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Brucella* and *Ochrobactrum **Taxonomic Updates for Laboratories**

Frequently Asked Questions (FAQ) for Clinical Laboratories

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Laboratory Practices Subcommittee

Background

In 2020, Hördt et al. proposed the reclassification of *Ochrobactrum* species to the genus *Brucella* based on recent gene-content analysis studies (1). The past taxonomic distinction between *Ochrobactrum* and *Brucella* was not based on 16S rRNA gene analysis, nor had phylogenetic analyses been conducted. Hördt et al. proposed including all *Ochrobactrum* species in the genus *Brucella* (1). This revised *Brucella* nomenclature follows the rules for nomenclature denoted by the International Code of Nomenclature of Prokaryotes (2008 Revision) for the 18 *Ochrobactrum* species (2, 3). As of the time of writing this FAQ, the original *Ochrobactrum* species and reclassified *Brucella* species names are therefore both considered to be ‘validly published.’ We will refer to the impacted organisms as *Brucella* (*Ochrobactrum*) species in this document to distinguish them from the classic *Brucella* species. The classic *Brucella* species are divided into those that are select agents and those that are not.

Some microbial identification systems, including matrix assisted laser/desorption ionization time-of-flight (MALDI-ToF) mass spectrometry, nucleic acid detection methods and automated phenotypic methods, have adopted the alternate *Brucella* genus designation for *O. anthropi* (i.e., *Brucella anthropi*), *O. intermedium* (i.e., *Brucella intermedia*) and other *Ochrobactrum* species. Application of the genus name *Brucella* to these organisms is thus likely occurring in clinical laboratories.

There are several implications of adopting the name of *Brucella* for these organisms. If laboratories report these organisms simply as *Brucella* species without including additional report comments or education around the name update, clinicians who are unaware of the alternate name may inappropriately treat patients as having brucellosis, which implies disease due to classic *Brucella* species. Laboratory implications are no less significant since some species are regulated select agents while others are not. The species of *Brucella* which are select agents according to the United States Department of Health and Human Services (HHS) and the United States Department of Agriculture (USDA) are *B. melitensis*, *B. abortus** and *B. suis**. The other classic *Brucella* species (including but not limited to *B. canis*, *B. pinnipedialis* and attenuated/vaccine strains of select agent species) are not select agents; neither are the *Brucella* (*Ochrobactrum*) species. Furthermore, cultures of certain species of *Brucella* must be handled and shipped as category A infectious substances, according to International Air Transport Association (IATA) and the United States Department of Transportation (DOT) (4). Variability of select agent designation within the genus *Brucella* can lead to confusion in laboratory handling and reporting.

**Brucella abortus and Brucella suis are considered synonyms of Brucella melitensis but the clinical and clinical laboratory implications of this classification are unclear at this point.*

Brucella and *Ochrobactrum* Taxonomic Updates for Laboratories

On December 19, 2022, the Centers for Disease Control and Prevention (CDC) released a brief laboratory update on this subject through the Laboratory Outreach Communication System (LOCS) entitled, “Reclassification of *Ochrobactrum* species into the *Brucella* genus” (5). This update guides laboratories to handle all organisms identified as *Brucella* in a Class II biological safety cabinet, and it states that “all bacterial isolates presumptively identified as *Brucella* species should be referred to [the applicable] public health laboratory for additional testing.” Given the recent *Ochrobactrum* taxonomic reclassifications, laboratories need further guidance concerning distinction of select agent *Brucella* species from non-select agent *Brucella* (*Ochrobactrum*) species and reporting of these organisms with clarity.

Which organism names are impacted?

<i>Ochrobactrum</i> species	Reclassification
<i>Ochrobactrum anthropi</i> [#]	<i>Brucella anthropi</i> [#]
<i>Ochrobactrum ciceri</i>	<i>Brucella ciceri</i>
<i>Ochrobactrum cytisi</i>	<i>Brucella cytisi</i>
<i>Ochrobactrum daejeonense</i>	<i>Brucella daejeonensis</i>
<i>Ochrobactrum endophyticum</i>	<i>Brucella endophytica</i>
<i>Ochrobactrum gallinifaecis</i>	<i>Brucella gallinifaecis</i>
<i>Ochrobactrum grignonense</i>	<i>Brucella grignonensis</i>
<i>Ochrobactrum haematophilum</i>	<i>Brucella haematophila</i>
<i>Ochrobactrum intermedium</i> [#]	<i>Brucella intermedia</i> [#]
<i>Ochrobactrum lupini</i>	<i>Brucella lupini</i>
<i>Ochrobactrum oryzae</i>	<i>Brucella oryzae</i>
<i>Ochrobactrum pecoris</i>	<i>Brucella pecoris</i>
<i>Ochrobactrum pituitosum</i>	<i>Brucella pituitosa</i>
<i>Ochrobactrum pseudointermedium</i>	<i>Brucella pseudointermedia</i>
<i>Ochrobactrum pseudogrignonense</i>	<i>Brucella pseudogrignonensis</i>
<i>Ochrobactrum rhizosphaerae</i>	<i>Brucella rhizosphaerae</i>
<i>Ochrobactrum thiophenivorans</i>	<i>Brucella thiophenivorans</i>
<i>Ochrobactrum tritici</i>	<i>Brucella tritici</i>

[#] Denotes organisms most commonly isolated from human specimens.

What are the Potential Clinical Implications of Using the Alternate *Brucella* Names?

If reporting the alternate *Brucella* genus names, clinical laboratories must thoughtfully manage the application and reporting of these results to minimize misunderstanding and confusion by providers of patient care. Misinterpretation of *Brucella* (*Ochrobactrum*) species as agents of brucellosis can lead to an inaccurate disease diagnosis and the unintentional selection of inappropriate or suboptimal antimicrobial therapy, as well as possible administration of post-exposure prophylaxis (e.g., for specimens collected with high risk of aerosolization) and other infection prevention and control concerns.

Prior to this taxonomic revision, any isolate identified as *Brucella* species in the clinical laboratory was considered a probable agent of brucellosis, a potentially life-threatening infectious disease. Organisms classically linked with brucellosis in humans include *B. melitensis*, *B. abortus*^{*}, *B. suis*^{*} and *B. canis*, and rarely *B. ceti* and *B. pinnipedialis* (6). On the other hand, *Brucella* (*Ochrobactrum*) species often represent microbial colonization when recovered from clinical specimens. As opportunistic pathogens, *Brucella* (*Ochrobactrum*) species cause infections with distinct transmission patterns and clinical presentations, and treatment may require antimicrobial regimens different from those used to treat brucellosis. In addition, infection control considerations for *Brucella* (i.e., antimicrobial prophylaxis following laboratory exposure) are different than for *Brucella* (*Ochrobactrum*) species.

Differences in Antimicrobial Therapy and Management

- Brucellosis: Doxycycline and rifampin combination therapy is recommended for at least 6-8 weeks. Doxycycline and streptomycin are alternatively recommended. These antibiotic regimens are well established, resistance is uncommon and *in vitro* antimicrobial susceptibility testing (AST) is not required to guide therapy. AST should only be performed at select, qualified laboratories, with select agent approvals. Additionally, serological monitoring is recommended over 24 weeks after laboratory exposure to an agent of brucellosis.
- Infection due to *Brucella* (*Ochrobactrum*) species: Successful treatment has been described with use of imipenem, fluoroquinolones, trimethoprim-sulfamethoxazole or aminoglycosides. Multidrug resistance has been described (7). AST can be performed on isolates for which the select agent *Brucella* species have been ruled out.

Importantly, if AST is performed on *Brucella* (*Ochrobactrum*) species, after the select agents are ruled out, these organisms should not be tested as *Brucella* species. Rather, the Clinical and Laboratory Standards Institute (CLSI) methods and interpretations recommended for 'Other Non-Enterobacterales' may be applied (8).

^{*}*Brucella abortus* and *Brucella suis* are considered synonyms of *Brucella melitensis* but the clinical and clinical laboratory implications of this classification are unclear at this point.

How Can Laboratory Personnel Distinguish *Brucella* (*Ochrobactrum*) Species From Agents of Brucellosis, Including Select Agent *Brucella* Species?

Laboratories in the U.S. should carefully screen all specimens for Gram stain and culture growth characteristics consistent with the U.S. select agent *Brucella* species. The reclassification of *Ochrobactrum* species does not change the requirements for select agent rule out. Readers may refer to the Sentinel Level Clinical Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases for further guidance (9).

The following characteristics are common to *Brucella* (*Ochrobactrum*) species and can be used to quickly rule out select agent *Brucella* species and non-select agent causes of brucellosis:

- Rapid colony growth on MacConkey agar (>0.5 mm after 24 h).
- Mucoid colony morphology.
- Positive motility (tube-based method recommended for safe handling).

Automated systems, including mass spectrometry technology, can misidentify *Brucella* (*Ochrobactrum*) species as select agent *Brucella* species or misidentify select agent species as non-select agent species (10,11). Alternatively, no organism identification may be obtained, particularly if a specialized database that includes select agent *Brucella* species is not used; notably, these databases are not available in most clinical laboratories. Laboratories can use key morphologic characteristics and phenotypic tests to distinguish between *Brucella* (*Ochrobactrum*) and select agent *Brucella* species, as summarized in Table 1 and shown in Figures 1 and 2.

Table 1: Morphologic and Phenotypic Characteristics of *Brucella* (*Ochrobactrum*) Species Versus Select Agent *Brucella* Species

	<i>Brucella</i> (<i>Ochrobactrum</i>) species	Select agent <i>Brucella</i> species
Gram Stain Reaction and Cellular Morphology	Gram-negative coccobacilli to rods (1.0-1.5x2.0 µm, approximately the length of <i>Escherichia coli</i>)	Small, faintly staining Gram-negative coccobacilli (0.4x0.8 µm, approximately the diameter of staphylococci and smaller)
Colony Growth <i>Blood^a and Chocolate Agars</i>		
24 hr	>0.5 mm	(-) to pinpoint
48-72 hr	>0.5 mm	0.5-1 mm
<i>MacConkey Agar</i>		
24 hr	>0.5 mm ^b , non-lactose fermenting	(-)
48-72 hr	>0.5 mm	(-) ^c
Colony Morphology (Blood Agar) <i>Mucoid^d</i>	Creamy, smooth, shiny, non-hemolytic (+/v)	Smooth, white or translucent, convex, non-hemolytic (-)
Phenotypic Tests <i>Motility^d</i>	(+/v)	(-)
<i>Urease^d</i>	(+/v)	(+) ^e

^a Blood agar: Tryptic soy agar w/ 5% sheep's blood.

^b Delayed growth on MacConkey agar is observed in some isolates of *Brucella* (*Ochrobactrum*) species.

^c Pinpoint colony growth on MacConkey agar is only rarely observed within 72 hr for select agent *Brucella* species and is most often seen if cultures are incubated for >7 d.

^d +, positive phenotypic results; -, negative phenotypic results; v, variable phenotypic results; +/v most isolates cultured from clinical specimens yield positive results, but some yield negative results.

^e Urease production by the agents of brucellosis can be very rapid (15 min-24 hr). Testing should be performed in a certified class II biological safety cabinet.

Brucella and *Ochrobactrum* Taxonomic Updates for Laboratories

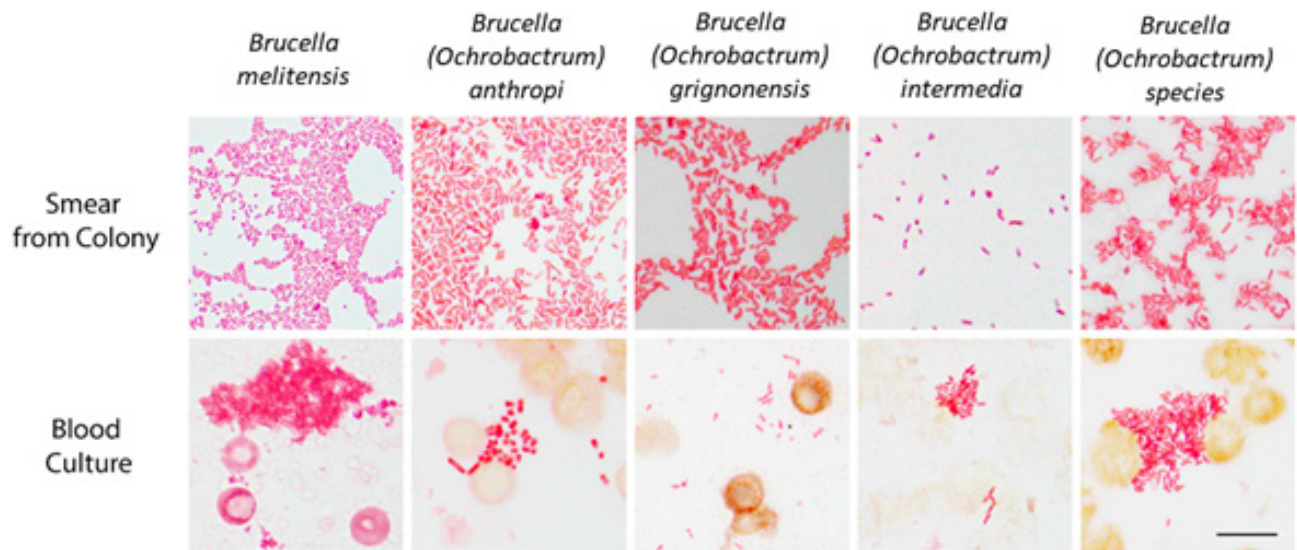


Figure 1. Gram stains of example *Brucella* and *Brucella (Ochrobactrum)* species made from colonies grown on agar plates (top row) and from positive blood culture broths (bottom row). Classical *Brucella* species (including select agents) such as *Brucella melitensis* are tiny, often faint-staining gram-negative coccobacilli, but are prone to resist decolorizing and may appear gram-positive, especially when stained of blood culture broths. The cells of *Brucella (Ochrobactrum)* species exhibit pleomorphy or form rod-shaped cells that are larger than those of select agent *Brucella* species. Scale bar, 10 μ m.

Brucella and *Ochrobactrum* Taxonomic Updates for Laboratories

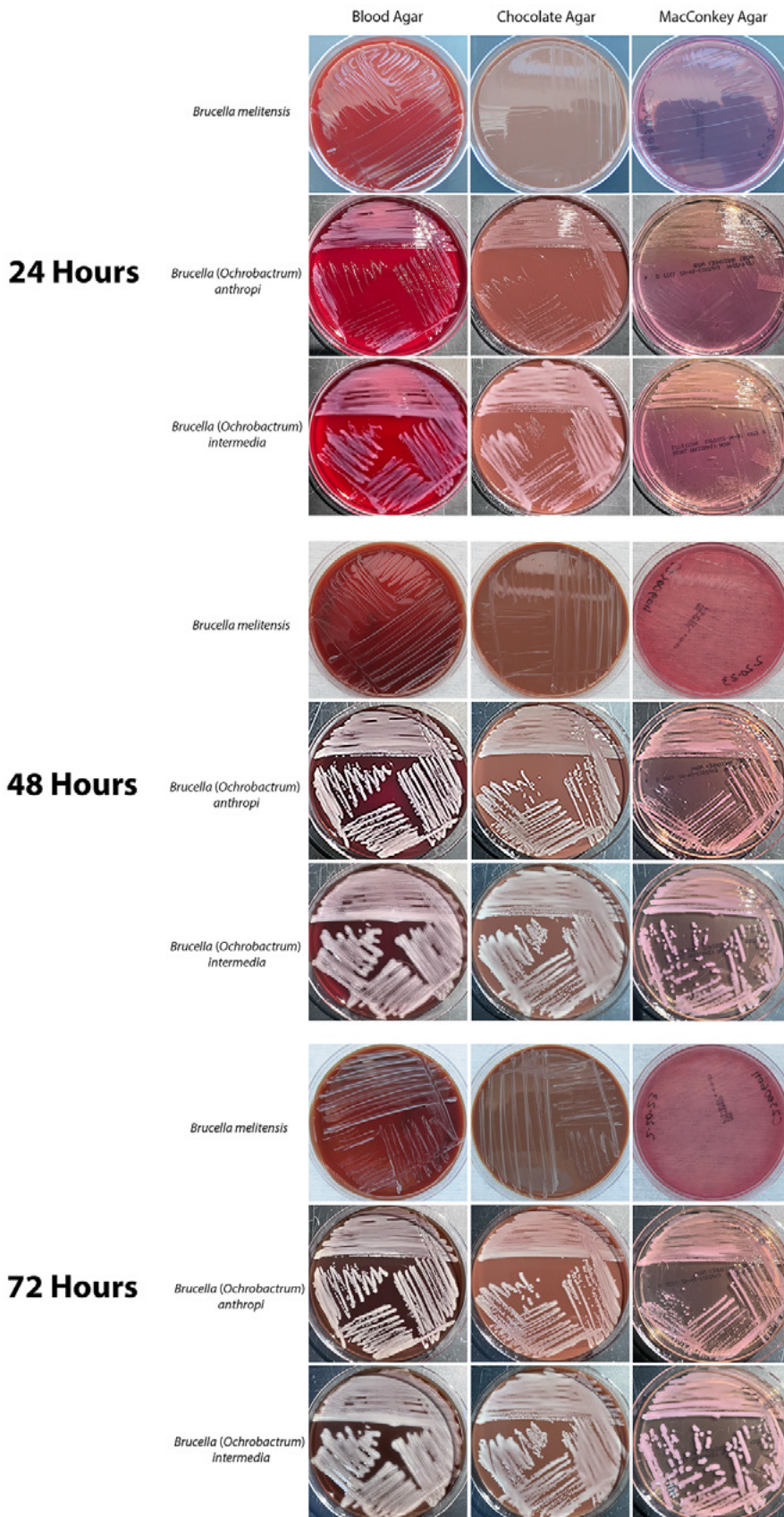


Figure 2. Growth characteristics of *B. melitensis*, compared with those of *Brucella (Ochrobactrum) anthropi* and *Brucella (Ochrobactrum) intermedia*, which are the *Brucella (Ochrobactrum)* species most commonly isolated from human clinical specimens. Cultures were grown on commercially prepared blood agar (tryptic soy agar + 5% defibrinated sheep's blood), chocolate agar and MacConkey agar, and photographs were taken after 24, 48 and 72 hours of incubation.

What Clinical Clues Can Aid in Distinguishing Infections Caused by Classic *Brucella* Species From Those Due to *Brucella* (*Ochrobactrum*) Species?

Patient exposure history, transmission risks and clinical history may support the differential identification of classic *Brucella* species versus *Brucella* (*Ochrobactrum*) species but does not replace the laboratory rule out process.

- Human disease due to classic *Brucella* species is often seen in those who work with animals or animal meat, individuals who eat unpasteurized animal products (dairy foods) contaminated with *Brucella* or individuals with travel history to regions where these products are available, and individuals who have had contact with wildlife in *Brucella* endemic areas.
- *Brucella* (*Ochrobactrum*) species are found in/on water, soil, plants and animals. Due to their low virulence, these organisms typically cause infections in immunocompromised individuals (e.g., line-associated infections). Healthcare-acquired infections typically can be traced back to contaminated invasive equipment (12). Isolation of *Brucella* (*Ochrobactrum*) species from clinical specimens often represents colonization (e.g., cultures of respiratory secretions, infection prevention and control surveillance cultures of stool). The specimen source, culture characteristics and clinical context should guide interpretation of the significance of these isolates.

How Can Laboratories Clearly Report These Organisms?

The clinical laboratory report should:

1. Accurately convey the organism detected to support appropriate treatment selection.
2. Provide clear communication of the clinical and public health risks of the organism.

Laboratories should consider interoperability of laboratory information systems, electronic medical records and patient access tools (i.e., laboratory report comments may not transfer to patient charts). Additionally, consider educating care providers if reclassified *Brucella* designations are used (e.g., laboratory bulletin about the clinical significance of a result of “*Brucella* (*Ochrobactrum*) *anthropi*” or “*Brucella* (*Ochrobactrum*) *intermedia*”). Emphasize that these organisms are distinct from species that cause brucellosis. Laboratories should also notify hospital Infection Prevention and Control, Infectious Diseases teams, local Public Health Officers and other stakeholders if the reclassified *Brucella* (*Ochrobactrum*) species names are used.

Scenario 1: Clinical Laboratories That Apply the Reclassified *Brucella* Species Names.

Consider providing the previously used name in parenthesis, appending an interpretive comment to the result, and reinforcing the presence of a report comment. Also consider consulting the clinical team by phone or email to explain the clinical significance of the results.

Example:

“*Brucella* (*Ochrobactrum*) *anthropi*, see comment below”

Patient Result/Report Comment: Recent taxonomic revisions have reassigned *Ochrobactrum* species to the genus *Brucella*. *B. anthropi* is not a select agent of bioterrorism and is not an agent of the disease brucellosis. When clinically indicated, infections should be treated according to *Ochrobactrum* antimicrobial therapy recommendations.

Scenario 2: Clinical Laboratories That Apply the *Ochrobactrum* Species Names.

Organism names can be reported to the genus or species-level according to laboratory procedures. Laboratory staff should document evidence that select agent *Brucella* was ruled-out, in the internal laboratory work-up (not in the patient results).

Example:

“*Ochrobactrum intermedium*”

Internal laboratory comment (not charted): Growth on MAC, >1 mm at 24 hr, mucoid. Select agent *Brucella* species ruled-out.

Scenario 3: Uncertainty in the Species-Level Identification but Select Agent *Brucella* Species Were Ruled Out.

Consider issuing a preliminary report delineating the status of the identification and clarify the nomenclature status.

Example:

“*Brucella* (*Ochrobactrum*) species, not select agent of bioterrorism *Brucella* species. Isolate referred for additional identification, see comment” or “*Brucella* (*Ochrobactrum*) species, not agent of brucellosis. Isolate referred for additional identification, see comment.”

Patient Result/Report Comment: Recent taxonomic revisions have assigned *Ochrobactrum* species to the genus *Brucella*. Species-level identification of this isolate was unsuccessful using the standard methods in our laboratory, but the agents that cause brucellosis (e.g., *B. melitensis*) were ruled out. This isolate is not a select agent of bioterrorism and is not an agent of the disease brucellosis.

Scenario 4: Select Agent *Brucella* Species Cannot Be Ruled Out.

Follow your current laboratory procedure for indicating a select agent is not ruled out.

Example:

“Gram-negative coccobacilli, unable to rule-out *Brucella* species. Isolate referred to [county/state LRN reference] laboratory for further testing.”



Brucella* and *Ochrobactrum **Taxonomic Updates for Laboratories**

How Does the 12/2022 CDC LOCS Announcement Affect Laboratory Approaches to These Organisms?

- The CDC LOCS notification “Reclassification of *Ochrobactrum* species into the *Brucella* genus” (5) provided an important reminder to laboratories that *Brucella* (*Ochrobactrum*) species and select agent *Brucella* species are closely related. When genus level identifications of *Brucella* are obtained, laboratories must scrutinize these isolates.
- On January 23, 2023, the CDC LOCS call further clarified that clinical laboratories should continue to rule out select agent *Brucella* species using the American Society for Microbiology (ASM) sentinel guidelines (9). Isolates for which rule out was not achieved by the ASM sentinel guidelines must be referred through the Laboratory Response Network (LRN; usually to the state laboratory) for further assessment (13).

