

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 μ m) 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:	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)			

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		

***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.



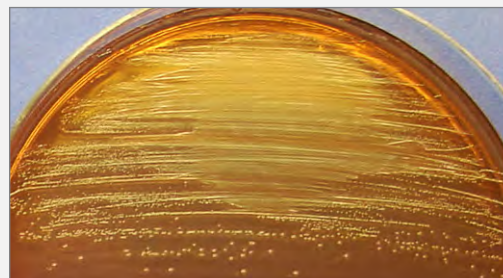
24h BAP, 25°C / 35°C



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	<i>Y. pestis</i>	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 ₂ S				
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	<i>Y. pestis</i>	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
<i>Acinetobacter</i> spp.	May appear as Gram negative coccobacilli, often in pairs. Glucose non-fermenter. Colony morphology.
<i>E. coli</i> , non-lactose fermenter	Faster growth rate. Indole positive (80%). Colony morphology.
<i>Pantoea</i> (formerly <i>Enterobacter</i>) <i>agglomerans</i>	Faster growth rate. May produce yellow pigment. ONPG positive (90%).
<i>Pasteurella multocida</i>	Faster growth rate. Oxidase positive (may be weak). Indole positive. Colony morphology may appear mucoid.
<i>Pseudomonas luteola</i>	May produce yellow pigment. Glucose non-fermenter
<i>Pseudomonas</i> spp.	Oxidase positive (except <i>P. luteola</i> and <i>P. oryzihabitans</i>). Glucose non-fermenter.
<i>Shigella</i> spp.	Faster growth rate. Colony morphology. <i>Shigella</i> antisera
<i>Salmonella</i> spp., H ₂ S(-)	Faster growth rate. Colony morphology. <i>Salmonella</i> antisera
<i>Yersinia enterocolitica</i>	Small Gram negative coccobacilli. Urease positive* Indole variable
<i>Yersinia pseudotuberculosis</i>	Urease positive*
* <i>Y. pseudotuberculosis</i> and <i>Y. enterocolitica</i> 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. <i>Y. pestis</i> is the only species of <i>Yersinia</i> 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., H₂S(-) *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: