Biothreat Organism Presumptive Identification Rule-out Evaluation Tool

JUNE 2020

APHL ASSOCIATION OF PUBLIC HEALTH LABORATORIES®

PURPOSE

APHL's Sentinel Laboratory Partnerships and Outreach Subcommittee of the Public Health Preparedness and Response Committee developed this tool to assist public health laboratories (PHLs) with evaluating biothreat organism presumptive identification rule-out procedures according to the American Society for Microbiology (ASM) Laboratory Response Network (LRN) Sentinel Level Clinical Laboratory Protocols for suspected biological threat agents and emerging infectious diseases. The tool is primarily intended for PHLs that are reference level members of the LRN for Biological Threats Preparedness (LRN-B) to evaluate Sentinel Clinical Laboratories in their jurisdiction submitting samples for rule-out testing but it may also be used for anyone evaluating the biothreat organism identification procedures for training and competency assessment purposes.

Individual rule-out evaluations are also available below and in the APHL Publications Library.

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ACRONYM GLOSSARY

APHL ..... Association of Public Health Laboratories
ASM ..... American Society for Microbiology
BAP ..... Blood Agar Plate
BCYE ..... Buffered Charcoal Yeast Extract
CDC ..... Centers for Disease Control and Prevention
CHA ..... Cysteine Heart Agar
CHOC ..... Chocolate Agar
CIN ..... Cefsulodin-Irgasan-Novobiocin
CNA ..... Columbia Naladixic Acid Agar
EMB ..... Eosin Methylene Blue Agar
LF ..... Lactose Fermenter
LRN ..... Laboratory Response Network
MAC ..... MacConkey Agar
MALDI TOF .... Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometer
MH ..... Mueller-Hinton Agar
MTM/TM ..... Modified Thayer-Martin / Thayer-Martin Agar
NLF ..... Non-lactose Fermenter
PDA ..... Phenylalanine-deaminase
SBA ..... Sheep Blood Agar
TSI ..... Triple Sugar Iron Agar

UNKNOWN BIOTHREAT AGENT 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:

Was another stain performed? ☐ No ☐ Yes If yes, from: ☐ Primary specimen ☐ Culture

What type of other stain:

Describe characteristics:

Media Growth Characteristics

Media	Growth Description			
	24h	48h	72h	No Growth
BAP/SBA (Blood Agar Plate / Sheep Blood Agar)	☐	☐	☐	☐
CHOC (Chocolate Agar)	☐	☐	☐	☐
MAC (MacConkey Agar)	☐	☐	☐	☐
Other Media:	☐	☐	☐	☐

Growth Descriptions

- ☐ Pinpoint
- ☐ Small
- ☐ Large
- ☐ Irregular
- ☐ Flat
- ☐ Convex
- ☐ Raised
- ☐ Round
- ☐ Rough
- ☐ Mucoid
- ☐ Waxy
- ☐ Smooth
- ☐ Ground glass
- ☐ Swarming
- ☐ Bulls-eye
- ☐ Fried egg
- ☐ Pigment on Mueller-Hinton Agar
- ☐ Grey
- ☐ White
- ☐ α (Alpha) hemolytic
- ☐ β (Beta) hemolytic
- ☐ ɣ (Gamma) non-hemolytic
- ☐ Lactose fermentation
- ☐ Non-lactose fermentation
- ☐ No growth

Susceptibility Tests

Antibiotics	Zone/ Susceptible	No Zone/ Resistant	Indeterminate	Not Performed
Amoxicillin-Clavulanate (20/10 µg)	☐	☐	☐	☐
Colistin (10 µg)	☐	☐	☐	☐
Doxycycline	☐	☐	☐	☐
Penicillin G (10 U)	☐	☐	☐	☐
Polymyxin-B (300 U)	☐	☐	☐	☐
Rifampin	☐	☐	☐	☐
Other:	☐	☐	☐	☐

Rule-out Tests

Rule-out Tests	Positive	Negative	Indeterminate	Not Performed
Catalase	☐	☐	☐	☐
Oxidase	☐	☐	☐	☐
Indole	☐	☐	☐	☐
Urease	☐	☐	☐	☐
Satellite	☐	☐	☐	☐
Beta-lactamase	☐	☐	☐	☐
Motility 25-28°C	☐	☐	☐	☐
Motility 35-37°C	☐	☐	☐	☐
BAP Growth at 25-30°C	☐	☐	☐	☐
BAP Growth at 35-37°C	☐	☐	☐	☐
BAP Growth at 42°C	☐	☐	☐	☐
CIN Agar Growth	☐	☐	☐	☐
CNA or PEA Agar Growth	☐	☐	☐	☐
MTM/TM Agar Growth	☐	☐	☐	☐
BCYE Agar Growth	☐	☐	☐	☐

Hemolysis: ☐ No hemolysis (Gamma) ☐ Alpha hemolysis ☐ Beta hemolysis ☐ Not Performed

TSI Reaction:

Final Results

LRN Ref. Lab test result: Date:

☐ Submitting lab followed LRN Sentinel Level Clinical Laboratory Protocols accordingly

☐ Submitting lab did not follow LRN Sentinel Level Clinical Laboratory Protocols accordingly

☐ No Follow up needed ☐ Follow up with submitting lab(s) needed. Recommended follow up:

BACILLUS ANTHRACIS 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

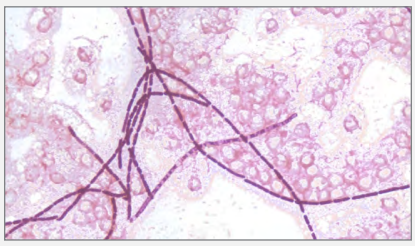
Was another stain performed? ☐ No ☐ Yes If yes, from: ☐ Primary specimen ☐ Culture

What type of other stain:

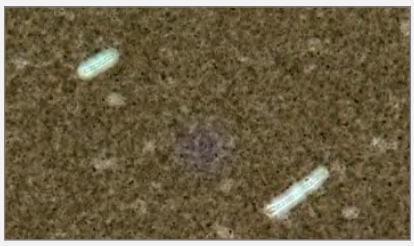
Describe characteristics:

Is the other stain result consistent with the rule-out organism? ☐ Yes ☐ No

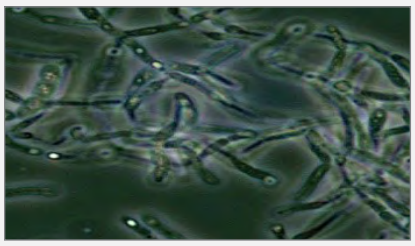
B. anthracis: Large Gram positive rods (1-1.5 µm x 3-5 µm). Direct smears of clinical specimens: Short chains (2-4 cells), capsule present, no spores present. Smears from culture (BAP or CHOC): Long chains, no capsule present, spores in older cultures: oval, central to subterminal, no swelling of cell wall. Note: Spores may be found in cultures grown in 5% CO_2 or ambient atmosphere but not usually observed in clinical samples. *B. anthracis* may appear Gram variable after 72 hours.



Gram stain from blood culture



Capsule with India ink stain



Spores with phase contrast

Culture Characteristics/Description

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

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Hemolysis:
- ☐ Alpha
- ☐ Beta
- ☐ Gamma (non-hemolytic)

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

CHOC (Chocolate Agar)

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

MAC (MacConkey Agar) ☐ Set up OR EMB (Eosin Methylene Blue Agar) ☐ Set up ☐ Neither Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Other: _____ ☐ Set up ☐ Not Setup

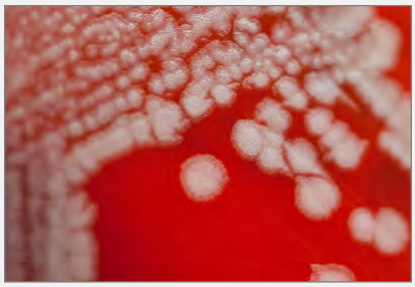
Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

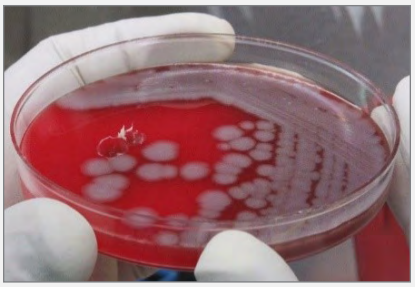
Colony description and other notes:

B. anthracis: Grows well on BAP and CHOC. Aerobic rapid growth as early as 4-8h. Colonies 2-5 mm on BAP and CHOC at 24h. No growth on MAC and EMB. Flat or slightly convex with irregular edges that may have comma-like projections. Ground-glass appearance. Non-pigmented, gamma hemolytic (non-hemolytic) on BAP. Tenacious, sticky colonies, adheres to agar surface. Note: Some strains of *B. cereus* biovar *anthracis* may be weakly hemolytic after 48h.

24h BAP



Irregular-edge colonies



Tenacious colonies

Is the culture consistent with the rule-out organism? ☐ **Yes** ☐ **No** ☐ **No cultures performed**

Rule-out Testing

Test	*Bacillus anthracis*	*Bacillus cereus* biovar *anthracis*	Rule-out Sample			
			Positive	Negative	Undetermined	Not Performed
Capsule Direct smears of clinical specimens	Positive	Positive	☐	☐	☐	☐
Capsule Smears from culture	Negative	Variable*	☐	☐	☐	☐
Catalase	Positive	Positive	☐	☐	☐	☐
Hemolysis	Non-hemolytic	Non-hemolytic	☐	☐	☐	☐
Motility 35°C	Non-motile	Variable (usually motile)	☐	☐	☐	☐
Other:			☐	☐	☐	☐

* Capsule expression dependent on growth in heart infusion broth (HIB) supplemented with horse serum and sodium bicarbonate enrichment and incubated at 37°C for 4-6 h.

Are the test results consistent with the rule-out organism? ☐ **Yes** ☐ **No** ☐ **No rule-out tests performed**

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**

Susceptibility Testing

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

Antibiotic	*B. anthracis*	*Bacillus cereus* biovar *anthracis*	Zone/ Susceptible	No Zone/ Resistant	Indeterminate	Not Performed
Penicillin G (10U)	Zone/ Susceptible	Variable	☐	☐	☐	☐
Other:			☐	☐	☐	☐

Are the test results consistent with the rule-out organism? ☐ **Yes** ☐ **No** ☐ **No susceptibility tests performed**

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 (e.g., associated travel or exposure history, or indicated by rule-out lab testing, etc.) the sample may be *B. cereus* biovar *anthracis*?	☐	☐	☐
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 *Bacillus anthracis*

Organism	Differential Test
Bacillus megaterium	Motility Positive (Note: 16% are non-motile)
Bacillus subtilis	Motility Positive
Bacillus cereus	Hemolytic*
Bacillus thuringiensis	Hemolytic and not a human pathogen

* Some strains of *Bacillus cereus* biovar *anthracis* are non-hemolytic.

Common Misidentifications
May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications include *Bacillus megaterium* and other *Bacillus* species.
Note: *Bacillus cereus* Group includes *B. anthracis*, but automated ID systems may not alert microbiologist beyond this group identification.

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:

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:

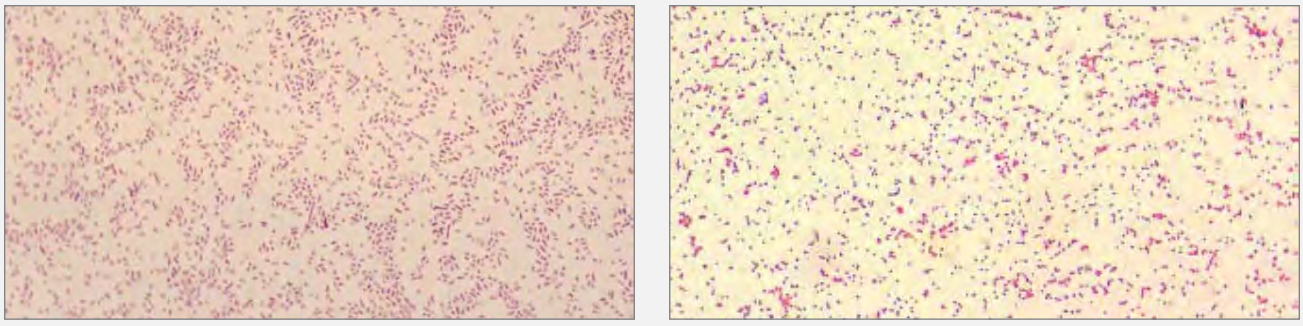
Is the Gram stain result consistent with the rule-out organism? ☐ Yes ☐ No

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:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Hemolysis:
- ☐ Alpha
- ☐ Beta
- ☐ Gamma (non-hemolytic)

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

CHOC (Chocolate Agar)

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

MAC (MacConkey Agar) ☐ Set up OR EMB (Eosin Methylene Blue Agar) ☐ Set up ☐ Neither Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

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

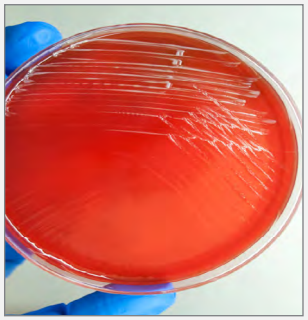
Other: _____ ☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

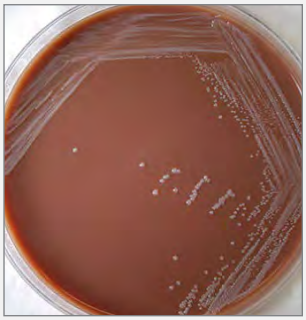
Colony description and other notes:

