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

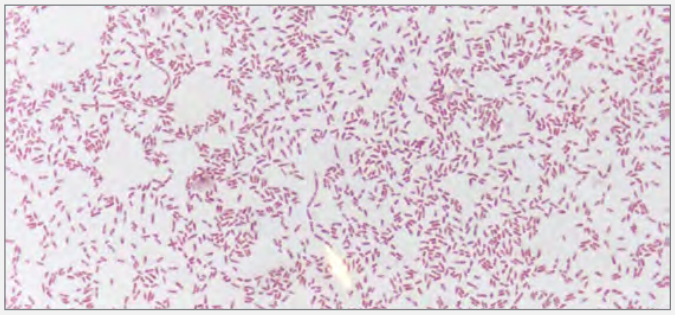
Was another stain performed? ☐ No ☐ Yes If yes, from: ☐ Primary specimen ☐ Culture

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) OR EMB (Eosin Methylene Blue Agar)

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

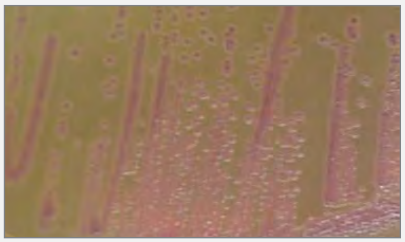
Temperature:
- ☐ 25°C
- ☐ 37°C
- ☐ 42°C

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: