

BRUCELLA SPP. RULE-OUT EVALUATION

General Sample Information

Evaluator(s):

Evaluation date(s):

Submitting Sentinel Clinical Laboratory:

Laboratorian being evaluated (if known):

Initial sample source:

Within Clinical Lab

Initial sample accessioning date/time:

Initial sample processing date/time:

Initial sample work-up complete date/time:

Notification to LRN Reference Laboratory

Notification sent Date:

Time:

No notification provided

Sample sent to LRN Ref. Lab:

Primary specimen

Isolate

Other:

Date shipped to LRN Ref. Lab:

Date received:

Sample shipped to LRN Ref. Lab as:

Category B

Category A

Unsure

Other:

Was sample shipped appropriately?

Yes

No

Unsure

Rule-out test requested:

Microscopy

Was a Gram stain performed?

No

Yes

If yes, from:

Primary specimen

Culture

Result:

Gram positive

Gram negative

Undetermined

Gram stain characteristics:

Is the Gram stain result consistent with the rule-out organism?

Yes

No

Was another stain performed?

No

Yes

If yes, from:

Primary specimen

Culture

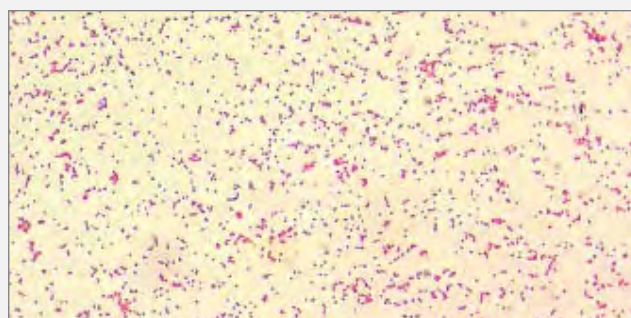
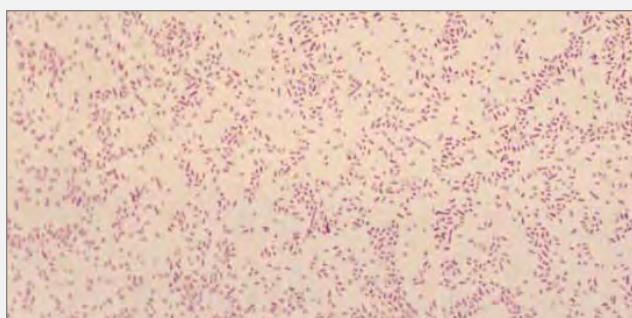
What type of other stain:

Describe characteristics:

Is the other stain result consistent with the rule-out organism?

Yes

No



Gram Stains

Culture Characteristics/Description

SBA/BAP (Sheep Blood Agar/Blood Agar Plate)

			Set up	Not Setup
Growth:	Hemolysis:	Temperature:	Colony description and other notes:	
24h growth	Alpha	25°C		
48h growth	Beta	37°C		
72h growth	Gamma	42°C		
No growth	(non-hemolytic)			

CHOC (Chocolate Agar)

		Set up	Not Setup
Growth:	Temperature:	Colony description and other notes:	
24h growth	25°C		
48h growth	37°C		
72h growth	42°C		
No growth			

MAC (MacConkey Agar)

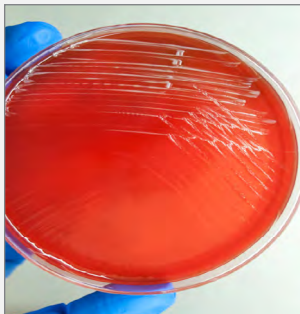
		Set up	OR	EMB (Eosin Methylene Blue Agar)	Set up	Neither Setup
Growth:	Temperature:	Colony description and other notes:				
24h growth	25°C					
48h growth	37°C					
72h growth	42°C					
No growth						

Note: *B. abortus* RB51 may show possible growth.

Other:

		Set up	Not Setup
Growth:	Temperature:	Colony description and other notes:	
24h growth	25°C		
48h growth	37°C		
72h growth	42°C		
No growth			

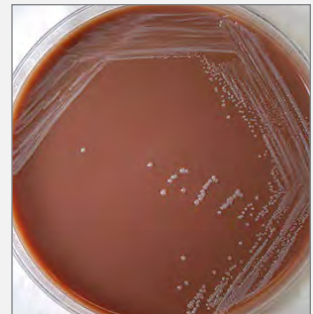
Brucella spp.: Aerobic, slow, poorly growing colonies after 24h incubation in 5-10% CO₂ at 35°C. Slow growth seen on BAP and CHOC (CO₂ may be required by some strains). Poor to variable growth on MAC. Pinpoint colonies may infrequently be observed with some strains after extended blood culture incubation (7 days). Non-mucoid. Pinpoint colonies at 24h, and easily visible, discrete, white, non-hemolytic colonies at 48h (0.5 mm-1 mm). Colonies on BAP have no distinguishing features. They will appear as white, non-pigmented and non-hemolytic. Colonies will appear as raised and convex with an entire edge and shiny surface.



48h BAP



24h CHOC



72h CHOC

Is the culture consistent with the rule-out organism?

Yes

No

No cultures performed

Rule-out Testing

Test	<i>Brucella</i> spp.	Rule-out Sample			
		Positive	Negative	Undetermined	Not Performed
Catalase	Positive				
Hemolysis	None (Gamma)				
Motility 35°C	Non-motile				
Oxidase	Positive (variable*)				
Urease	Positive				
Staph-streak Satellite	Negative or no growth (not required)				
X & V Factor	Negative or not required				
Other:					

* Oxidase may be variable and test should be performed on fresh cultures (18-24h).

Are the test results consistent with the rule-out organism?	Yes	No	No rule-out tests performed
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Rapid, Automated and Molecular Testing

Test/Platform	Yes	No	Result
API			
BioFire			
MALDI TOF			
Microscan			
PCR			
Phoenix			
VITEK			
Other:			

Are the test results consistent with the rule-out organism?	Yes	No	None performed
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Susceptibility Testing

Note: Routine susceptibility testing is not a recommended sentinel clinical laboratory procedure.

Antibiotic (if performed)	<i>Brucella</i> spp.	Zone/Susceptible	No Zone/Resistant	Indeterminate	Not Performed

Note: *B. abortus* RB51 is resistant to rifampin and penicillin.

Are the test results consistent with the rule-out organism?	Yes	No	No susceptibility tests performed
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Other Items of Consideration

	Yes	No	Unsure
Were the LRN Sentinel Level Clinical Laboratory Protocols followed?			
Are the patient symptoms / clinical presentation consistent with rule-out organism sent for testing?			
Is there a concern for <i>B. abortus</i> RB51 strain?			
If any samples remained at the submitting clinical laboratory, were they kept secured?			
If any samples remained at the submitting clinical laboratory, were they sealed and marked with a precautionary hazard warning (e.g., brightly colored distinguishing tape)?			
Were all remaining samples transferred from submitting laboratory(ies) to LRN Reference Laboratory?			
Were all remaining samples at submitting laboratory(ies) destroyed?			
Was the related sample waste handled and disposed of properly?			
Are there any safety or exposure concerns at the submitting laboratory(ies)? All potential exposures to a select agent require an APHIS/CDC Form 3 .			
Has the LRN Reference Laboratory completed Sections A and B of the required CDC/APHIS Form 4? All identified select agents require an APHIS/CDC Form 4 .			
Has the submitting laboratory(ies) completed Sections C and D of the required CDC/APHIS Form 4? All identified select agents require an APHIS/CDC Form 4 .			

Possible Misidentifications for *Brucella* spp.

Organism	Differential Test
<i>Haemophilus</i> species Both this organism and <i>Brucella</i> appear as tiny coccobacillus in Gram stain.	Catalase, urease and oxidase variable. Will not grow on blood agar. Will demonstrate satellite growth around <i>S. aureus</i> on blood agar while <i>Brucella</i> growth is present on blood agar and is not limited to the area around the <i>Staphylococcus</i> .
<i>Oligella ureolytica</i> Both appear as tiny coccobacillus in Gram stain, are catalase, urease and oxidase positive.	<i>O. ureolytica</i> will show delayed motility. Since <i>Oligella</i> has poor motility reaction, it would be better ruled-out with PDA (<i>Oligella</i> is PDA positive and <i>Brucella</i> is PDA negative) which is included in some rapid urea tests. Motility is also not necessary since <i>O. ureolytica</i> is rarely found in the blood or sterile sites where <i>Brucella</i> is more likely to be found, as <i>O. ureolytica</i> is a rare urinary pathogen.
<i>Psychrobacter phenylpyruvicus</i> Both are catalase, urease and oxidase positive.	<i>Psychrobacter phenylpyruvicus</i> has plump (not tiny) rods or coccobacillus and is PDA positive.
<i>Psychrobacter immobilis</i> Both are catalase, urease and oxidase positive.	<i>P. immobilis</i> will prefer to grow at 25°C. May have an odor of roses (however, do NOT sniff cultures). Variable growth on MAC.
<i>Bordetella bronchiseptica</i> Both are catalase, urease and oxidase positive.	<i>Bordetella bronchiseptica</i> is motile, and <i>Brucella</i> is non-motile. Motility testing is not needed to rule-out <i>Bordetella</i> since <i>B. bronchiseptica</i> grows on MAC, and is a rarely-encountered organism in sterile site specimens.
<i>Paracoccus yeei</i> Both are catalase, urease and oxidase positive.	<i>P. yeei</i> will appear mucoid on BAP.

Common Misidentifications

May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Moraxella* spp., *Micrococcus* spp., *Corynebacterium* spp., "slow growing" *Staphylococcus* spp., *Oligella ureolytica*, *Bordetella bronchiseptica*, *Haemophilus* spp., *Pasteurella* spp., *Psychrobacter phenylpyruvicus* and *Psychrobacter immobilis*.

Final Results

LRN Reference Laboratory test result:

LRN Reference Laboratory test result date:

Protocol Adherence

Submitting lab followed [LRN Sentinel Level Clinical Laboratory Protocols](#) accordingly

Submitting lab did not follow [LRN Sentinel Level Clinical Laboratory Protocols](#) accordingly

Overall Assessment

No follow up needed

Follow up with submitting lab(s) needed. Recommended follow up: