

# Michigan 2014 CAP LPX – B Survey Analysis

## **Introduction**

The College of American Pathologist (CAP) Laboratory Preparedness Exercise (LPX) survey provides clinical laboratories with an educational exercise that can be used to help prepare for the detection of pathogens of public health importance, including pathogens that might be used as biothreat (BT) agents. Another purpose of the LPX is to prepare participant laboratories for effective and efficient communication of critical information related to the detection of potential BT agents to public health authorities.

The LPX survey consists of organism identification (rule out) plus a notification component to test communications between Laboratory Response Network (LRN) Sentinel Laboratories and LRN Reference Labs. In these exercises, LRN Sentinel Labs are required to contact their LRN Reference Lab if, after following the established ASM Sentinel Level Clinical Laboratory Guidelines on a challenge isolate, they are unable to rule out an agent of bioterrorism.

Approximately 40% of Michigan sentinel labs participated in the 2014 LPX exercises. We congratulate participating laboratories for a job well done and encourage all laboratories to consider enrolling in this worthwhile educational activity.

This report includes a detailed laboratory analysis and growth characteristics for each LPX challenge sample and also summarizes the results of the Michigan LRN Sentinel Laboratories on the 2014 LPX-B survey panel in aggregate and compares participating labs throughout the country.

The **2014 LPX-B** survey contained the following samples:

LPX-04 *Yersinia enterocolitica*, in pure culture

LPX-05 *Yersinia pestis*, in pure culture

LPX-06 *Francisella tularensis*, in pure culture

Correct Result Reporting LPX-B N = 37	
Sample Number	% of MI Labs with Intended Response
LPX-04	100.0% (34/34)
LPX-05	67.6% (25/37)
LPX-06	94.6% (35/37)

## **2014 LPX-B-04**

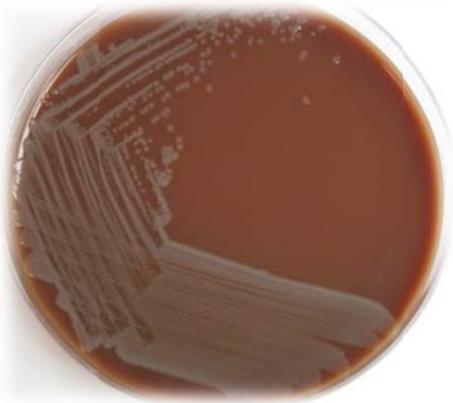
This challenge was a simulated stool specimen from a 10-year old with watery diarrhea and fever after attending a picnic.

### **CULTURE CHARACTERISTICS AT 24 HOURS:**



#### **5% SHEEP BLOOD AGAR:**

Small, shiny gray opaque colonies with greening of the agar at 24 hours.



#### **CHOCOLATE AGAR:**

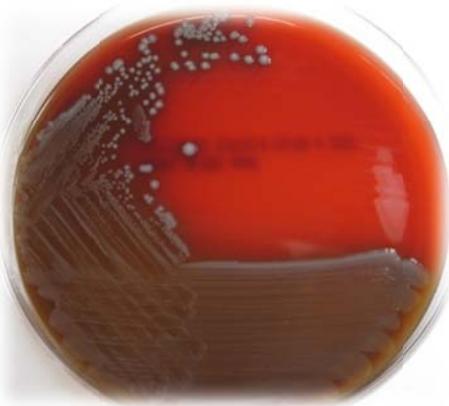
As blood agar, but slightly larger colonies, at 24 hours.



#### **MACCONKEY AGAR:**

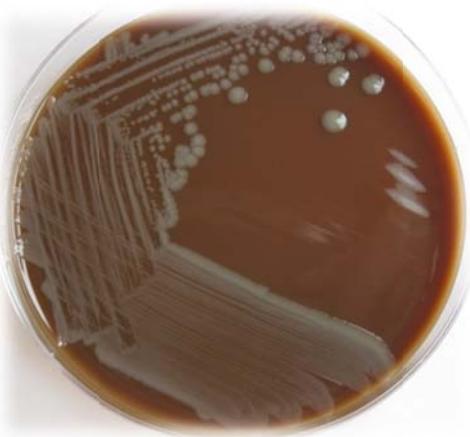
Pinpoint non-lactose fermenter at 24 hours.

