Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases

Brucellosis-causing *Brucella* species (BBS)

Revised August 2025

ASM Subject Matter Experts

Carrie Anglewicz, MS
Michigan Department of Health and Human Services
AnglewiczC@michigan.gov

Kurt H. Jerke, Ph.D., D(ABMM), MS, LTC
United States Military Academy
kurt.jerke@westpoint.edu

ASM Sentinel Level Laboratory Protocol Working Group

Laura Filkins, Ph.D., D(ABMM)
University of Texas Southwestern Medical Center
Laura.Filkins@utsouthwestern.edu

Maria E. Navas M.D.
VA Northeast Ohio Healthcare System, Cleveland
Maria.navas@va.gov

Logan Patterson, Ph.D.
University of North Carolina
Logan.Patterson@unchealth.unc.edu

Frances Valencia-Shelton, Ph.D., D(ABMM), SM(ASCP)CM
Baptist Health Medical Center, Jacksonville FL
Frances.valencia-shelton@bmcjax.com

Elizabeth L. Palavecino, M.D.
Wake Forest University School of Medicine
Epalave@wakehealth.edu

Rosemary C. She, M.D.
City of Hope
rshe@coh.org

Paige M.K. Larkin, Ph.D., D(ABMM), M(ASCP)
American Society for Microbiology
PLarkin@asmusa.org

APHL Advisory Committee

Patricia Blevins, MPH
Centers for Disease Control and Prevention
qty9@cdc.gov

Erin Bowles
Wisconsin State Laboratory of Hygiene
bowlesej@mail.slh.wisc.edu

Chris N. Mangal, MPH
Association of Public Health Laboratories
Chris.Mangal@aphl.org

Amanda Moore, BS
South Carolina Department of Health and Environmental Control
mooreal@dhec.sc.gov

Amity L. Roberts, Ph.D., D(ABMM)
Hartford Hospital Ancillary Microbiology Laboratory
amity.roberts@hhchealth.org

Maureen Sullivan, MPH
Association of Public Health Laboratories
maureen.sullivan@aphl.org

Tanya Swanson
Massachusetts State Public Health Laboratory
tanya.swanson@mass.gov

CDC Subject Matter Experts

Rebekah Tiller, LCDR, MPH
Centers for Disease Control and Prevention
Eto3@cdc.gov

Elke Saile, Ph.D.
Centers for Disease Control and Prevention
Csx2@cdc.gov

Summary of Major Changes in this Update

- Background updated with recent taxonomic changes to genus *Brucella* and further defined clinically significant *Brucella* species.
- Emphasis for sentinel level laboratories is shifted from performing presumptive identification to rule-out of previous select agent *Brucella* spp, now referred to as Brucellosis-causing *Brucella* species (BBS).
- Case definition of brucellosis updated to reflect the 2025 CSTE Case Definition (1).
- Additional specifications added to specimen collection information, including discontinuation of Isolator lysis-centrifugation system.
- Recognition of gram-variable staining of *Brucella* spp.
- As of December 17th, 2024, *Brucella* was removed as a select agent. Guidance updated on biosecurity and other public health requirements for rule-out BBS cases after removal of BBS as select agent.

Preanalytical Considerations

I. Principle

A. Introduction

The *Brucella* genus has historically been comprised of highly homogenous coccobacilli species which are fastidious, aerobic, small, gram-negative and neither motile nor spore forming (2, 3). In 2020, the *Ochrobactrum* genus, comprised of ~18 species, was reclassified into the *Brucella* genus which now comprises non-pathogenic, environmental bacteria and highly pathogenic, intracellular bacterial species as one taxonomic group (4, 5). The naming and reporting of *Brucella* (*Ochrobactrum*) species may differ between laboratories due to nomenclature differences in rapid microbial identification systems and lack of specificity for these opportunistic pathogens. Prior to the taxonomic expansion of the *Brucella* genus, any isolate identified as *Brucella* species was considered a probable brucellosis causing *Brucella* species (BBS, including *B. melitensis, B. suis, B. abortus,* and *B. canis*), a potentially life-threatening infectious disease, with the organism considered a select agent. *Brucella* (*Ochrobactrum*) species, non-brucellosis causing *Brucella* species (nBBS), are generally opportunistic pathogens in immunocompromised individuals and are morphologically and phenotypically different from BBS. Clinical laboratories should handle all organisms identified as *Brucella* in a Class II biological safety cabinet until BBS are ruled-out. As of December 17th, 2024, *Brucella* was removed as a select agent. Deregulation of a select agent changes the biosecurity requirements (e.g., documentation, required forms, and destruction) but does not change the biosafety precautions necessary. BBS remains highly infectious and remains a reportable disease. Potential isolates should still undergo rule-out procedures in sentinel level clinical laboratories and be referred to the designated Laboratory Response Network (LRN) Reference Laboratory for definitive identification.

This procedure describes the steps to recognize, and rule-out potential brucellosis causing *Brucella* species isolated from 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.

Consult with the designated Laboratory Response Network Reference Laboratory or Centers for Disease Control and Prevention (CDC) Division of Regulatory Science and Compliance for questions.

B. Disease and Geographic Distribution

Brucellosis is a zoonotic infection, with four species recognized as causing infection in humans: *B. abortus* (cattle), *B. melitensis* (goats, sheep, and camels), *B. suis* (pigs), and *B. canis* (dogs) (6). *Brucella* species have been discovered in many wildlife reservoirs including swine, marine mammals, rodents, bats, foxes, voles, amphibians and bats (7). Human cases caused by *B. pinnipedialis* and *B. ceti* (marine species), have rarely been reported (8, 9). There has been one reported human case of brucellosis caused by an Amphibian-type *Brucella* species which is genetically related to *B. inopinata*, recovered from an infected breast implant (10, 11). *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 (3, 6, 12).

There are about 100 to 200 cases of *Brucella* infection reported in humans each year in the United States, with the highest reported incidence from California and Texas (3, 13). Efforts to reduce the number of infections have focused on vaccination of cattle herds. *Brucella* continues to be present in the swine, bison and elk population of the United States (14, 15).

Populations most at risk for *Brucella* infection are those who have contact with infected animals (e.g., farmers, veterinarians, hunters and slaughterhouse workers) or animal products (e.g., consuming unpasteurized milk products), or those living in or visiting endemic areas (e.g., rural areas of Latin America and the Middle East) (3).

Laboratory workers can also acquire the disease from direct exposure to cultures of the organism (6, 16-20). A case of nosocomial transmission to hospital colleagues from a pregnant patient with brucellosis at the time of delivery has been reported (21).

C. Clinical Presentation and Council of State and Territorial Epidemiologists Case Definition

BBS can cause both acute and chronic infections. The CDC describes brucellosis as "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)" (22). Gastrointestinal symptoms, present in about half of patients, include abdominal pain, constipation, diarrhea, and vomiting (23). 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 (3). 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 can range from a few days to 6 months. It has a mortality of 5% in untreated individuals, usually due to endocarditis (24).

The CSTE case definition for clinically compatible cases includes the following laboratory criteria for diagnosis:

1. **Definitive Diagnosis**

   A definitive diagnosis requires that bacteria be identified from clinical specimens using a method specific for BBS (i.e., PCR assay with documented specificity for BBS and/or biochemical tests and/or whole genome sequencing of *Brucella* isolate). Alternatively, a fourfold rise in *Brucella* antibody titer between acute and convalescent serum collected at least two weeks apart is confirmatory.

2. **Presumptive Diagnosis**

   A presumptive diagnosis can be made by demonstrating serum antibody titers of at least 1:160 by standard tube agglutination test (SAT) or *Brucella* microagglutination test (BMAT) collected after onset of symptoms. Detection of *Brucella* IgG antibodies by ELISA in a sample collected at least 2 weeks after onset of symptoms is considered to be supportive laboratory evidence of brucellosis infection. Results of enzyme immunoassay must be confirmed by a reference method (BMAT or SAT) to accurately interpret antibody titers according to this definition. False-positive results can occur in some assays due to cross-reacting antibodies with other gram-negative bacilli, especially when testing IgM antibodies and when using some non-reference methods (25, 26).

II. Safety Considerations

A. BSL-3 practices are recommended for all manipulations of specimens and cultures suspected of containing BBS (27). When available, specimens and cultures suspected of containing BBS should be handled in a BSL-3 laboratory space. When not available, BSL-2 laboratory facilities with BSL-3 practices may be used. Appropriate practices include wearing gloves and gowns and performing all sample and culture handling in a biosafety cabinet (BSC). All plates should be taped closed and incubated in 5 to 10% CO_2. Additional protection(s) may be applied according to individual laboratory risk assessments, especially for pregnant people and individuals with other immunocompromising conditions (27).

B. Do not process nonclinical or nonhuman (environmental or animal) specimens in hospital laboratories. Nonclinical or nonhuman specimens should be discussed with and, if approved, directed to the designated LRN Reference Laboratory.

C. The laboratory should have a safety risk assessment plan in place to handle additional specimens from patients with known current BBS infection (e.g., additional blood samples received) to minimize potential laboratory personnel exposures.

D. Plates and specimens should be destroyed as directed by the LRN reference laboratory when the identification is confirmed.

III. Materials

A. Media: Standard liquid blood culturing system with manual or instrumented detection (28-30), can be used for blood specimens. For laboratories not utilizing blood culture bottles for body fluids, heart infusion broth can be used for liquid culture of BBS.

NOTE: If the blood culture system and culture vials are FDA-cleared for use or the laboratory has validated testing as a laboratory developed test for culturing body fluids and/or bone marrow, the above reagents may also be used for testing these specimen types.

1. Media used for subculturing of positive blood culture bottles and inoculation of other primary specimens (e.g., CSF, wounds, body fluids).
    a. Blood Agar Plates (BAP)
    b. Chocolate Agar (CHOC)
    c. MacConkey (MAC)

B. Reagents

1. Appropriate disinfectant, such as 10% bleach
2. Gram stain reagents
3. Catalase (3% hydrogen peroxide)
4. Oxidase (0.5 tetramethyl-p-phenylenediamine)
5. Urea agar (Christensen's) or rapid urea disks (31) (Remel, Inc; Key Scientific; or Hardy Diagnostics)
6. Culture of *Staphylococcus aureus* ATCC 25923 for satellite test

C. Equipment and supplies

1. Class II Type A2 Biosafety cabinet
2. PPE (gloves, solid front gown)
3. 35-37°C incubator (5-10% CO_2)
4. Light microscope with 100x objective and 10x eyepiece
5. Microscope slides and cover slips
6. Pipettes, inoculating loops and swabs
7. Blood culture instrument (optional)

IV. Quality Control

Perform quality control of media and reagents according to manufacturer's instructions for use (IFU) and CLIA standards, using positive and negative controls. Do not use BBS 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. For frequency of quality control, refer to manufacturer guidelines and state and federal regulations.

V. Specimen Collection

A. Collection and Transport of Clinical Specimens for Laboratory Rule-Out Testing	
Bone marrow or whole blood	• Blood cultures and bone marrow are the primary specimen types recommended for diagnosis of acute brucellosis but are less sensitive in chronic cases. Culture positivity rates are typically reported to be higher from bone marrow than blood, but other specimen types, such as from focally infected sites, may be helpful to optimize recovery (32-34). • Blood: Collect directly into an appropriate blood culture bottle(s). Preferably, collect the maximum amount of blood acceptable per the blood culture bottle manufacturer. Transport bottles at room temperature as soon as possible. • Bone marrow: Collect directly into an appropriate blood culture bottle or other sterile collection devices (such as a sodium polyanethol sulfonate (SPS)), as directed by the receiving laboratory. Transport at room temperature as soon as possible.
Joint or abdominal fluid	• Collect in a sterile tube, container, or syringe (transport to the laboratory with the needle removed and capped), or if directed by the receiving laboratory, these fluids may be collected directly into an appropriate blood culture bottle. Transport bottles at room temperature as soon as possible following collection.
Spleen, liver abscesses	• Tissue pieces (at least the size of a pea) should be collected and kept moist. • Abscess fluid should be collected in a sterile syringe (transport to the laboratory with the needle removed and capped) or sterile sealed cup. • Transport at room temperature as soon as possible after collection.
Other specimens for culture	• Other sample types may be appropriate to culture depending on the patient's clinical presentation, such as wounds, cerebrospinal fluid (CSF), and abscess. • Collect specimens according to local laboratory protocols. Specimen collection using swab devices is strongly discouraged. • Transport to the laboratory at room temperature as soon as possible following collection.
Serum	• Collect at least 1 ml without anticoagulant for serologic diagnosis.* • Store at 4°C until testing is performed. CDC states that serum may be stored at refrigerated temperature (2-8°C) for up to 14 days post-collection and frozen (-20°C or lower) for up to 2 months. • Acute specimen is collected as soon as possible after onset of disease. • Convalescent-phase should be collected >14 days after the acute specimen.

* Tests include the Brucella microagglutination test (BMAT), serum agglutination test (SAT) and ELISA IgM and IgG, all of which vary in their sensitivity and specificity (35).

B. Specimen Rejection

1. Use established laboratory rejection criteria for specimens received for culture.
2. Environmental or nonclinical samples are not processed by Sentinel laboratories; contact your designated LRN Reference Laboratory directly.

Analytical Considerations

VI. Specimen Processing

NOTE: BBS is infectious from primary specimens. See safety precautions above.

A. Blood and bone marrow

1. Blood is inoculated into a blood culture bottle by the phlebotomist following standard precautions and standard protocols. Laboratory colleagues should receive the bottle following standard laboratory protocol and immediately load them into the automated instrument.
2. For manual methods such as lysis centrifugation, following processing, inoculate the pellet to BAP, CHOC and MAC. Incubate plates at 35°C in a humidified incubator with 5 to 10% CO_2.

B. For all other specimens (e.g. tissue, body fluid), inoculate BAP, CHOC and MAC and incubate at 35°C in a humidified incubator with 5 to 10% CO_2. 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 may not be incubated in CO_2, but this should be confirmed with the manufacturer instructions for use (IFU).

VII. Incubation and Examination of Cultures

NOTE: Recovery of BBS 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 times of 21 days with weekly or terminal blind subculture are advocated, careful studies in *Brucella*-endemic areas using automated culture systems suggest that an incubation time of 10 days is sufficient for reliable recovery of this organism (36). Additionally, in some studies, blind or terminal subculture at 7 or 10 days increased the total detection of blood specimens positive for BBS. Therefore, laboratories should consider subculturing negative blood culture vials when brucellosis is suspected (28).

A. Blood culture bottles

1. Incubate blood culture vials in automated systems for 10 days. To increase yield, perform blind or terminal subcultures at 7 or 10 days (28).
2. For blind subculturing, inoculate to CHOC and BAP and incubate plates at 35 - 37°C in a humidified incubator with 5 to 10% CO_2.
3. 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% CO_2 until growth appears.

B. Plate culture incubation times

1. Recommended incubation of BAP and CHOC is at 35 - 37°C in a humidified incubator with 5 to 10% CO_2
   a. Incubate plates for 7 days if inoculated directly with the patient specimen.
   b. Incubate at least 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% CO_2.
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-Based Rule-Out

Sentinel laboratories should NOT intentionally use automated systems for identification of possible select agents or BBS but should use the established sentinel laboratory procedures instead. Using automated systems, including MALDI-TOF MS technology, may result in exposure to dangerous pathogens, and could result in erroneous identification (37), Ex: *Yersinia pestis* misidentified as *Y. pseudotuberculosis* or *Bacillus anthracis* misidentified as *B. cereus*. However, from a biosafety and biosecurity perspective, in addition to patient management, it is critical that the microbiologist is knowledgeable of these differences and takes the appropriate action when interpreting such a result. Laboratorians should also consider the possibility of isolate variability (such as atypical Gram stain reactions), and ensure an isolate is thoroughly ruled-out before attempting identification.

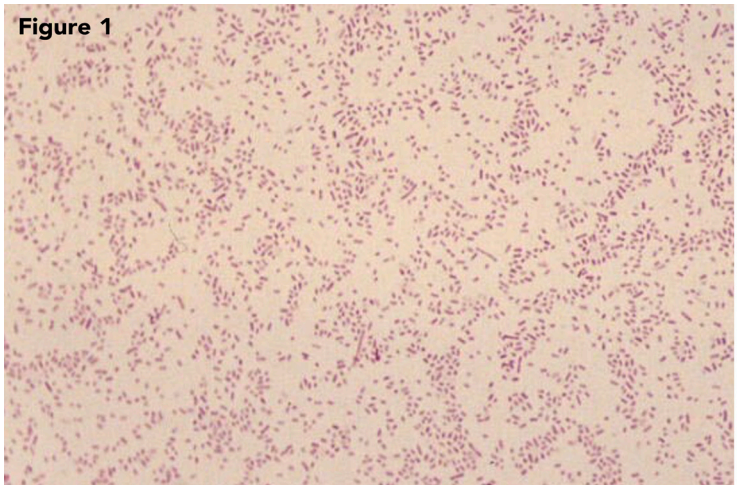
The rule-out or referral of BBS is further accomplished by utilizing the BBS Rule-Out Flow Chart and the following tests:

A. Gram stain

1. Gram stain suspicious colonies from agar plates and positive blood culture bottles in a BSC. To minimize potential exposure events, slides should be fixed before being removed from the BSC. Fixed slides may be removed from the BSC for staining. After staining is complete, slides may be read using a microscope on the open benchtop (38).
2. *Brucellae* are classically tiny (0.4 by 0.8 µm), gram-negative coccobacilli that stain faintly (Fig. 1), but they may stain gram-variable.

NOTE: BBS has been responsible for many laboratory-acquired infections (6, 16-20). Although they are gram-negative organisms, in some cases the cells retain crystal violet and appear gram-variable or gram-positive (6, 19). If BBS is suspected or the Gram stain shows a tiny coccobacillus, tape shut all plates, avoid aerosols and perform all subcultures and further rule-out testing in a Class II BSC following Biosafety level 3 practices (27).

Figure 1



B. Growth Morphology

1. BBS grows on both BAP and CHOC but not on MAC. [Note, pinpoint colonies have been infrequently observed on MAC after extended incubation times (7 days)].
2. The colonies typically show "dust-like" growth after overnight incubation, are pinpoint at 24 h, and are easily visible (0.5 to 1 mm in diameter) after 48 h.
3. On BAP, colonies are raised, convex white with an entire edge and shiny surface (Fig 2). Colonies are not mucoid, are non-pigmented, are non-hemolytic and have no distinct odor **(do not intentionally smell plates).**
4. BBS do not grow in anaerobic conditions, including anaerobic blood culture vials.

Figure 2. BBS colony morphology on BAP, 48 h incubation. Courtesy Larry Stauffer, Oregon State Public Health Laboratories, Image #1902.

C. Phenotypic Biochemical Reactions

Perform the following biochemical tests in a BSC, if the above criteria are met.

1. Oxidase reaction: BBS are positive
2. Catalase reaction: BBS are positive
3. Urease reaction: BBS are urease positive between 5 min – 72 h. Reactions of a small number of strains are delayed up to 72 h on Christensen's agar.

D. Additional information to help differentiate BBS from organisms with similar characteristics

Identification using an automated system or MALDI-TOF-MS should not be attempted for any isolate for which BBS, or any select agent, is suspected. However, given the recent classification of *Ochrobactrum* into the *Brucella* genus, laboratories may increasingly encounter results from automated systems indicating the identification of a possible BBS species or their phylogenetic relative, nBBS. If *Brucella* or a related organism is detected by any method, laboratories should consider the possibility of a BBS and complete the rule-out and/or referral process before reporting the identification or performing susceptibility testing.

The following characteristics are common to *Brucella* (*Ochrobactrum*) species and can be used to distinguish BBS and nBBS. Any one of the following is sufficient to rule-out BBS.

- Rapid colony growth on MAC agar (>0.5mm after 24h) (note, if high clinical suspicion for brucellosis and borderline colony growth rate on any medium, consider using additional tests to confirm rule-out)
- Mucoid colony morphology
- Positive motility (tube-based method recommended for safe handling).

Haemophilus species can be confused with BBS; however, many *Haemophilus* species do not grow on BAP, and those species that do grow often form beta-hemolytic colonies. For example, *H. influenzae and H. aegypticus* only grow on CHOC or on BAP associated with *Staphylococcus* colony while *H. haemolyticus* and *H. parahaemolyticus* grow and produce hemolysis on horse or sheep blood agar. The satellite test can be used to help differentiate these two genera. Inoculate a BAP, followed by cross-streaking or spotting with *Staphylococcus aureus* ATCC 25923. After 24-48 h of incubation in 5% CO_2, *Haemophilus* demonstrates satellite growth around the *S. aureus*, while BBS growth is present on the BAP but is not limited to the area around the *Staphylococcus*.

Other organisms that can be confused with *Brucella* species because they are urease positive include *Oligella ureolytica* (usually found in the urine; delayed positive motility), *Psychrobacter phenylpyruvicus (non motile), Psychrobacter immobilis (non motile),* and *Bordetella bronchiseptica* (positive motility) (39).

The Gram stain of BBS from isolated colonies is distinct in that the organisms are small, stain poorly (gram-negative or gram-variable) and not clustered (though often are seen in aggregates from positive blood culture).

Colonies of BBS are odorless **(do not intentionally smell plates)**, non-pigmented, non-hemolytic, and non-mucoid on BAP, which further differentiates them from other genera. See Table 1 for a phenotypic comparison of these species and BBS.

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

	(BBS) *Brucella* spp.[a]	*Psychrobacter immobilis*	*Paracoccus yeei*	*Psychrobacter phenylpyruvicus*	*Methyloba cterium* spp.	*Oligella ureolytica*[b]	*Bordetella bronchiseptica, B. hinzii, Cupriavidus pauculus*	*Haemophilus* spp.	*Brucella (Ochrobactrum) anthropi* and *intermedium (nBBS)*
Gram stain morphology	tiny ccb, stains faintly	ccb, rods	cocci in packets	ccb, rods, retains crystal violet	Vacuolated rods	tiny ccb	rods	tiny ccb	ccb, rods
Catalase	+	+	+	+	+	+	+	v	+
Oxidase	+	+	+	+	+	+	+	v	+
Urea	+	v	+	+	v	+	+	v	+
Motility	-	-	-	-	-	+,delayed	+	-	-
BAP distinctions	-	Prefers 20°C, Odor of roses	Mucoid	-	Pink, mucoid	-	-	v[c]	Mucoid (v)
MAC-48 h	-	-	-	-	-	-	+	-	+

Abbreviations; v, variable; ccb, coccobacilli. BAP, blood agar plate, MAC, MacConkey agar plate

[a] *Brucella melitensis, B. abortus, B. suis* and *B. canis*

[b] Reactions extracted from reference 19; *O. ureolytica* is primarily a uropathogen.

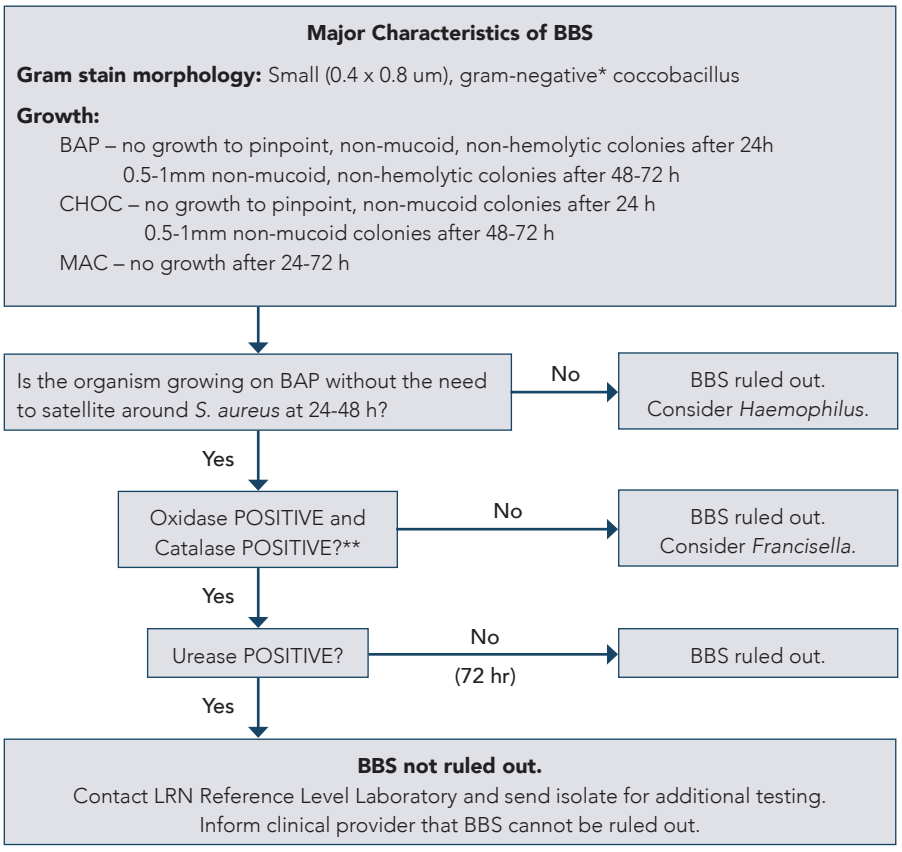
[c] Species-dependent. Examples: *H. influenzae, H. aegypticus* only grow on CHOC or on BAP associated with *Staphylococcus* colony; *H. haemolyticus, H. parahaemolyticus* grow and produce hemolysis on horse or sheep blood agar.

Note: While motility is not part of the BBS Rule-Out Flow Chart, if *Brucella* (*Ochrobactrum*) species, *Oligella ureolytica,* or *Bordetella bronchiseptica* is suspected, sentinel laboratories may perform tube-based motility to supplement the routine BBS rule-out procedure.

See flowchart below for summary of major characteristics for rule-out of BBS.

BBS Rule-Out Flowchart

SAFETY: As soon as BBS is suspected, perform ALL further work in a Class II Biosafety Cabinet (BSL-3 or BSL-2 with additional precautions if BSL3 is not available)



* BBS may stain gram-variable.

** *B. canis* can be oxidase-variable.

Post Analytical Considerations

IX. Reporting, Notification, and Transfer

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

- Faintly staining tiny gram-negative coccobacillus (caution, see note above about potential for variable Gram stain reaction).
- Growth on BAP without the need to satellite around *S. aureus*, as non-hemolytic, non-pigmented, odorless, white colonies.
- Minimal, faint or no growth on MAC within 72 h.
- Oxidase-positive, catalase-positive, and urease-positive. Urease production can be delayed.

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

1. Generate a report to the physician that BBS cannot be ruled out.
2. Do not attempt full identification and/or susceptibility testing in the Sentinel Clinical Laboratory.
3. Immediately notify the 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. Do not ship specimens or cultures to LRN Reference Laboratories without prior arrangements.
5. Notify other public health authorities (e.g. state public health department epidemiologist/health officer) as required by local and state communicable disease reporting requirements.
6. Within the hospital setting, immediately notify the infection preventionists and/or infectious disease service so that the patient can be treated appropriately, infectious precautions taken, and a further investigation of the patient's history made.
7. Consult with the LRN Reference Lab about additional clinical specimens that may be submitted for testing.

C. Sentinel Laboratories should consult with the designated LRN Reference Laboratory prior to or concurrent with testing, if BBS is strongly suspected by the physician.

D. If BBS is ruled out, proceed with efforts to identify using established procedures.

E. If other cases are suspected or there is a laboratory exposure, consult with the state public health laboratory and epidemiologist to determine exposure risk classifications of exposed individuals. After the risk assessment, collect samples, as indicated, to submit to the designated LRN Reference Laboratory for serological testing.

IX. Summary/Special Considerations

A. Antimicrobial susceptibility

1. Antimicrobial susceptibility testing of BBS is neither needed nor appropriate for Sentinel Laboratories to perform. Susceptibility may be performed, when appropriate, in public health laboratories equipped to handle agents that require higher biosafety levels. Contact the appropriate public health laboratories for more information.
2. Consult the CDC website for the most up-to-date information on treatment for *Brucella* infection or postexposure prophylaxis (40). Treatment varies by age, pregnancy status, and other clinical considerations such as whether the strain causing infection is *B. abortus* RB51 which is resistant to rifampin.

B. Reporting and compliance

1. Reporting of BBS is required as it is a nationally notifiable disease.

E. Destruction

1. Follow the laboratory policies for specimen destruction of highly pathogenic organisms. These policies may include autoclaving, incinerating on-site, soaking contaminated items in 10% bleach or 10% formalin for 24 h, or submitting to the designated LRN Reference Laboratory for disposal, if unable to sufficiently destroy the organism.