Note, this recommendation applies to suspect isolates detected prior to attempting identification, as well as those identified to the genus or species level as *Ochrobactrum* or *Brucella* species by automated systems.

- Additional testing, including AST, should not be performed while pending results from the LRN laboratory.
- Once the select agent of bioterrorism organisms are ruled-out, clinical laboratories are not required to refer the isolate, unless otherwise directed by their local public health partners or their LRN laboratory.

Example: If a laboratory is using either the *Ochrobactrum* or *Brucella* nomenclature and an isolate is identified as *O. anthropi* or *B. anthropi*, respectively, the laboratory should first rule out the select agent *Brucella* species using the sentinel laboratory guidelines. After confirming the isolate is not a select agent, the laboratory may release the isolate identification in accordance with the manufacturer’s instructions and/or individual laboratory protocol and may perform AST if applicable.

What Are the Biosafety Requirements for Handling Isolates That Require Select Agent *Brucella* Rule-Out Testing?

Handling of clinical specimens and cultures suspected of containing high risk, select agent *Brucella* species (*B. melitensis*, *B. suis** or *B. abortus**) should be conducted using BSL-3 or BSL-2 with BSL-3 precautions as per the ASM Sentinel Guidelines and BMBL (14).

Brucella (*Ochrobactrum*) species do not cause brucellosis and are not considered high-risk agents. After ruling out select agent *Brucella* species, these isolates can be handled using standard BSL-2 clinical laboratory precautions.

When a select agent or agent of brucellosis cannot be ruled out, laboratories should follow the *Brucella* species Sentinel Level Clinical Laboratory Guidelines (9) for further guidance.

**Brucella abortus* and *Brucella suis* are considered synonyms of *Brucella melitensis* but the clinical and clinical laboratory implications of this classification are unclear at this point.



Brucella and *Ochrobactrum* Taxonomic Updates for Laboratories

What Are the Suggested Next Steps for Clinical Laboratories?

1. Review the current databases and reporting rules for automated identification systems your laboratory uses to determine which names the system currently reports. Note: regardless of which name a laboratory chooses to report, it is important to know what the identification system uses and incorporate this into laboratory procedures.
2. Determine if your laboratory is reporting the original validly published names (*Ochrobactrum*) or the reclassified names (*Brucella*).

The following factors must be considered when determining which name to use:

- Potential clinical impact of either naming choice (see reporting section above).
 - Clarity of procedures for laboratory staff.
 - Recommendations from the identification system vendor(s).
 - Requirements and/or recommendations from your laboratory's accrediting agency.
 - Consistency in use of nomenclature across different identification systems in the laboratory.
 - Additional information systems builds, procedure updates and staff competency that will be required. Note: these will likely be required whether you determine to use your current reporting or change your reporting methods.
3. Review laboratory protocols for additional tests which can be safely performed to distinguish *Brucella* (*Ochrobactrum*) from select agent *Brucella* species.
 4. Ensure your laboratory staff understand the naming situation and are competent in any updated laboratory practices and procedures.
 5. Notify applicable stakeholders such as Infection Prevention and Control providers, Infectious Diseases, Public Health Officers and others of applicable updated nomenclature changes.
 6. Ensure the correct antimicrobial breakpoints are applied to *Ochrobactrum* ('Other Non-Enterobacterales') if the *Brucella* nomenclature is adopted for these isolates. Select agent *Brucella* species should not be tested for antimicrobial resistance.

Conclusions

In summary, work-up and reporting practices will vary based on whether a laboratory has chosen to adopt the reclassified, validly published nomenclature for the impacted species. The Laboratory Practices Subcommittee (LPS) of ASM will work with the Association of Public Health Laboratories (APHL) to update the *Brucella* Sentinel Laboratory Guidelines by providing specific identification clues to differentiate non-select agent *Brucella* species and *Brucella* (*Ochrobactrum*) species from select agent *Brucella* species, particularly those that can cause human disease. It is recognized that the existence of two names for an organism is suboptimal for clinical care and laboratory workflow/processes, and efforts are underway to encourage a standardized approach to organism name adoption.

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