**CULTURE CHARACTERISTICS AT 48 HOURS:**



**5% SHEEP BLOOD AGAR:**

Non-hemolytic, white opaque colonies with entire edge at 48 hours. (slight greening of the agar)



**CHOCOLATE AGAR:**

Large, shiny, white, opaque colonies with entire edge at 48 hours.



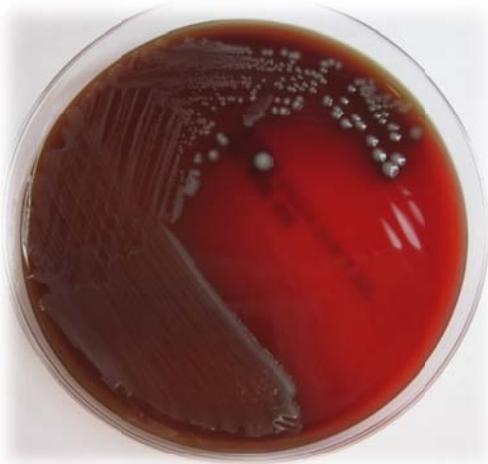
**MACCONKEY AGAR:**

Slightly pink, non-lactose fermenting colonies at 48 hours.

**CULTURE CHARACTERISTICS AT 72 HOURS:**

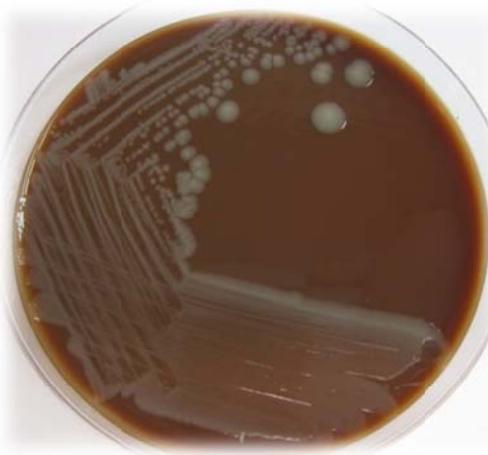
**5% SHEEP BLOOD AGAR:**

Large, white, opaque colonies with entire edge at 72 hours.



**CHOCOLATE AGAR:**

As 48 hour chocolate agar, but larger at 72 hours.



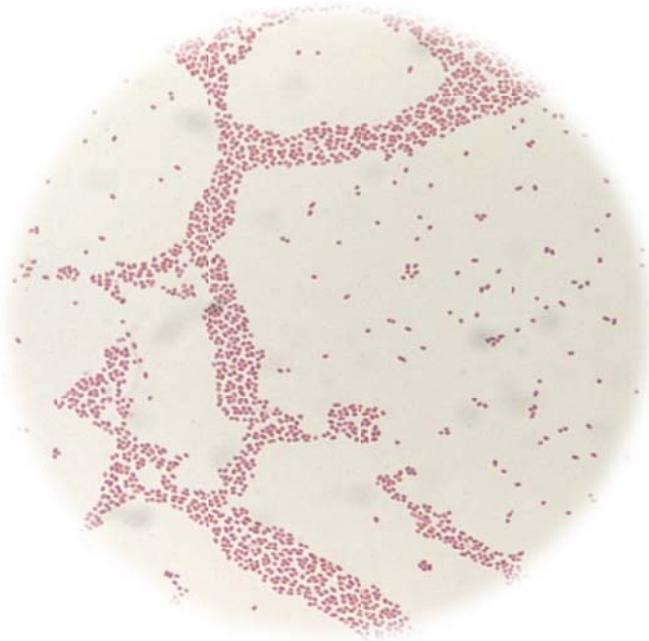
**MACCONKEY AGAR:**

As 48 hour MacConkey agar, but larger at 72 hours.



## **GRAM STAIN:**

Short, straight, Gram-negative rods.



This Gram stain, as well as the growth present on MacConkey agar is not consistent with what would be expected with either a *Brucella* species or *Francisella tularensis*. The other potential select agent Gram-negative rods would need to be ruled-out with further testing.

## **LABORATORY TESTING:**

### **CATALASE (3% HYDROGEN PEROXIDE) TEST:**

A catalase test was performed in the BSC and was positive, as indicated by the formation of bubbles upon the addition of 3% hydrogen peroxide. (SAFETY NOTE: the catalase test on any suspect agent of bioterrorism should always be performed in a Biological Safety Cabinet due to the potential of aerosol creation.)

OXIDASE TEST:

An oxidase test was performed and was oxidase negative. This rules out *Burkholderia pseudomallei* and further rules out a *Brucella* species.



Oxidase positive control



Oxidase negative control



Oxidase negative LPX-B-04

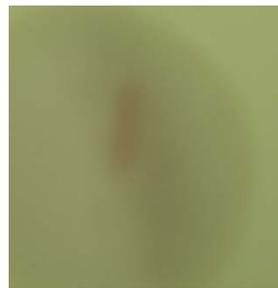
Further testing following the ASM Sentinel Lab procedures were performed, specifically the spot indole test, the urease test, the motility test, and growth characteristics at 42°C.

SPOT INDOLE TEST:

A spot indole test was performed and was positive, as indicated by the development of a blue color around the smear of the isolate on filter paper upon addition of the reagent. This result rules out both *Burkholderia mallei* and *Burkholderia pseudomallei*, as well as *Yersinia pestis*.



Spot indole positive control



Spot indole negative control



Spot indole positive LPX-B-04

UREA TEST:

The test for urease was performed and was urease positive. This result further rules out *Yersinia pestis*.



Urease positive  
control

Urease negative  
control

Urease positive  
LPX-B-04

MOTILITY TEST: (performed at 25°C)

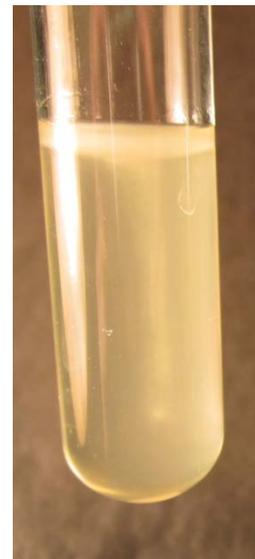
A semi-solid medium was used to detect motility and was positive, indicated by noticeable growth diffusion in the medium surrounding the stab line. This provides yet another test to rule out *Yersinia pestis*.



Motility positive control



Motility negative control



Motility positive LPX-B-04

42°C GROWTH:

A 5% sheep blood agar plate was inoculated and incubated at 42°C, and growth was visible within 24 hours. This test further rules out *Burkholderia mallei*.

SUMMARY:

2014 LPX-B-04 was catalase positive, oxidase negative, motile, non-hemolytic, MacConkey positive, non-lactose fermenting Gram-negative bacillus. These results, along with the positive spot indole, positive urea slant, and growth at 42°C, rule out all of the gram-negative agents of bioterrorism.

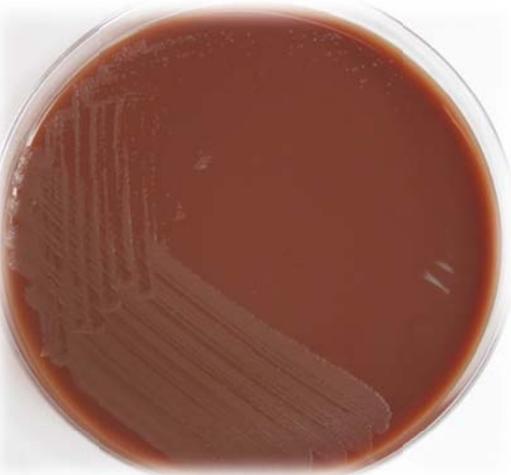
**2014 LPX-B-05**

This challenge was a simulated blood specimen from a 58-year old farmer with fever.

**CULTURE CHARACTERISTICS AT 24 HOURS:**



5% SHEEP BLOOD AGAR:  
Pinpoint colonies at 24 hours.



CHOCOLATE AGAR:  
As blood agar at 24 hour.



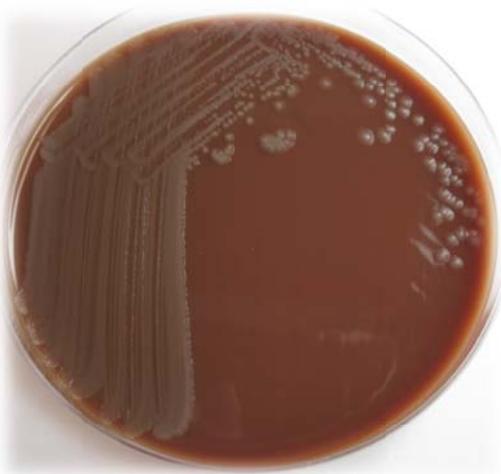
MACCONKEY AGAR:  
Pinpoint non-lactose fermenter at 24 hours.

**CULTURE CHARACTERISTICS AT 48 HOURS:**



**5% SHEEP BLOOD AGAR:**

Non-hemolytic, small, shiny, white colonies with a slight greening of the agar at 48 hours.



**CHOCOLATE AGAR:**

As blood agar, but slightly larger colonies, at 48 hours.



**MACCONKEY AGAR:**

Pinpoint non-lactose fermenter at 48 hours.

**CULTURE CHARACTERISTICS AT 72 HOURS:**

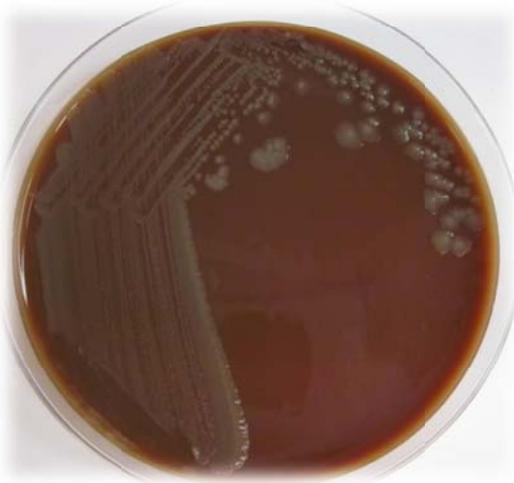
**5% SHEEP BLOOD AGAR:**

Non-hemolytic, shiny, white colonies with greening of the agar at 72 hours.



**CHOCOLATE AGAR:**

As blood agar at 72 hours.

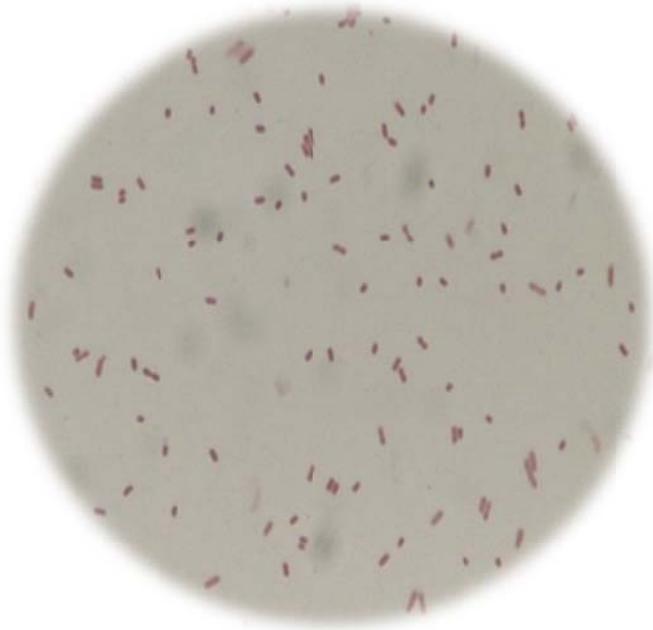


**MACCONKEY AGAR:**

Small, non-lactose fermenter at 72 hours.



### **GRAM STAIN:**



Short, Gram-negative rods and Gram-negative coccobacilli.

This Gram stain, along with growth on MacConkey agar, is not consistent with what would be expected with either a *Brucella* species or *Francisella tularensis*. The other potential select agent Gram-negative rods would need to be ruled out with further testing.

### **LABORATORY TESTING:**

#### **CATALASE (3% HYDROGEN PEROXIDE) TEST:**

A catalase test was performed in the BSC and was positive, as indicated by the formation of bubbles upon the addition of 3% hydrogen peroxide. (SAFETY NOTE: the catalase test on any suspect agent of bioterrorism should always be performed in a Biological Safety Cabinet due to the potential of aerosol creation.)

OXIDASE TEST:

An oxidase test was performed and was oxidase negative. This rules out *Burkholderia pseudomallei* and further rules out a *Brucella* species.



Oxidase positive control



Oxidase negative control



Oxidase negative LPX-B-04

Further testing following the ASM Sentinel Lab procedures were performed, specifically the spot indole test, the urease test, the motility test, and growth characteristics at 42°C.

SPOT INDOLE TEST:

A spot indole test was performed and was negative, as indicated by the lack of development of a blue color around the smear of the isolate on filter paper upon addition of the reagent. This result supports the potential identification of *Yersinia pestis*.



Indole positive control

Indole negative control

Indole negative LPX-B-05

## UREA TEST:

The test for urease was performed and was urease negative. This result also helps rule out a *Brucella* species, and provides further support of the potential identification of *Yersinia pestis*.



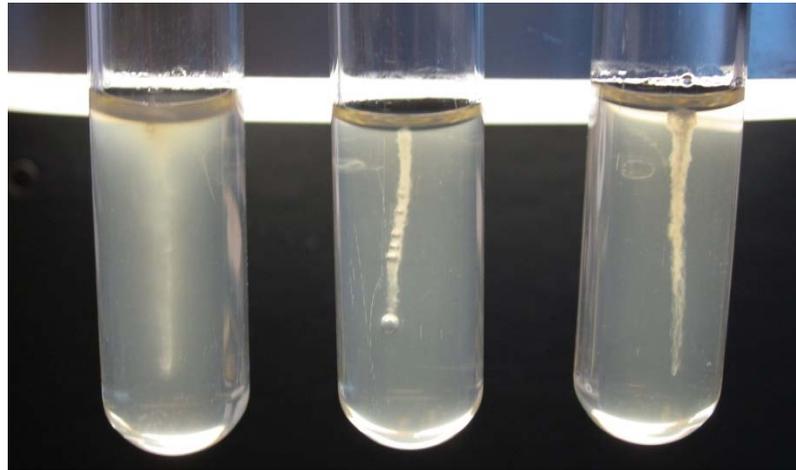
Urea positive  
control

Urea negative  
control

Urea negative  
LPX-B-05

## MOTILITY TEST: (performed at 25°C)

A semi-solid medium was used to detect motility and was negative, with no growth diffusion in the medium surrounding the stab line. This result provides an additional rule-out test for *Burkholderia pseudomallei*, and provides further support for the potential identification of *Yersinia pestis*.



Motility positive  
control

Motility negative  
control

Motility negative  
LPX-B-05

#### 42°C GROWTH:

A 5% sheep blood agar plate was inoculated and incubated at 42°C, and growth was visible, albeit poorly, within 24 hours. This result helps rule out *Burkholderia mallei*, and provides further support for the potential identification of *Yersinia pestis*.

#### SUMMARY:

2014 LPX-B-05 was a catalase positive, oxidase negative, non-motile, non-hemolytic, MacConkey positive non-lactose fermenting Gram-negative bacillus. These results, along with the negative indole test, the negative urea slant, and growth at 25°C and 35°C cannot rule out the possibility of *Yersinia pestis* based on the ASM Sentinel Laboratory Guidelines.

The intended response for Sentinel Laboratories was either “Suspect *Yersinia pestis*, refer for confirmation,” “*Yersinia* sp., refer to rule out *Yersinia pestis*,” or “Gram-negative bacillus, refer to rule out *Yersinia pestis*.”

Participants in the LPX exercises were required to contact their LRN Reference Laboratory on this isolate.

The CAP Laboratory Preparedness Exercise Final Critique identified LPX-B-05 as *Yersinia pestis* in pure culture.

## **2014 LPX-B-06**

This challenge was a simulated wound specimen from a 38-year-old hunter from Texas with an ulcerated skin lesion on his right arm and a low-grade fever.

### **CULTURE CHARACTERISTICS AT 24 HOURS:**



5% SHEEP BLOOD AGAR:  
Poor/scant growth at 24 hours



CHOCOLATE AGAR:  
Poor/scant growth at 24 hours



MACCONKEY AGAR:  
No growth at 24 hours

**CULTURE CHARACTERISTICS AT 48 HOURS:**



**5% SHEEP BLOOD AGAR:**  
Pinpoint growth at 48 hours



**CHOCOLATE AGAR:**  
Best growth at 48 hours: shiny, flat, 1 mm. diameter colonies, white to gray-white, opaque, with entire edges



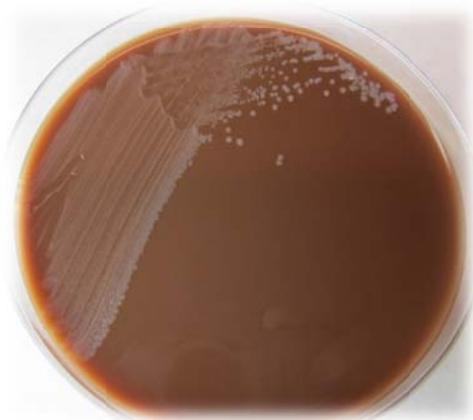
**MACCONKEY AGAR:**  
No growth at 48 hours

**CULTURE CHARACTERISTICS AT 72 HOURS:**



**5% SHEEP BLOOD AGAR:**

Shiny, flat, 1 mm. diameter colonies, white to gray-white, opaque, with entire edges at 72 hours



**CHOCOLATE AGAR:**

Shiny, flat, 2-3 mm. diameter colonies, white to gray-white, opaque, with entire edges at 72 hours

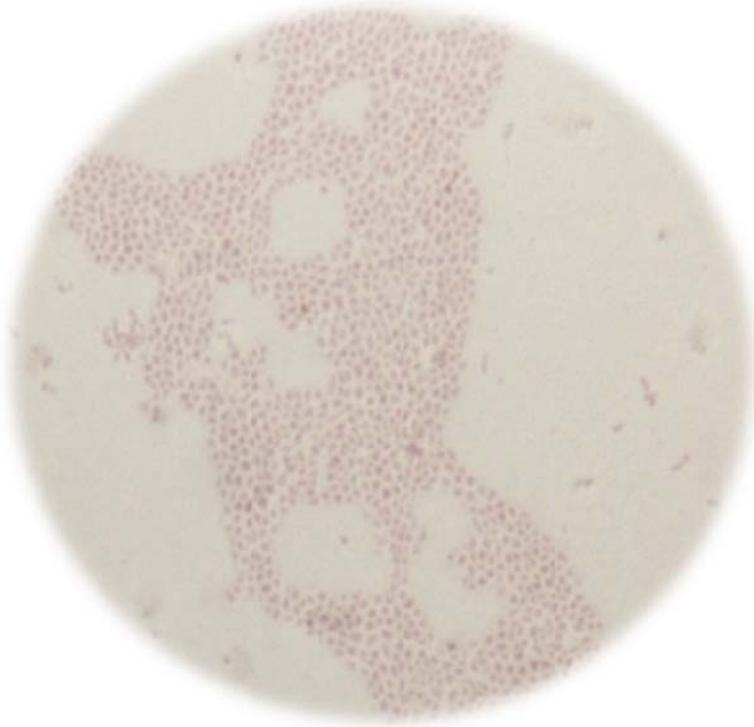


**MACCONKEY AGAR:**

No growth at 72 hours

**GRAM STAIN:** (of 48 hour chocolate agar)

Very small (0.2 to 0.5  $\mu\text{m}$  x 0.7 to 1.0  $\mu\text{m}$ ), faint-staining, pleomorphic Gram-negative coccobacilli.



This Gram stain could be interpreted to resemble either *Francisella tularensis* or *Brucella* species. Further testing is needed.

**LABORATORY TESTING:**

**CATALASE (3% HYDROGEN PEROXIDE) TEST:**

A catalase test was performed in the BSC and was weakly positive, with only a small number of bubbles observed. (SAFETY NOTE: the catalase test on any suspect agent of bioterrorism should always be performed in a Biological Safety Cabinet due to the potential of aerosol creation.)

## OXIDASE TEST:

An oxidase test was performed and was oxidase negative. This rules out a possible *Brucella* sp. The negative oxidase test supports a potential identification of *Francisella tularensis*.



Oxidase  
Positive  
control



Oxidase  
negative  
control



Oxidase  
negative  
LPX-B-06

Further testing following the ASM Sentinel Lab procedures for *Francisella tularensis* were performed. This includes urea, satellite test, and beta-lactamase.

UREA TEST:

The test for urease was performed and was urease negative. This result supports the potential identification of *Francisella tularensis*.



Urease positive control

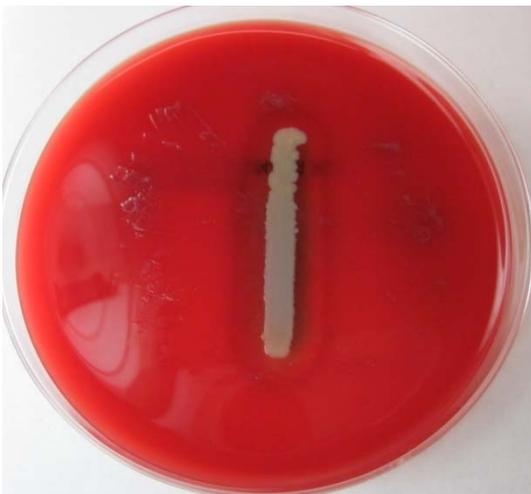


Urease negative control



Urease negative LPX-B-06

SATELLITE TEST:



Satellite test negative. This isolate is growing on Sheep Blood Agar well away from the *Staphylococcus aureus* ATCC 25923 streak. There is no enhanced growth along the Staph streak as would be seen with a *Haemophilus* species. This result supports the potential identification of *Francisella tularensis*.

NOTE: per ASM Sentinel guidelines, Sheep Blood Agar can support the growth of *Francisella* on initial culture but not upon subculture. This isolate continued to grow on SBA upon subculture but the best growth was upon chocolate agar.

### BETA-LACTAMASE TEST:

A beta-lactamase test (nitrocefin-based) was performed and was beta-lactamase positive. This result supports the potential identification of *Francisella tularensis*. *Aggregatibacter* species (a common misidentification of *F. tularensis*) would be beta-lactamase negative.



$\beta$ -lactamase  
positive  
control



$\beta$ -lactamase  
negative  
control



$\beta$ -lactamase  
positive  
LPX-B-06

### SUMMARY:

2014 LPX-B-06 was oxidase negative, weakly catalase positive, urease negative, satellite test negative, and beta-lactamase positive. Combined with the Gram stain, colony morphology and growth characteristics, as well as the clinical diagnosis, these results cannot rule out the possibility of *Francisella tularensis* based on the ASM Sentinel Laboratory Guidelines.

The intended response for Sentinel Laboratories was “Suspect *Francisella tularensis*, refer for confirmation,” “*Francisella* sp., refer to rule out *Francisella tularensis*,” or “Gram-negative bacillus/coccobacilli, refer to rule out *Francisella tularensis*”.

Participants in the LPX exercises were required to contact their LRN Reference Laboratory on this isolate.

The CAP Laboratory Preparedness Exercise Final Critique identified LPX-B-06 as *Francisella tularensis*, the live vaccine strain (LVS).

## Aggregate Performance Summary:

### Michigan Laboratory Results Compared to All Participants

LPX-04 <i>Yersinia enterocolitica</i>		
Submitted Answers	Michigan Participants	All Participants
* Non-BT Culture	32/34 94.1%	1267/1361 93.1%
* <i>Yersinia</i> sp., refer to rule out <i>Yersinia pestis</i>	1/34 2.9%	59/1361 4.3%
*Gram-negative bacillus, refer to rule out <i>Yersinia pestis</i>	1/34 2.9%	26/1361 1.9

\*Acceptable responses for Sentinel Laboratories

LPX-05 <i>Yersinia pestis</i>		
Submitted Answers	Michigan Participants	All Participants
* <i>Yersinia pestis</i> , confirmed	0/37 0.0%	57/1382 4.1%
*Suspect <i>Yersinia pestis</i> , refer for confirmation	7/37 18.9%	302/1382 21.9%
* <i>Yersinia</i> sp., refer to rule out <i>Yersinia pestis</i>	6/37 16.2%	173/1382 12.5%
*Gram-negative bacillus, refer to rule out <i>Yersinia pestis</i>	12/37 32.4%	532/1382 38.5%
Non-BT Culture	7/37 18.9%	203/1382 14.7%
<i>Burkholderia</i> sp.	5/37 13.5%	

\*Acceptable responses for Sentinel Laboratories

LPX-06 <i>Francisella tularensis</i>		
Submitted Answers	Michigan Participants	All Participants
* <i>Francisella tularensis</i> , confirmed	0/37 0.0%	64/1387 4.6%
*Suspect <i>Francisella tularensis</i> , refer for confirmation	9/37 24.3%	383/1387 27.6%
* <i>Francisella</i> sp., refer to rule out <i>Francisella tularensis</i>	6/37 16.2%	123/1387 8.9%
*Gram-negative bacillus/ coccobacillus, refer to rule out <i>Francisella tularensis</i>	26/37 70.3%	733/1387 52.9%
Non-BT Culture	2/37 5.4%	67/1387 4.8%

\*Acceptable responses for Sentinel Laboratories

### Notification Drill

Michigan Laboratories Notifying their LRN Reference Laboratory N = 37			
Sample Number	Notification Required?	%MI Labs Indicating Notification to the LRN Reference Lab	% MI Labs Actually Notified the LRN Reference Lab
LPX-04	No	Not required	-
LPX-05	Yes	100.0%	72.2%
LPX-06	Yes	100.0%	80.6%

### Final Comments

Public health happens every day in clinical laboratories in Michigan. The work you do effects not just the patients in your facilities but can impact the lives of all the citizens in your local community, Michigan and the nation. It is your continued preparedness that ensures the health and safety of Michigan. Thank you for participating in this CAP LPX exercise.

Michigan Department of Community Health  
Bureau of Laboratories