C. Packing and shipping

1. Refer to the ASM Packing and Shipping Sentinel Guidelines (41).
2. All materials sent to the designated LRN Reference Laboratory must be shipped in compliance with IATA and DOT regulations

X. Limitations

1. BBS (*B. abortus, B. melitensis, B. suis, B. canis*) 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 5 min incubation with *B. suis* and *B. canis* 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 coccobacilli or 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 urease-positive, fastidious tiny gram-negative rods, the definitive identification of BBS is generally performed by a reference or state health department laboratory. However, isolation of an organism with the characteristics of BBS listed in this procedure from a blood or normally sterile site is most likely BBS.

References

1. CDC. 2025. Brucellosis (Brucella spp.) 2025 Case Definition https://ndc.services.cdc.gov/case-definitions/brucellosis/. Accessed January 2025.
2. Mantur BG, Amarnath SK, Shinde RS. 2007. Review of clinical and laboratory features of human brucellosis. Indian J Med Microbiol 25:188-202.
3. Pappas G, Akritidis N, Bosilkovski M, Tsianos E. 2005. Brucellosis. N Engl J Med 352:2325-36.
4. Hördt A, López MG, Meier-Kolthoff JP, Schleuning M, Weinhold LM, Tindall BJ, Gronow S, Kyrpides NC, Woyke T, Göker M. 2020. Analysis of 1,000+ Type-Strain Genomes Substantially Improves Taxonomic Classification of Alphaproteobacteria. Front Microbiol 11:468.
5. Munson E, Carroll KC. 2023. Update on Accepted Novel Bacterial Isolates Derived from Human Clinical Specimens and Taxonomic Revisions Published in 2020 and 2021. J Clin Microbiol 61:e0028222.
6. Yagupsky P, Baron EJ. 2005. Laboratory exposures to brucellae and implications for bioterrorism. Emerg Infect Dis 11:1180-5.
7. Whatmore AM, Foster JT. 2021. Emerging diversity and ongoing expansion of the genus Brucella. Infect Genet Evol 92:104865.
8. McDonald WL, Jamaludin R, Mackereth G, Hansen M, Humphrey S, Short P, Taylor T, Swingler J, Dawson CE, Whatmore AM, Stubberfield E, Perrett LL, Simmons G. 2006. Characterization of a Brucella sp. strain as a marine-mammal type despite isolation from a patient with spinal osteomyelitis in New Zealand. J Clin

Microbiol 44:4363-70.

9. Brew SD, Perrett LL, Stack JA, MacMillan AP, Staunton NJ. 1999. Human exposure to Brucella recovered from a sea mammal. Vet Rec 144:483.
10. De BK, Stauffer L, Koylass MS, Sharp SE, Gee JE, Helsel LO, Steigerwalt AG, Vega R, Clark TA, Daneshvar MI, Wilkins PP, Whatmore AM. 2008. Novel Brucella strain (BO1) associated with a prosthetic breast implant infection. J Clin Microbiol 46:43-9.
11. Rouzic N, Desmier L, Cariou ME, Gay E, Foster JT, Williamson CHD, Schmitt F, Le Henaff M, Le Coz A, Lorléac'h A, Lavigne JP, O'Callaghan D, Keriel A. 2021. First Case of Brucellosis Caused by an Amphibian-type Brucella. Clin Infect Dis 72:e404-e407.
12. Laine CG, Scott HM, Arenas-Gamboa AM. 2022. Human brucellosis: Widespread information deficiency hinders an understanding of global disease frequency. PLoS Negl Trop Dis 16:e0010404.
13. CDC. 2024. Nationally Notifiable Infectious Diseases and Conditions, United States: Weekly Tables, on Office of Public Health Data, Surveillance, and Technology. https://wonder.cdc.gov/nndss/static/2024/49/2024-49-table340.html. Accessed December 17.
14. National Academies of Sciences E, and Medicine. Revisting Brucellosis in the Greater Yellowstone Area. https://www.nationalacademies.org/our-work/revisiting-brucellosis-in-the-greater-yellowstone-area. Accessed 04/02/2025.
15. USDA. 2010. Brucellosis Class Free States and Certified Brucellosis-Free Herds; Revisions to Testing and Certification Requirements. https://www.federalregister.gov/documents/2010/12/27/2010-32371/brucellosis-class-free-states-and-certified-brucellosis-free-herds-revisions-to-testing-and. Accessed
16. Fiori PL, Mastrandrea S, Rappelli P, Cappuccinelli P. 2000. Brucella abortus infection acquired in microbiology laboratories. J Clin Microbiol 38:2005-6.
17. Sewell DL. 1995. Laboratory-associated infections and biosafety. Clin Microbiol Rev 8:389-405.
18. Staszkiewicz J, Lewis CM, Colville J, Zervos M, Band J. 1991. Outbreak of Brucella melitensis among microbiology laboratory workers in a community hospital. J Clin Microbiol 29:287-90.
19. Noviello S, Gallo R, Kelly M, Limberger RJ, DeAngelis K, Cain L, Wallace B, Dumas N. 2004. Laboratory-acquired brucellosis. Emerg Infect Dis 10:1848-50.
20. Memish ZA, Mah MW. 2001. Brucellosis in laboratory workers at a Saudi Arabian hospital. Am J Infect Control 29:48-52.
21. Mesner O, Riesenberg K, Biliar N, Borstein E, Bouhnik L, Peled N, Yagupsky P. 2007. The many faces of human-to-human transmission of brucellosis: congenital infection and outbreak of nosocomial disease related to an unrecognized clinical case. Clin Infect Dis 45:e135-40.
22. CDC. 2021. Brucellosis (Brucella spp.) 2010 Case Definition. https://ndc.services.cdc.gov/case-definitions/brucellosis-2010/. Accessed December 16.
23. Hassouneh L, Quadri S, Pichilingue-Reto P, Chaisavaneeyakorn S, Cutrell JB, Wetzel DM, Nijhawan AE. 2019. An Outbreak of Brucellosis: An Adult and Pediatric Case Series. Open Forum Infect Dis 6:ofz384.
24. Peery TM, Belter LF. 1960. Brucellosis and heart disease. II. Fatal brucellosis: a review of the literature and report of new cases. Am J Pathol 36:673-97.
25. Al Dahouk S, Tomaso H, Nöckler K, Neubauer H, Frangoulidis D. 2003. Laboratory-based diagnosis of brucellosis--a review of the literature. Part II: serological tests for brucellosis. Clin Lab 49:577-89.
26. Young EJ. 1991. Serologic diagnosis of human brucellosis: analysis of 214 cases by agglutination tests and review of the literature. Rev Infect Dis 13:359-72.
27. Anonymous. 2020. Biosafety in Microbiological and Biomedical Laboratories, 6th ed. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Institutes of Health.
28. Yagupsky P. 1999. Detection of Brucellae in blood cultures. J Clin Microbiol 37:3437-42.

29. Yagupsky P, Peled N, Press J, Abramson O, Abu-Rashid M. 1997. Comparison of BACTEC 9240 Peds Plus medium and isolator 1.5 microbial tube for detection of Brucella melitensis from blood cultures. J Clin Microbiol 35:1382-4.

30. Bannatyne RM, Jackson MC, Memish Z. 1997. Rapid diagnosis of Brucella bacteremia by using the BACTEC 9240 system. J Clin Microbiol 35:2673-4.

31. Ederer GM, Chu JH, Blazevic DJ. 1971. Rapid test for urease and phenylalanine deaminase production. Appl Microbiol 21:545.

32. Gotuzzo E, Carrillo C, Guerra J, Llosa L. 1986. An evaluation of diagnostic methods for brucellosis--the value of bone marrow culture. J Infect Dis 153:122-5.

33. Mantur BG, Mulimani MS, Bidari LH, Akki AS, Tikare NV. 2008. Bacteremia is as unpredictable as clinical manifestations in human brucellosis. Int J Infect Dis 12:303-7.

34. Yagupsky P, Morata P, Colmenero JD. 2019. Laboratory Diagnosis of Human Brucellosis. Clin Microbiol Rev 33.

35. Gómez MC, Nieto JA, Rosa C, Geijo P, Escribano MA, Muñoz A, López C. 2008. Evaluation of seven tests for diagnosis of human brucellosis in an area where the disease is endemic. Clin Vaccine Immunol 15:1031-3.

36. Baysallar M, Aydogan H, Kilic A, Kucukkaraaslan A, Senses Z, Doganci L. 2006. Evaluation of the BacT/ALERT and BACTEC 9240 automated blood culture systems for growth time of Brucella species in a Turkish tertiary hospital. Med Sci Monit 12:Br235-8.

37. Keller PM, Bruderer V, Müller F. 2016. Restricted Identification of Clinical Pathogens Categorized as Biothreats by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry. J Clin Microbiol 54:816.

38. Anonymous. 08/25/2020 2017. Federal Select Agent Program: Regulatory Interpretation: Gram Stained Slides Containing Select Agents, on CDC and USDA. https://www.selectagents.gov/regulations/interpretations/stain.htm. Accessed

39. Weyant RS, C. W. Moss, R. E. Weaver, D. G. Hollis, J. G. Jordan, E. C. Cook, and M. I. Daneshvar. 1995. Identification of unusual pathogenic Gram-negative aerobic and facultatively anaerobic bacteria. Williams & Wilkins, Baltimore, MD.

40. CDC. 2024. Clinical Overview of Brucellosis. https://www.cdc.gov/brucellosis/hcp/clinical-overview/index.html. Accessed

41. ASM. 2021. Guideline: Packing and Shipping Infectious Substances. https://asm.org/Guideline/Packing-and-Shipping-Infectious-Substances. Accessed