

BACILLUS ANTHRACIS RULE-OUT EVALUATION

General Sample Information

Evaluator(s):

Evaluation date(s):

Submitting Sentinel Clinical Laboratory:

Laborarian 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

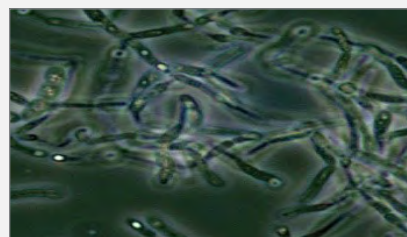
***B. anthracis*:** Large Gram positive rods (1-1.5 μm x 3-5 μm). Direct smears of clinical specimens: Short chains (2-4 cells), capsule present, no spores present. Smears from culture (BAP or CHOC): Long chains, no capsule present, spores in older cultures: oval, central to subterminal, no swelling 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:	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							

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				

***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	<i>Bacillus anthracis</i>	<i>Bacillus cereus</i> biovar <i>anthracis</i>	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
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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	<i>B. anthracis</i>	<i>Bacillus cereus</i> biovar <i>anthracis</i>	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
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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 (e.g., associated travel or exposure history, or indicated by rule-out lab testing, etc.) the sample may be <i>B. cereus</i> biovar <i>anthracis</i> ?			
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
<i>Bacillus megaterium</i>	Motility Positive (Note: 16% are non-motile)
<i>Bacillus subtilis</i>	Motility Positive
<i>Bacillus cereus</i>	Hemolytic*
<i>Bacillus thuringiensis</i>	Hemolytic and not a human pathogen
* Some strains of <i>Bacillus cereus</i> biovar <i>anthracis</i> 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: