laboratories. Such laboratories are defined as those certified to perform high complexity testing under the Clinical Laboratory Improvement Amendments of 1988 (CLIA) by the Centers for Medicare and Medicaid Services for the applicable Microbiology specialty. Laboratory in-house testing includes Gram stains, and at least one of the following: lower respiratory tract, wound or blood cultures.

Sentinel Clinical Laboratories are not required to register with the Select Agent Program to conduct diagnostic testing for Select Agents, both Tier I and non-Tier 1. Testing for Select Agents may be performed by laboratories as long as the laboratory follows the policies listed in the reporting section of this document when a Select Agent cannot be ruled out. Consult with your designated LRN Reference Laboratory or refer to the CDC Division of Select Agents and Toxins website at <http://www.selectagents.gov> for questions.

NOTE: To identify *Brucella* to the genus level some biochemical testing is needed, however, automated systems and manual multi-test kit identifications have no place in the identification of the organism, due to its minimal reactivity, creation of aerosols in performance of the testing, the danger of misidentification due to its close relation to other organisms that are positive for only a few tests, and the easy, rapid method to presumptively identify the organism without use of system identifications. Clinically, rapid identification to the genus level is adequate to initiate therapy, and the type of *Brucella* species involved does not alter the therapy.

B. Geographic distribution

There are between 80 and 120 cases of *Brucella* infection in humans each year in the United States, with the highest reported incidence from California (4). Efforts to reduce the number of infections have focused on vaccination of cattle herds. In 2008, the USDA reported that all 50 states were *Brucella* Class Free, although sporadic cases have altered the status of some states since then. *Brucella* continues to be present in ferel swine, bison and the elk population of this country.

Infections are seen in essentially two patient populations. The first is individuals who

work with animals, which have not been vaccinated against brucellosis. This patient population includes farmers, veterinarians, and slaughterhouse workers. *B. abortus* (cattle) and *B. suis* (pigs) are the agents most likely to cause infections in this group of individuals. The dangers of acquiring brucellosis among hunters represents another risk factor, they can acquire the disease by direct contact with or aerosolization from infected animal tissues.

Brucellosis is secondarily seen in individuals who ingest unpasteurized dairy products contaminated with *Brucella*. This is most likely to occur in individuals who travel to or migrate from rural areas of Latin American and the Middle East, where disease is endemic in dairy animals, particularly in goats and camels. *B. melitensis* is the most common agent seen in this patient population.

Laboratory workers can also acquire the disease from direct exposure to cultures of the organism (9, 15, 17). A case of nosocomial transmission to hospital staff from a patient who was infected at the time of delivery has recently been reported (13).

C. Diseases and Clinical Presentation

Brucellosis is a zoonotic infection, with four species being recognized as causing infection in humans: *Brucella abortus* (cattle), *Brucella melitensis* (goats, sheep, and camels), *Brucella suis* (pigs), and *Brucella canis* (dogs). Case reports in humans of *B. pinnipediae* and *B. cetaceae*, normally found in marine species, have rarely been reported (14). A new species, *Brucella inopinata* that caused a breast implant infection, has been described recently (6). Before the development of molecular techniques, species identification was difficult and, in most cases, was not performed. Species were usually differentiated by their natural host reservoir (14). *B. melitensis* is thought to be the most virulent and causes the most severe and acute cases of brucellosis. *B. melitensis* is also the most prevalent worldwide.

The disease has been known by several terms, including Malta fever, undulant fever, Rock of Gibraltar fever, and Bang's disease. Brucellosis is named after David Bruce, a British army medical doctor, who isolated *Brucella melitensis* from the spleen of a dead British soldier on the island of Malta in 1887. Following the institution of measures to prohibit the consumption of goat milk, the number of cases of brucellosis declined. Alice Evans, an American scientist who did landmark work on pathogenic bacteria in dairy products, was central in gaining acceptance of the pasteurization process to prevent brucellosis.

In 1954, *Brucella suis* became the first biological agent to be weaponized by the United States in the days of its offensive biological warfare program. The infective dose for these organisms is very low if acquired via the inhalation route, which makes them a potentially effective bioterrorism agent and also makes them a hazard in the clinical microbiology laboratory.

Brucella can cause both acute and chronic infections. The symptoms of brucellosis are non-specific and systemic, with intermittent fever, sweats, headache, anorexia, fatigue, weakness, malaise, back pain, and weight loss being frequent. Gastrointestinal

symptoms, present in 50% of patients, include abdominal pain, constipation, diarrhea, and vomiting. The chronic form of the disease can mimic miliary tuberculosis. Chronic untreated brucellosis can lead to abscesses in the liver, spleen, heart valves, brain, or bone; osteoarticular complications; and, in rare cases, death. The organism is often included in the differential diagnosis of fevers of unknown origin. The average incubation period for brucellosis is 2 to 10 weeks but ranges from a few days to 6 months. It has a mortality of 5% in untreated individuals, usually from endocarditis.

D. CDC CASE DEFINITION

The CDC case definition of brucellosis is “an illness characterized by acute or insidious onset of fever and one or more of the following: night sweats, arthralgia, headache, fatigue, anorexia, myalgia, weight loss, arthritis/spondylitis, meningitis, or focal organ involvement (endocarditis, orchitis/epididymitis, hepatomegaly, splenomegaly).” (<http://www.bt.cdc.gov/Agent/Brucellosis/CaseDef.asp>). A definitive diagnosis requires that bacteria be cultured from clinical specimens.

E. PRESUMPTIVE DIAGNOSIS

A presumptive diagnosis can be made by demonstrating high or rising titers of specific antibodies in the serum, however false positive results can occur in some assays due to cross-reacting antibodies with other gram negative bacilli (3). Results of EIA tests must be confirmed by a reference method such as *Brucella* microagglutination test (BMAT), which is quantitative and provides evidence of rising antibody titers when paired sera are tested.

II. SAFETY CONSIDERATIONS

- A. *Brucella* is an infectious pathogen that requires the use of biological safety level BSL-3 or BSL-2 with BSL-3 precautions (18) for all manipulations of specimens and cultures. Do not process nonclinical (environmental or animal specimens) in hospital laboratories. Restrict processing to human clinical specimens only. Veterinary laboratories are equipped to handle animal specimens. Nonclinical specimens should be directed to the designated LRN Reference Laboratory.
- B. All patient specimens should be handled as BSL-2, while wearing gloves and gowns and working in a biosafety cabinet (BSC). Subcultures should be performed in a Class II BSC. Plates should be taped shut, and incubated in 5 to 10% CO₂. All further testing should be performed only in the BSC while wearing gloves to protect from infections through the skin.
- C. Decontamination of laboratory surfaces is easily accomplished using a fresh solution of 10% bleach. Plates and specimens should be destroyed as directed by the LRN reference laboratory when the identification is confirmed.

III. MATERIALS

A. Media

1. One of the following blood culture systems should be used for body fluids, bone marrow aspirates and blood specimens.
 - a. Standard liquid blood culturing system with manual or instrument detection (1, 20, 21).
 - b. Castañeda (2) biphasic system with both agar and liquid in the bottle, such as PML biphasic (PML Microbiologicals, Inc., Wilsonville, OR)
 - c. Lysis-centrifugation system of ISOLATOR (Wampole Laboratories, Cranbury, NJ) has been shown to be more sensitive than the Castañeda system to detect the organism when culture for *Brucella* is specifically requested (8), but less sensitive than automated liquid blood cultures (21). Lysis-centrifugation has been shown to be less sensitive than broth-based systems for pediatric specimens (21).
2. Media used for subculturing of positive blood culture bottles and inoculation of pellet from lysis-centrifugation system.
 - a. BAP
 - b. CHOC
 - c. MAC or EMB
3. Reagents
 - a. Appropriate disinfectant such as 10% bleach
 - b. Gram stain reagents

NOTE: Refer to General Introduction and Recommendations section for a listing of biochemical test and associated procedures.

- c. Catalase (3% hydrogen peroxide)
 - d. Oxidase (0.5 tetramethyl-p-phenylenediamine)
 - e. Urea agar (Christensen's) or rapid urea disks (7) (Remel, Inc; Key Scientific; or Hardy Diagnostics)
 - f. Culture of *Staphylococcus aureus* ATCC 25923 for satellite test.
4. Equipment and supplies
 - a. Biosafety cabinet
 - b. PPE (gloves, solid front gown)
 - c. 35-37°C incubator (5-10% CO₂)
 - d. Light microscope with 100x objective and 10x eyepiece
 - e. Microscope slides and cover slips
 - f. Pipettes, inoculating loops and swabs
 - g. Blood culture instrument (optional)

IV. QUALITY CONTROL

Perform quality control of media and reagents according to package inserts, most recent CLSI document M22, and CLIA standards, using positive and negative controls. Do not use *Brucella* spp. as a control organism, due to its infectious nature. Examine culture plates for contamination, poor hemolysis, cracks, and drying. Confirm the ability of

CHOC to support growth of fastidious organisms. Quality control of biochemical tests using a positive and negative reacting organism should generally be performed on each lot of reagent. For frequency of quality control, refer to manufacturer guidelines and state and federal regulations. Refer to the biochemical test section for procedures and quality control organisms for each test.

It is desirable for Sentinel laboratories to prescribe to a proficiency program designed to test the competency of Sentinel Laboratories in detection of agents of bioterrorism. Should the laboratory identify a select agent, the laboratory is required to fill out and submit Form 4b within 90 days of receipt of the sample (<http://www.selectagents.gov>).

V. SPECIMEN COLLECTION

A. Collection and Transport of Clinical Specimens for Laboratory Rule-Out Testing	
Bone marrow or whole blood	<ul style="list-style-type: none"> • Considered the best specimen for culture but can be limited in chronic cases. • Collect directly into an appropriate blood culture bottle • Transport bottles at room temperature as soon as possible to obtain the diagnosis
Joint or abdominal fluid	<ul style="list-style-type: none"> • Collect directly into an appropriate blood culture bottle • Transport bottles at room temperature as soon as possible to obtain the diagnosis
Spleen, liver abscesses	<ul style="list-style-type: none"> • Tissue pieces (at least the size of a pea) should be collected and kept moist • Transport in sterile container at room temperature within 1 hour of collection
Serum	<ul style="list-style-type: none"> • Collect at least 1 ml without anticoagulant for serologic diagnosis^a • Store at 4°C until testing is performed. • Acute specimen is collected as soon as possible after onset of disease • Convalescent-phase should be collected >14 days after the acute specimen.

^a Tests include the titrated Rose Bengal test, microagglutination test, microtiter-adapted Coombs test, and immunocapture-agglutination test, all of which are more sensitive than EIA tests, which vary in their sensitivity and specificity (3,10).

B. Rejection of specimens

1. Use established laboratory criteria for rejection of cultures
2. Environmental or non-clinical samples are not processed by Sentinel laboratories; contact your designated LRN Reference Laboratory state public health laboratory directly.

ANALYTICAL CONSIDERATIONS

VI. SPECIMEN PROCESSING

NOTE: *Brucella* can be spread from infected specimens. See safety precautions above.

- A. Blood and bone marrow
 1. Aseptically inoculate liquid blood culture bottles with maximum amount of blood or body fluid per manufacturers' instructions. Incubate at 35°C.
 2. Alternatively, follow the manufacturer's instructions for the lysis-centrifugation method and inoculate pellet to BAP, CHOC and MAC. Incubate plates at 35°C in a humidified incubator with 5 to 10% CO₂

- B. For tissues, inoculate BAP, CHOC and MAC and incubate at 35°C in a humidified incubator with 5 to 10% CO₂. Humidity may be maintained by placing a pan of water in the bottom of the incubator or by wrapping the plates with gas permeable tape. MAC need not be incubated in CO₂.

VII. INCUBATION AND EXAMINATION OF CULTURES

NOTE: Isolation of *Brucella* is often delayed compared to other bloodstream pathogens, with peak isolation occurring at 3 to 4 days compared to 6 to 36 h for most other pathogens. Although incubation time of 21 days with weekly or terminal blind subculture are advocated, careful studies in *Brucella*-endemic areas using automated culture systems suggest that a maximal incubation time of 10 days is sufficient for reliable recovery of this organism (1). Terminal subcultures at 7 days have been reported to increase yield (20).

- A. Blood culture bottles
 1. Incubate non-automated broth blood cultures for 21 days, with direct observations for turbidity daily and blind subculturing every 7 days, followed by terminal subculturing of negative blood cultures.
 2. Incubate automated systems for 10 days and perform terminal subcultures at 7 days to increase yield (20).
 3. Daily observation for growth in the bottles is either automated or visual, depending on the system.
 4. For blind subculturing, inoculate to CHOC and BAP and incubate plates at 35°C in a humidified incubator with 5 to 10% CO₂.
 5. For positive broth cultures, inoculate to BAP, CHOC and MAC. Place a dot or streak of *Staphylococcus aureus* ATCC 25923 culture on the BAP in the first quadrant of the plate. Incubate plates at 35°C in a humidified incubator with 5 to 10% CO₂ until growth appears.

- B. Plate culture incubation times:
 1. Recommended incubation of BAP and CHOC is at 35°C in a humidified incubator with 5 to 10% CO₂

- i. 7 days for tissue specimens
 - ii. A minimum of 3 days for blind blood culture subculture plates, but incubation can be extended up to 7 days.
2. MAC need only be incubated for 3 days at 35°C in ambient air or 5 to 10% CO₂.
3. All plates either from direct inoculation of specimens or from subculture of broths should be examined daily for growth of tiny colonies.

VIII. CULTURE IDENTIFICATION

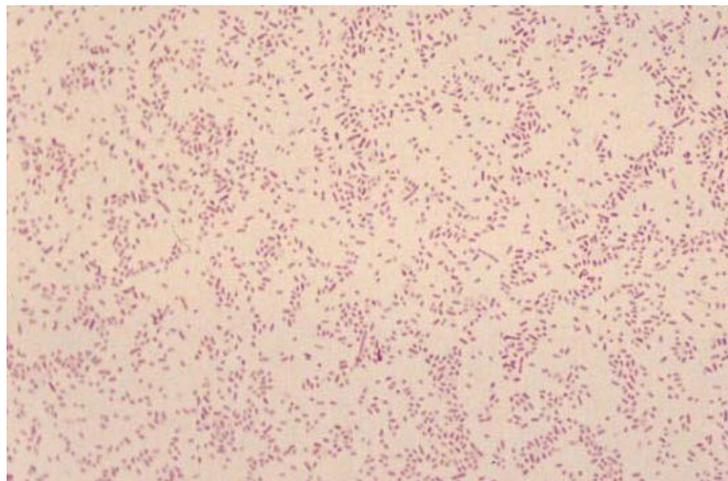
If your laboratory uses Mass Spectrometry (MALDI-TOF or bioMerieux) for bacterial identification and if the manufacturer provides your facility with an alternate tube extraction method, it is recommended that the resulting extract be filtered using a 0.2 μ (or less) filter. This additional step is recommended to reduce the risk of laboratory contamination with viable bacteria and spores.

Using automated systems, including Mass Spectrometry (MALDI-TOF, bioMerieux) technology may result in exposure to dangerous pathogens, and could result in erroneous identification, Ex: *Bacillus anthracis* misidentified as *B. cereus*; *Yersinia pestis* misidentified as *Y. pseudotuberculosis*.

The rule out or referral of *Brucella* is accomplished by utilizing the flow diagram and the following tests.

- A. Gram stain suspicious colonies from agar plates and positive blood culture bottles in a BSC. *Brucellae* are tiny (0.4 by 0.8 μm), gram-negative coccobacilli that stain faintly. See photograph below.

NOTE: *Brucella* has been responsible for many laboratory-acquired infections (9, 15, 17). If *Brucella* is suspected or the Gram stain shows a tiny, gram-negative coccobacillus, avoid aerosols and perform all subcultures in a Class II BSC (18). Plates should be taped shut, and all further testing should be performed only in the BSC, using Biosafety level III practices (18).



B. *Brucella* grows on both BAP and CHOC but not on MAC. Colonies are pinpoint at 24 h and are easily visible as white, non-hemolytic colonies at 48 h.



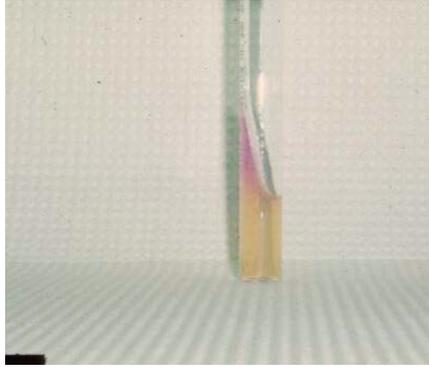
Courtesy Larry Stauffer, Oregon State Public Health Laboratories, Image #1902

NOTE: If a gram negative rod is isolated in a Sentinel Laboratory and it does not grow on MacConkey agar, it should not be processed for identification by a multi-test kit or automated system.

- C. Perform the following biochemical tests in a BSC, if the above criteria are met.
1. Oxidase-should be positive
 2. Catalase-should be positive

WARNING: If characteristic Gram stain is seen with growth on blood agar of oxidase-positive and catalase-positive colonies with no growth on MAC, it is likely to be *Brucella* (19). Further identification should not be attempted with commercial automated or kit identification systems, because of the danger of aerosol production due to preparing high concentrations of organisms. In addition, the identification by these systems can produce false results due to biochemically related organisms in the database.

3. Urea – See section on biochemical tests for procedure. Urea agar is easily available and does not require user quality control with positive and negative reacting organisms when purchased commercially.
 - a) Inoculate broth or slant
 1. Observe for color change to pink at 15 min, 2 h and up to 72 h.
 2. Reactions of small numbers of strains are delayed up to 72 h on Christensen's agar



b) Disk method

- 1) Inoculate saline with heavy suspension in BSC while wearing gloves.
- 2) Incubate aerobically at 35°C and observe for development of an intense magenta to bright pink in 15 min to 24 hr as seen in the right tube below.



Negative

Positive

- D. *Haemophilus* can be confused with *Brucella*; however *Haemophilus* do not grow on BAP. When in doubt, differentiate between these two genera by performing a satellite test. Inoculate a blood agar plate, followed by cross-streaking or spotting with *Staphylococcus aureus* ATCC 25923. After 24-48 h of incubation in 5% CO₂, *Haemophilus* demonstrate satellite growth around the *S. aureus*, while *Brucella* growth is present on the BAP but is not limited to the area around the staphylococcus.
- E. Other organisms that can be confused with *Brucella* species because they are urease positive include *Oligella ureolytica* (usually found only in the urine), *Psychrobacter phenylpyruvicus*, *Psychrobacter immobilis*, and *Bordetella bronchiseptica* (motile) (19). The Gram stain of *Brucella* is unique in that the organisms are small, stain poorly and not clustered (See Table 1). Colonies are odorless, non-pigmented, non-hemolytic, and non-mucoid on BAP, which further differentiates them from other genera.

F. Presumptive identification of *Brucella* species

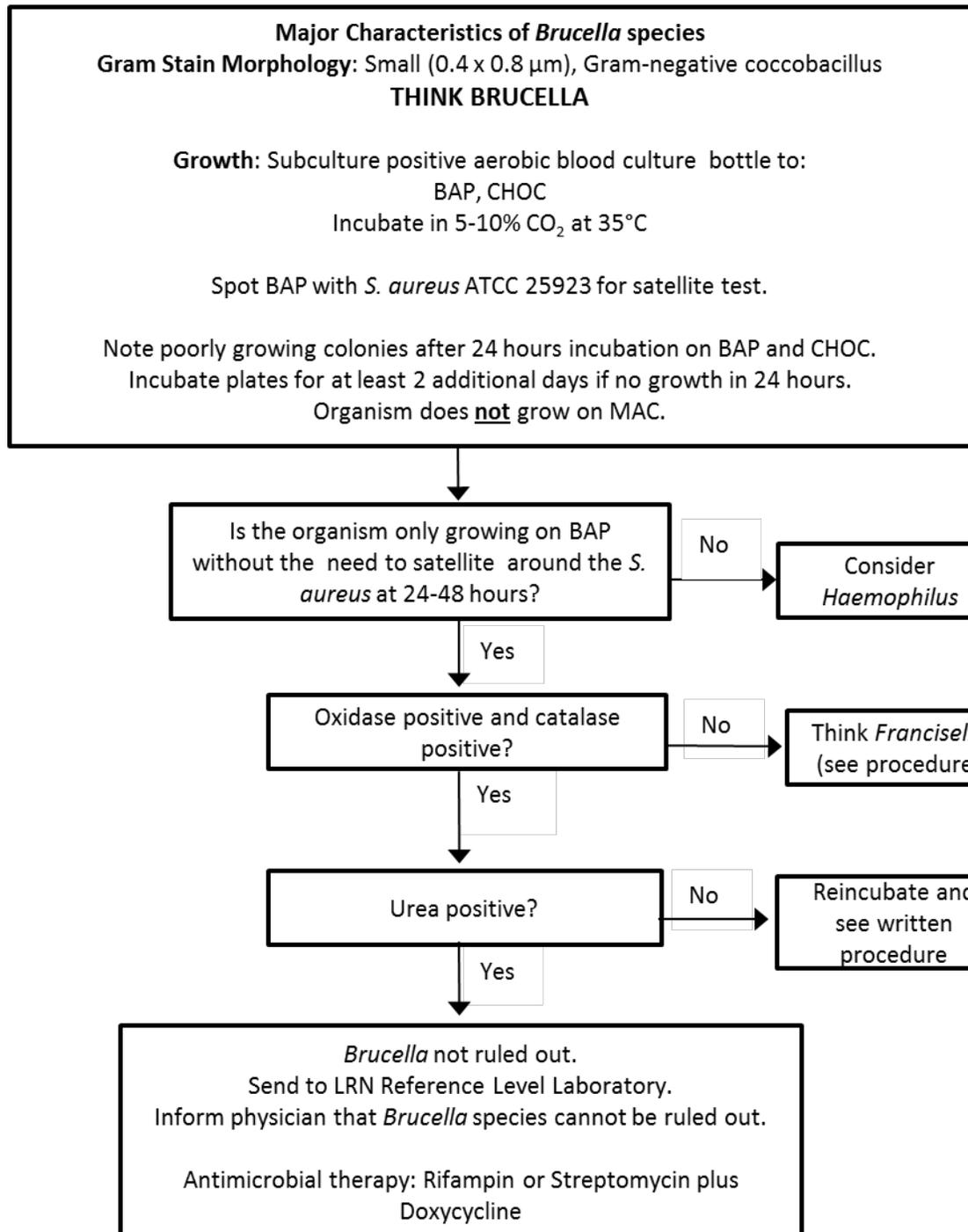
NOTE: Confirmatory identification is made by an LRN Reference Level Laboratory; refer

to <http://www.bt.cdc.gov/lrn/biological.asp>

1. *Brucella* species will grow only in aerobic blood culture bottles after 2-4 days and on subculture after 48 h of incubation on CHOC and BAP.
2. The organism typically does not grow on MAC and will not show the typical gram negative rod colony morphology on MAC within 48 hours. This observation will allow it to be separated from other gram-negative coccobacilli. Pinpoint colonies have been infrequently observed on MAC after extended incubation times (7 days).
3. The colonies typically show “dust-like” growth after overnight incubation, and a minimum of 48 h is necessary to get sufficient growth for further identification. Colonies on BAP have no distinguishing features.
 - i. *Brucella* will appear as raised, convex white colonies with an entire edge and shiny surface. Colonies are 0.5 to 1 mm in diameter after 48 h incubation.
 - ii. Colonies are not mucoid, are non-pigmented, are non-hemolytic and have no distinct odor.
4. Gram stain shows tiny gram-negative coccobacilli that stain faintly. They should not be confused with poorly staining gram positive cocci, because of their tiny size.
5. Colonies are positive for oxidase, catalase and urease.
6. See flowchart below for summary of major characteristics for presumptive diagnosis of *Brucella*.

Brucella Identification Flowchart

SAFETY: As soon as *Brucella* is suspected, perform ALL further work in a Class II Biosafety Cabinet (BSL3)



Note: Biochemical test procedures and quality control instructions can be found at the end of the *General Recommendation and Biochemical Testing Procedures* document.

POST ANALYTICAL CONSIDERATIONS

IX. REPORTING, NOTIFICATION, AND TRANSFER

A. *Brucella* is suspected and cannot be ruled out if the isolate fulfills the following characteristics:

- Faintly staining tiny gram negative coccobacillus
- Growing on BAP without the addition of a staphylococcus streak, as non-hemolytic, non-pigmented, odorless, white colonies.
- Not growing on MAC in 48 h
- Oxidase-positive, catalase-positive, and urease-positive.

Note: Urease production can be delayed but and is not required to make the presumptive diagnosis. If all the other characteristics are present the organism should be suspected.

B. Notifications and submission of cultures if *Brucella* cannot be ruled out by above characteristics.

1. Generate a report to the physician that *Brucella* species cannot be ruled out.
2. Do not attempt full identification and susceptibility testing in the Sentinel Clinical Laboratory.
3. Immediately notify your designated LRN Reference Laboratory, which will provide the referring laboratory with guidance and recommendations for retaining the specimen or isolate and submission for confirmative identification.
4. Preserve original specimens pursuant to a potential criminal investigation and transfer to your designated LRN Reference Laboratory in accordance with state and local requirements. In particular, the appropriate material, including blood culture bottles, tubes and plates, and actual clinical specimens (aspirates, biopsies, sputum specimens) should be documented, and either submitted to the LRN Reference Laboratory or saved until the Reference Laboratory confirms the identification.
5. Do not ship specimens or cultures to LRN Reference Laboratories without prior arrangements.
6. Notify other public health authorities (e.g. state public health department epidemiologist/health officer) as required by local and state communicable disease reporting requirements. The state public health laboratory/state public health department will notify law enforcement officials (state and federal), such as local FBI agents, as appropriate.
7. Within the hospital setting, immediately notify the infection preventionists and/or infectious disease service so that the patient can be treated appropriately, infectious precautions can be taken, and a further investigation of the patient's history can be made.
8. Consult with the LRN Reference Level Lab about additional clinical specimens that may be submitted for testing
9. Initiate documentation, showing the specimen identification control, notification

and transfer to the designated LRN Reference Laboratory, and documentation of all plates and tube cultures, which will need to be destroyed or transferred once identification has been completed.

- C. Sentinel Laboratories should consult with the designated LRN Reference Laboratory prior to or concurrent with testing, if *Brucella* species is requested by the physician or a bioterrorist event is suspected. Obtain guidance from the state public health laboratory as appropriate (e.g., requests from local law enforcement or other local government officials). FBI and state public health laboratory/state public health department will coordinate the transfer of isolates/specimens to a higher-level LRN laboratory as appropriate.
- D. If *Brucella* species is ruled out, proceed with efforts to identify using established procedures.
- E. If other cases are suspected or there is a laboratory exposure, collect samples to submit to the designated LRN Reference Laboratory for serological testing.

X. SUMMARY/SPECIAL CONSIDERATIONS

A. Antimicrobial susceptibility

1. Antimicrobial susceptibility testing of *Brucella* is neither needed nor appropriate for Sentinel Laboratories to perform. CLSI lists “susceptible only” breakpoints meaning that resistance rarely occurs and should be confirmed in a reference laboratory equipped to test agents of bioterrorism in a BSL level 3 facility (5, 12).
2. Tetracyclines (doxycycline) are the most active drugs and should be used in combination with streptomycin (or gentamicin or rifampin, if streptomycin is unavailable) to prevent relapse.
3. Many antimicrobial agents are active against *Brucella* species; however, clinical efficacy does not always correlate with *in vitro* susceptibility. The treatment recommended by the World Health Organization for acute brucellosis in adults is rifampin 600 to 900 mg and doxycycline 100 mg twice daily for a minimum of six weeks. Rifampin with or without a combination of cotrimoxazole has proved safe to treat brucellosis during pregnancy.
4. Post-exposure prophylaxis for laboratory workers includes either doxycycline 100 mg twice daily plus rifampin 600 mg once daily for 3 weeks. Trimethoprim-sulfamethoxazole (160mg/800 mg) is an alternative for patients that cannot take doxycycline.
5. Azithromycin in combination with gentamicin has been reported to be successful in patients that cannot tolerate tetracyclines or rifampin (16).

B. Select Agent reporting and compliance

1. Reporting of all identified Select Agents is still required, even though Sentinel laboratories are not required to register under the Select Agent Rule.
2. The laboratory must complete Form 2 within one week (7 days) following notification of the confirmed identification. For further guidance and access to the necessary forms, consult with your designated LRN Reference Laboratory or refer to the CDC Division of Select Agents and Toxins website at www.selectagents.gov
3. Reporting all identified Select Agents is required by completing Form 4 A within 7 days of confirmed identification. If the isolate is from a Proficiency test sample, Form 4 B is to be completed within 90 days of receipt of the sample.
4. Your designated LRN Reference Laboratory will advise you with completion of required forms (e.g., Forms 2, 3, and 4). Always refer to www.selectagents.gov for the latest guidance and versions of these forms.

D. Destruction

1. Once the identification of the isolate is confirmed, the Sentinel Laboratory Select Agent regulations require that the residual specimen and cultures of the isolate be destroyed or transferred to an approved Select Agent entity within 7 days of confirmed identification. Your designated LRN Reference Laboratory must advise you on destruction or transfer of isolates.
2. Generally, all plates and clinical material that contain the organism should be autoclaved, incinerated on-site or submitted to the designated LRN Reference Laboratory for disposal.
3. Alternatively, contaminated items should be soaked in 10% bleach or 10% formalin for 24 h.

C. Packing and shipping

1. Refer to the ASM Packing and Shipping Sentinel Guidelines.
2. All materials sent to your designated LRN Reference Laboratory must be shipped in compliance with IATA and DOT regulations

X. LIMITATIONS

1. *B. abortus*, *B. melitensis*, and *B. suis* are all oxidase-positive organisms. *B. canis* isolates may be oxidase-variable.
2. Using the Christensen's tube test, urea hydrolysis can be observed in as early as 15 min incubation with *B. suis* strains and within 1 day of incubation with most strains of *B. abortus*, and *B. melitensis*. Some *B. melitensis* strains take even longer to be positive.
3. Do not attempt to identify tiny gram-negative rods that do not grow on MAC using a commercial identification system because of their lack of accuracy and danger of aerosols.
4. Because there are a number of urea-positive, fastidious tiny gram-negative rods, the definitive identification of *Brucella* is generally performed by a reference or state health department laboratory. However, isolation of an organism with the characteristics of *Brucella* listed in this procedure from a blood or normally sterile

site is most likely *Brucella*.

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Table 1. **Differentiation of *Brucella* from other urea-positive, oxidase-positive gram-negative coccobacilli^a**

	<i>Brucella</i> spp.	<i>Psychrobacter</i> <i>immobilis</i>	<i>Paracoccus yeei</i>	<i>Psychrobacter</i> <i>phenylpyruvicus</i>	<i>Methylobac</i> <i>terium</i> spp.	<i>Oligella</i> <i>ureolytica</i> ^a	<i>Bordetella</i> <i>bronchiseptica</i> , <i>B. hinzii</i> , <i>Cupriavidus</i> <i>pauculus</i>	<i>Haemophilus</i> spp. ^b
Gram stain morphology	tiny ccb, stains faintly	ccb, rods	cocci in packets	ccb, rods, retains crystal violet	Vacuolated rods	tiny ccb	rods	Tiny ccb
Catalase	+	+	+	+	+	+	+	v
Oxidase	+	+	+	+	+	+	+	v
Urea	+	v	+	+	v	+	+	v
Motility	-	-	-	-	-	+,delayed	+	-
BAP distinctions	-	Prefers 20°C, Odor of roses	Mucoid	-	Pink, mucoid	-	-	No growth
MAC-48 h	-	-	-	-	-	-	+	-

^a Reactions extracted from reference 19; NA, not applicable; v, variable; ccb, coccobacilli. *O. ureolytica* is primarily a uropathogen.

^b Only grows on CHOC; or on BAP associated with staphylococcus colony.