***Brucella* spp.:** Aerobic, slow, poorly growing colonies after 24h incubation in 5-10% CO_2 at 35°C. Slow growth seen on BAP and CHOC (CO_2 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	*Brucella* 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

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

Susceptibility Testing

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

Antibiotic (if performed)	*Brucella* 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

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

BURKHOLDERIA MALLEI 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

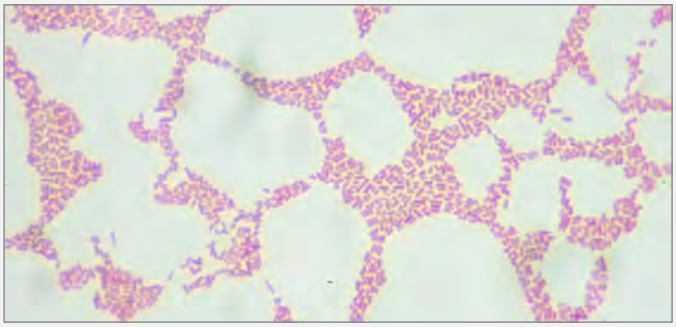
Was another stain performed? ☐ No ☐ Yes If yes, from: ☐ Primary specimen ☐ Culture

What type of other stain:

Describe characteristics:

Is the other stain result consistent with the rule-out organism? ☐ Yes ☐ No

B. mallei: Small straight or slightly curved Gram negative coccobacilli (1.5 µm-3 µm x 0.5-1 µm) with rounded ends. Cells arranged in pairs, parallel bundles, or the Chinese letter form.



Gram Stain

Culture Characteristics/Description

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

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Hemolysis:
- ☐ Alpha
- ☐ Beta
- ☐ Gamma (non-hemolytic)

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

CHOC (Chocolate Agar)

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

MAC (MacConkey Agar) OR EMB (Eosin Methylene Blue Agar)

☐ Set up ☐ Set up ☐ Neither Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Ashdown Agar

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Burkholderia cepacia Selective Agar

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Other: _____

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Is the culture consistent with the rule-out organism? ☐ **Yes** ☐ **No** ☐ **No cultures performed**

B. mallei: Aerobic. Poor growth at 24h on all media. No pigment, even on Mueller Hinton Agar. No distinctive odor (from closed plate).

On BAP: Pinpoint to small grey colonies at 24h that may become smooth, grey and translucent at 48h. Non-hemolytic.

On MAC: No growth at 42˚C. Poor or no growth at 48h. No growth or pinpoint colorless colonies after 48h.

24h BAP

48h BAP

Rule-out Testing

Test	*B. mallei*	Rule-out Sample			
		Positive	Negative	Undetermined	Not Performed
Arginine	Positive	☐	☐	☐	☐
Catalase	Positive	☐	☐	☐	☐
Hemolysis	None (Gamma)	☐	☐	☐	☐
Indole	Negative	☐	☐	☐	☐
Motility 35°C	Non-motile	☐	☐	☐	☐
Growth at 42°C	No growth	☐	☐	☐	☐
Nitrate	Positive (without gas)	☐	☐	☐	☐
Oxidase	Variable (most are negative)	☐	☐	☐	☐
Urease	Variable	☐	☐	☐	☐
Other:		☐	☐	☐	☐

Are the test results consistent with the rule-out organism? ☐ Yes ☐ No ☐ No rule-out tests performed

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

Susceptibility Testing

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

Antibiotic	*B. mallei*	Zone/ Susceptible	No Zone/ Resistant	Indeterminate	Not Performed
Penicillin (10 U)	No Zone/Resistant	☐	☐	☐	☐
Amoxicillin clavulanate (20/10 µg)	Zone/Susceptible	☐	☐	☐	☐
Colistin (10 µg)	No Zone/Resistant	☐	☐	☐	☐
Polymyxin-B (300 U)	No Zone/Resistant	☐	☐	☐	☐
Gentamicin	Zone/Susceptible	☐	☐	☐	☐
Other:		☐	☐	☐	☐

Are the test results consistent with the rule-out organism? ☐ **Yes** ☐ **No** ☐ **No susceptibility tests performed**

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?	☐	☐	☐
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 *B. mallei*

Organism	Differential Test
Burkholderia cepacia	Resistant to amoxicillin-clavulanic acid, lactose fermenter on MacConkey and EMB, motile, arginine negative.
Chromobacterium violaceum	Hemolysis, violet pigment on BAP, motile
Pseudomonas stutzeri	Growth on MacConkey, arginine negative
Stenotrophomonas maltophilia	Growth on MacConkey, arginine negative
Bacillus spp. may appear Gram negative	Sensitive to penicillin
Pandoraea spp.	Growth on MacConkey
Ralstonia spp.	Growth on MacConkey
Acinetobacter spp.	MAC positive, oxidase negative

Common Misidentifications

May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Burkholderia cepacia, Chromobacterium violaceum, Pseudomonas stutzeri, Bacillus* spp., *Pandoraea* spp., *Ralstonia* spp. other nonfermenting Gram negative bacilli.

Note: *B. pseudomallei* and *B. mallei* are arginine positive, unlike other *Burkholderia*; the arginine test may be in kit or identification systems.

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:

BURKHOLDERIA PSEUDOMALLEI 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

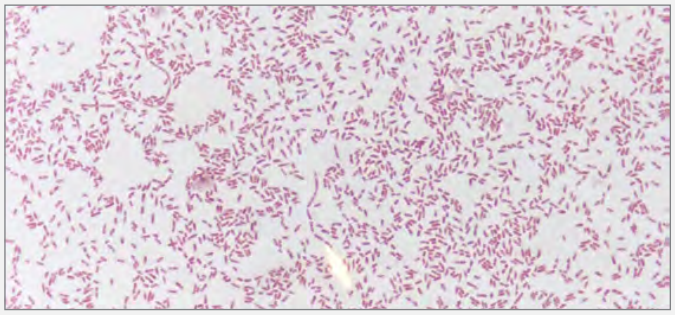
Was another stain performed? ☐ No ☐ Yes If yes, from: ☐ Primary specimen ☐ Culture

What type of other stain:

Describe characteristics:

Is the other stain result consistent with the rule-out organism? ☐ Yes ☐ No

B. pseudomallei: Straight, or slightly curved Gram negative rod (2-5 µm x 0.4-0.8 µm). Colonies may demonstrate bipolar morphology in direct specimens and peripheral staining in older cultures, which can mimic endospores.



Gram Stain

Culture Characteristics/Description

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

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Hemolysis:
- ☐ Alpha
- ☐ Beta
- ☐ Gamma (non-hemolytic)

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

CHOC (Chocolate Agar)

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

MAC (MacConkey Agar) ☐ Set up OR EMB (Eosin Methylene Blue Agar) ☐ Set up ☐ Neither Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Ashdown Agar

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Burkholderia cepacia Selective Agar

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Other: _____

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

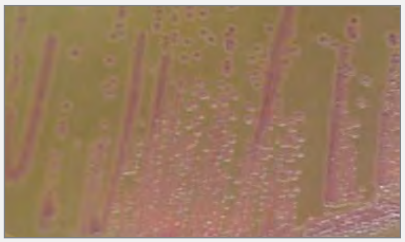
Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

B. pseudomallei: Aerobic. Growth on BAP: Small, smooth, creamy colonies in the first 1-2 days, that may gradually change in time to dry, wrinkled colonies (similar to *Pseudomonas stutzeri*). Poor growth at 24h, good growth at 48h. Colonies are non-hemolytic and not pigmented on BAP or Mueller Hinton Agar. Grows on MAC (may uptake pink dye). Distinctive musty, earthy odor is apparent without sniffing or opening plate. Growth at 42˚C.

24h BAP

48h BAP



48h MAC

Is the culture consistent with the rule-out organism? ☐ Yes ☐ No ☐ No cultures performed

Rule-out Testing

Test	*B. pseudomallei*	Rule-out Sample			
		Positive	Negative	Undetermined	Not Performed
Arginine	Positive	☐	☐	☐	☐
Catalase	Positive	☐	☐	☐	☐
Hemolysis	Non-hemolytic	☐	☐	☐	☐
Indole	Negative	☐	☐	☐	☐
Motility 35°C	Motile	☐	☐	☐	☐
Growth at 42°C	Growth	☐	☐	☐	☐
Nitrate	Positive (with gas)	☐	☐	☐	☐
Oxidase	Positive	☐	☐	☐	☐
TSI	Red butt (no change)/ variable slant	☐	☐	☐	☐
Other:		☐	☐	☐	☐

Are the test results consistent with the rule-out organism? ☐ Yes ☐ No ☐ No rule-out tests performed

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

Susceptibility Testing

Antibiotic	*B. pseudomallei*	Zone/ Susceptible	No Zone/ Resistant	Indeterminate	Not Performed
Penicillin (10 U)	No Zone/Resistant	☐	☐	☐	☐
AmoxicillinClavulanate (20/10µg)	Zone/Susceptible	☐	☐	☐	☐
Colistin (10 µg)	No Zone/Resistant	☐	☐	☐	☐
Polymyxin-B (300 U)	No Zone/Resistant	☐	☐	☐	☐
Gentamicin	No Zone/Resistant	☐	☐	☐	☐
Other:		☐	☐	☐	☐

Are the test results consistent with the rule-out organism? ☐ **Yes** ☐ **No** ☐ **No susceptibility tests performed**

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?	☐	☐	☐
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 *B. pseudomallei*

Organism	Differential Test
*Burkholderia cepacia**	Resistant to amoxicillin-clavulanic acid, lactose fermenter on MacConkey and EMB, arginine negative.
Chromobacterium violaceum	Hemolysis, violet pigment on BAP.
Pseudomonas aeruginosa	Colonial morphology, grape odor (do NOT sniff plates)
Pseudomonas stutzeri	Arginine negative, susceptible to polymyxin B
Stenotrophomonas maltophilia	Arginine negative

Common Misidentifications

May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Burkholderia cepacia*,* *Chromobacterium violaceum*, *Pseudomonas aeruginosa*, *Pseudomonas stutzeri*, *S. maltophilia* and other nonfermenting Gram negative bacilli.

B. pseudomallei and *B. mallei* are arginine positive, unlike other *Burkholderia*; arginine test may be in kit identification systems. Also, unlike *B. mallei*, *B. pseudomallei* grows at 42°C in 48h and is motile.

* *B. pseudomallei* is separated from *B. cepacia* by a susceptible amoxicillin-clavulanate test. Although rare in *B. pseudomallei*, resistance cannot rule out the identification.

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:

FRANCISELLA TULARENSIS 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

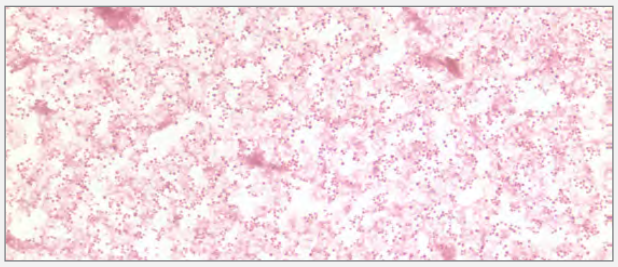
Was another stain performed? ☐ No ☐ Yes If yes, from: ☐ Primary specimen ☐ Culture

What type of other stain:

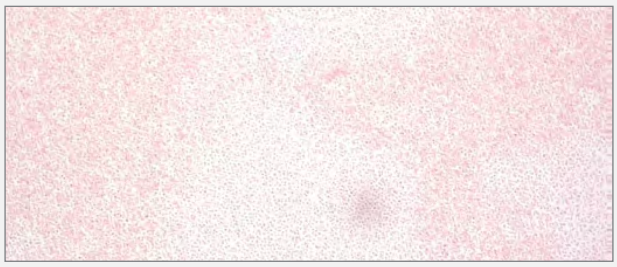
Describe characteristics:

Is the other stain result consistent with the rule-out organism? ☐ Yes ☐ No

F. tularensis: Tiny, Gram negative coccobacilli (0.2-0.5 µm x 0.7-1.0 µm). Poor counterstaining with safranin (basic fuchsin counterstain may increase resolution). Pleomorphic. Mostly single cells.



Gram Stain



Gram Stain of Blood Culture

Culture Characteristics/Description

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

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Hemolysis:
- ☐ Alpha
- ☐ Beta
- ☐ Gamma (non-hemolytic)

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

CHOC (Chocolate Agar)

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

MAC (MacConkey Agar) ☐ Set up OR EMB (Eosin Methylene Blue Agar) ☐ Set up ☐ Neither Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

TM/MTM (Thayer Martin/Modified Thayer Martin Agar)

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

BCYE/*Legionella* (Buffered Charcoal Yeast Extract Agar)

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Cysteine Heart Agar (CHA)

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Other: _____

☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

F. tularensis: Aerobic, fastidious, does not require CO_2. No growth on MAC or EMB. Scant or no growth on BAP; may grow on primary culture, not well on subculture. Slow growing on CHOC, TM or BCYE: 1-2 mm after 48h. Colonies are opaque, grey-white, butyrous with smooth and shiny surface.

48h BAP

48h CHOC

Is the culture consistent with the rule-out organism? ☐ Yes ☐ No ☐ No cultures performed

Rule-out Testing

Test	*F. tularensis*	Rule-out Sample			
		Positive	Negative	Undetermined	Not Performed
Beta-lactamase	Positive	☐	☐	☐	☐
Catalase	Negative or weakly positive	☐	☐	☐	☐
Motility	Non-motile	☐	☐	☐	☐
Oxidase	Negative	☐	☐	☐	☐
Urease	Negative	☐	☐	☐	☐
Staph-streak Satellite	Negative or no growth	☐	☐	☐	☐
X & V Factor	Not required	☐	☐	☐	☐
Other:		☐	☐	☐	☐

Are the test results consistent with the rule-out organism? ☐ Yes ☐ No ☐ No rule-out tests performed

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

Susceptibility Testing

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

Antibiotic	*F. tularensis*	Zone/ Susceptible	No Zone/ Resistant	Indeterminate	Not Performed
Beta-lactamase	Positive	☐	☐	☐	☐
Other:		☐	☐	☐	☐
Other:		☐	☐	☐	☐

Are the test results consistent with the rule-out organism? ☐ **Yes** ☐ **No** ☐ **No susceptibility tests performed**

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?	☐	☐	☐
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 *Francisella tularensis*

Organism	Differential Test
Acinetobacter spp.	Growth on MAC, oxidase negative
Aggregatibacter spp.	Catalase positive, β-lactamase negative
Haemophilus spp.	Oxidase positive, requires X & V factors
Haemophilus influenzae	Satellite or X&V positive
Bordetella Grp. IV	Inert, urease positive
Pasteurella spp.	Non-sticky, growth on MAC
Dysgonomonas spp.	Colonies measure 1 to 2 mm in diameter after 24 h of growth, have a distinct strawberry-like odor (do NOT sniff plates).
Brucella spp.	Oxidase, urease and catalase positive
Psychrobacter phenylpyruvicus	Oxidase positive
Oligella ureolytica	Oxidase positive

Common Misidentifications

May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Aggregatibacter actinomycetemcomitans*, *Haemophilus influenzae*, *Oligella* spp. and *Psychrobacter* spp.

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:

YERSINIA PESTIS 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

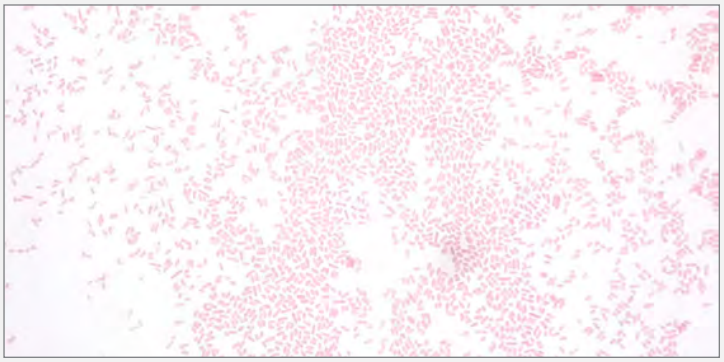
Was another stain performed? ☐ No ☐ Yes If yes, from: ☐ Primary specimen ☐ Culture

What type of other stain:

Describe characteristics:

Is the other stain result consistent with the rule-out organism? ☐ Yes ☐ No

Y. pestis: Plump, Gram negative rods (0.5 x 1-2 µm) seen mostly as single cells or pairs and may demonstrate short chains in liquid media. May exhibit bipolar, "safety-pin" appearance that is not seen on Gram stain, may be exhibited by Giemsa stain or Wright's stain.



Gram stain

Culture Characteristics/Description

SBA/BAP (Sheep Blood Agar/Blood Agar Plate) ☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Hemolysis:
- ☐ Alpha
- ☐ Beta
- ☐ Gamma (non-hemolytic)

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

MAC (MacConkey Agar) ☐ Set up **OR** **EMB (Eosin Methylene Blue Agar)** ☐ Set up ☐ Neither Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

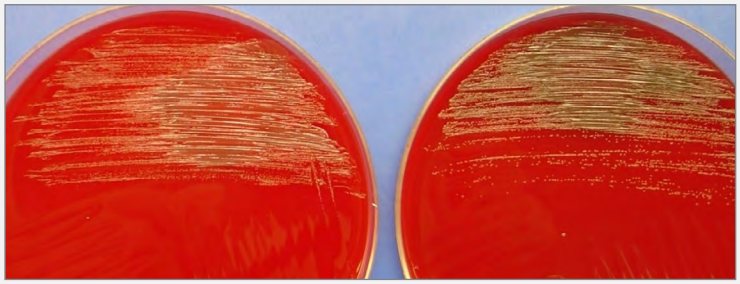
Other: _____ ☐ Set up ☐ Not Setup

Growth:
- ☐ 24h growth
- ☐ 48h growth
- ☐ 72h growth
- ☐ No growth

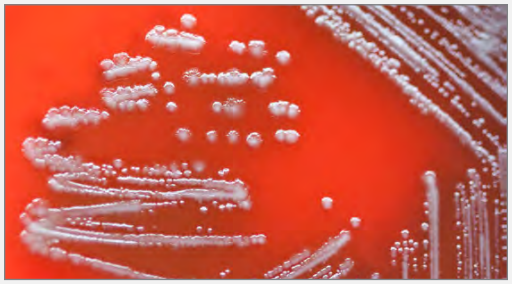
Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

Colony description and other notes:

Y. pestis: Facultative anaerobe. Slow growing at 35˚C, better growth at 25-28˚C. Grey-white, translucent pinpoint colonies at 24h, usually too small to be seen. On BAP: After 48h: colonies approximately 1-2 mm in diameter, gray-white to slightly yellow and opaque. Older cultures (~96h): "Fried egg" or "hammered copper" appearance (under magnification). Little to no hemolysis. Lactose non-fermenter at 48h on MAC or EMB.

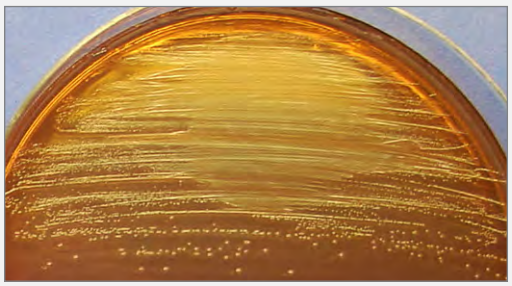


24h BAP, 25ºC / 35ºC



48h BAP

"Fried Egg" 96h BAP



48h MAC

Is the culture consistent with the rule-out organism? ☐ **Yes** ☐ **No** ☐ **No cultures performed**

Rule-out Testing

Test	*Y. pestis*	Rule-out Sample			
		Positive	Negative	Undetermined	Not Performed
Catalase	Positive	☐	☐	☐	☐
Hemolysis	None (gamma)	☐	☐	☐	☐
Indole	Negative	☐	☐	☐	☐
Oxidase	Negative	☐	☐	☐	☐
TSI	Alkaline slant / acid butt (K/A) without gas or H_2S	☐	☐	☐	☐
Urease	Negative	☐	☐	☐	☐
Other:		☐	☐	☐	☐

Are the test results consistent with the rule-out organism? ☐ Yes ☐ No ☐ No rule-out tests performed

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

Susceptibility Testing

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

Antibiotic	*Y. pestis*	Zone/ Susceptible	No Zone/ Resistant	Indeterminate	Not Performed
		☐	☐	☐	☐
		☐	☐	☐	☐

Are the test results consistent with the rule-out organism? ☐ Yes ☐ No ☐ No susceptibility tests performed

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?	☐	☐	☐
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 *Yersinia pestis*

Organism	Differential Test
***Acinetobacter* spp.**	May appear as Gram negative coccobacilli, often in pairs. Glucose non-fermenter. Colony morphology.
***E. coli*, non-lactose fermenter**	Faster growth rate. Indole positive (80%). Colony morphology.
Pantoea* (formerly *Enterobacter*) *agglomerans	Faster growth rate. May produce yellow pigment. ONPG positive (90%).
Pasteurella multocida	Faster growth rate. Oxidase positive (may be weak). Indole positive. Colony morphology may appear mucoid.
Pseudomonas luteola	May produce yellow pigment. Glucose non-fermenter
***Pseudomonas* spp.**	Oxidase positive (except *P. luteola* and *P. oryzihabitans*). Glucose non-fermenter.
***Shigella* spp.**	Faster growth rate. Colony morphology. *Shigella* antisera
***Salmonella* spp., H_2S(-)**	Faster growth rate. Colony morphology. *Salmonella* antisera
Yersinia enterocolitica	Small Gram negative coccobacilli. Urease positive* Indole variable
Y*ersinia pseudotuberculosis*	Urease positive*

* *Y. pseudotuberculosis* and *Y. enterocolitica* give stronger reactions in urea agar or broth when incubated at 25-28°C, but incubation at this temperature is not necessary to demonstrate urease production. *Y. pestis* is the only species of *Yersinia* that is non-motile at room temp.

Common Misidentifications

May not be identified in common automated ID systems, including MALDI TOF, and possible misidentifications may include: *Shigella* spp., H_2S(-) *Salmonella* spp., *Acinetobacter* or *Pseudomonas* spp. and *Yersinia pseudotuberculosis*.

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:

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