

Michigan 2015 CAP LPX – A 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 2015 LPX survey. We congratulate participating laboratories for a job well done and encourage all laboratories to consider enrolling in this worthwhile educational exercise.

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 2015 LPX-A survey panel in aggregate and compares participating labs throughout the country.

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

- LPX-01 *Francisella tularensis*, live vaccine strain
- LPX-02 *Yersinia enterocolitica*, in pure culture
- LPX-03 *Bacillus anthracis*, in pure culture

Correct Result Reporting LPX-A N = 35	
Sample Number	% of MI Labs with Intended Response
LPX-01	97.1% (34/35)
LPX-02	100.0% (33/33)
LPX-03	97.1% (34/35)

2015 LPX-A-01

This challenge was a simulated wound specimen from a rabbit hunter in Wyoming with an ulcerated lesion on his left arm.

CULTURE CHARACTERISTICS AT 24 HOURS:



5% SHEEP BLOOD AGAR:

Poor/scant growth at 24 hours



CHOCOLATE AGAR:

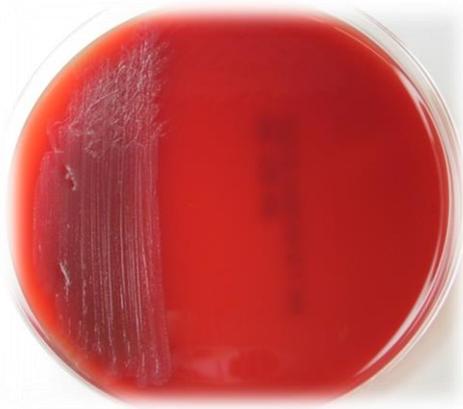
Poor/scant growth at 24 hours



MACCONKEY AGAR:

No growth at 24 hours

CULTURE CHARACTERISTICS AT 48 HOURS:



5% SHEEP BLOOD AGAR:

Pinpoint growth at 48 hours



CHOCOLATE AGAR:

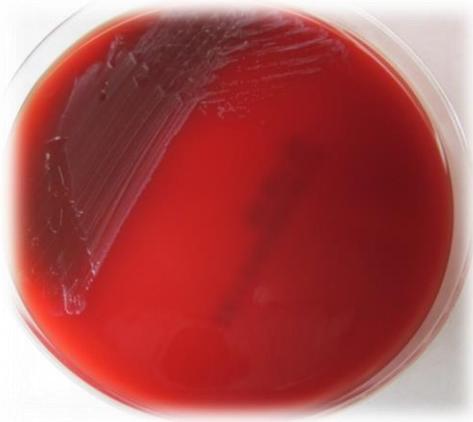
Best growth at 48 hours: shiny, flat, 1 mm. diameter colonies, white to gray-white, opaque, with entire edges



MACCONKEY AGAR:

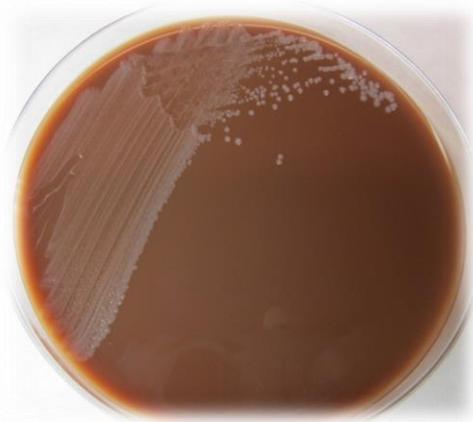
No growth at 48 hours

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:

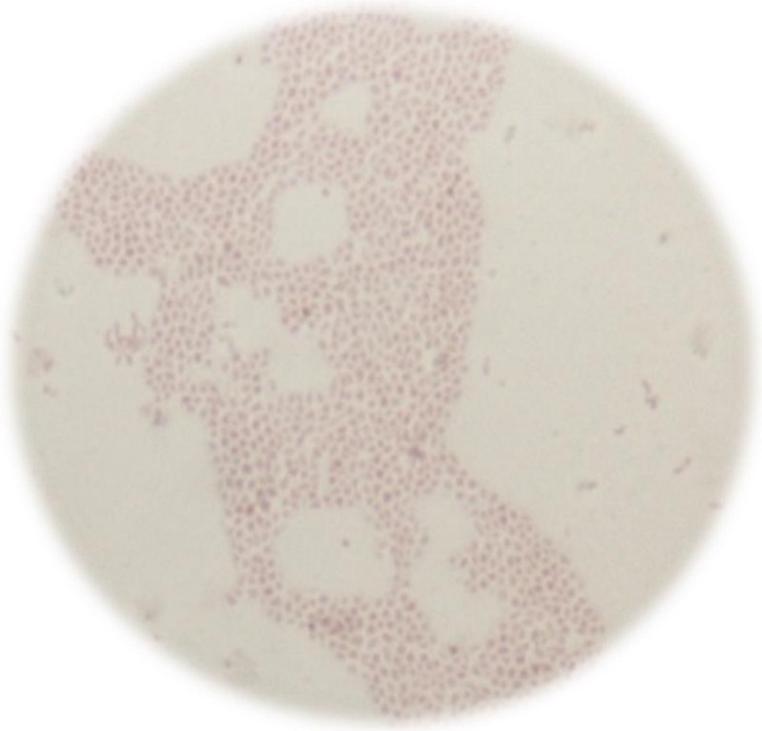
Shiny, flat, 2-3 mm. diameter colonies, white to gray-white, opaque, with entire edges at 72 hours



MACCONKEY AGAR:

No growth at 72 hours

GRAM STAIN: (of 48 hour chocolate agar)



Very small (0.2 to 0.5 μm x 0.7 to 1.0 μm), faint-staining, pleomorphic Gram-negative coccobacilli.

This Gram strain could be interpreted to resemble either *Francisella tularensis* or *Brucella* species. Further testing 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-A-01

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*.



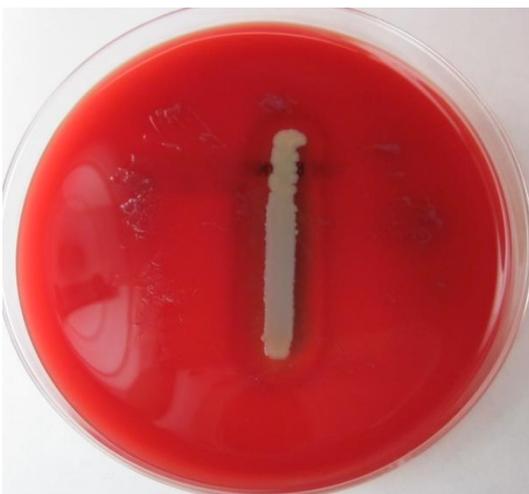
Urease
positive
control



Urease
negative
control



Urease
negative
LPX-A-01



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.



β-lactamase
positive
control



β-lactamase
negative
control



β-lactamase
positive
LPX-A-01

SUMMARY:

2015 LPX-A-01 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-A-01 as *Francisella tularensis*, the live vaccine strain (LVS).

2015 LPX-A-02

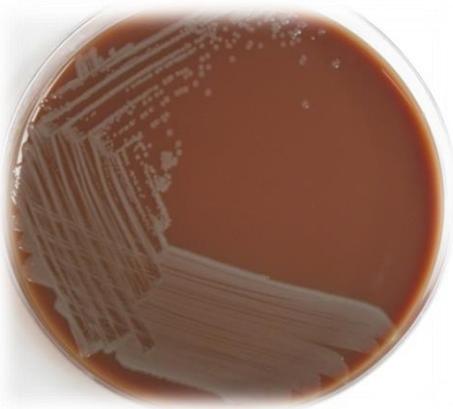
This challenge was a simulated stool specimen from a 30-year old with watery diarrhea.

CULTURE CHARACTERISTICS AT 24 HOURS:



5% SHEEP BLOOD AGAR:

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



CHOCOLATE AGAR:

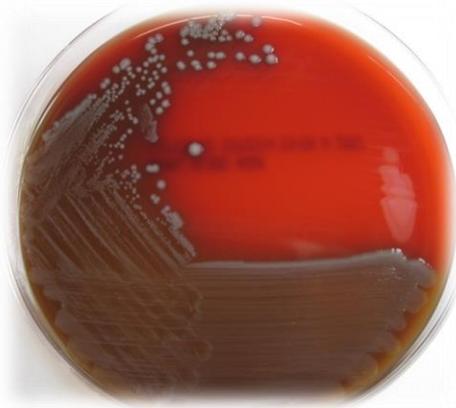
As blood agar, but slightly larger colonies, at 24 hours.



MACCONKEY AGAR:

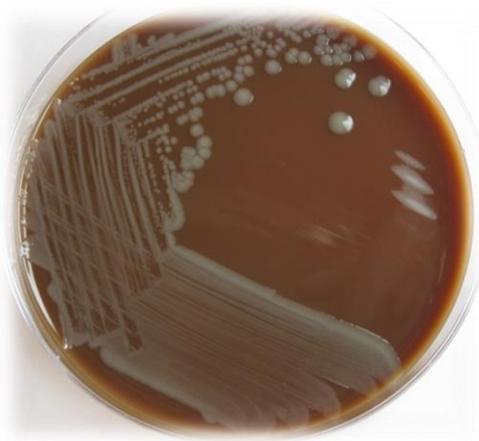
Pinpoint non-lactose fermenter at 24 hours.

CULTURE CHARACTERISTICS AT 48 HOURS:



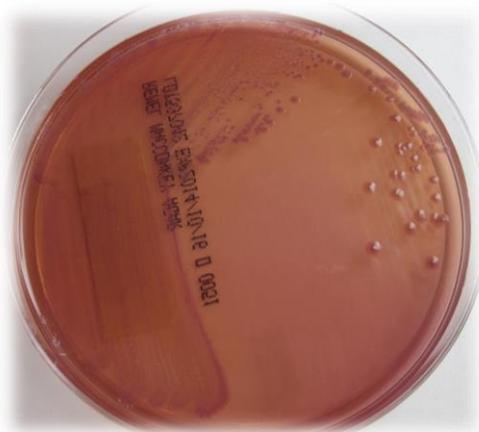
5% SHEEP BLOOD AGAR:

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



CHOCOLATE AGAR:

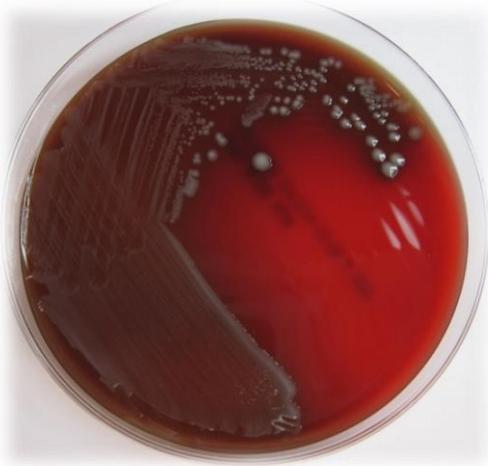
Large, shiny , white, opaque colonies with entire edge at 48 hours.



MACCONKEY AGAR:

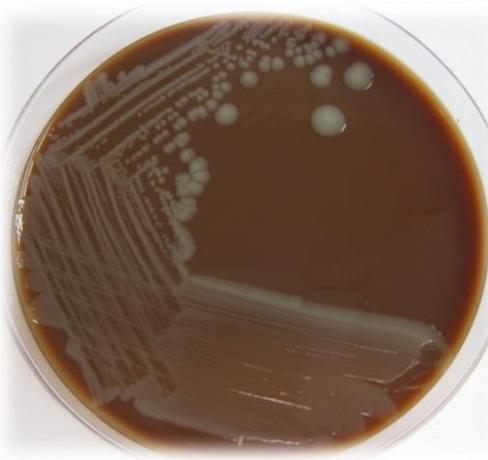
Slightly pink, non-lactose fermenting colonies at 48 hours.

CULTURE CHARACTERISTICS AT 72 HOURS:



5% SHEEP BLOOD AGAR:

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



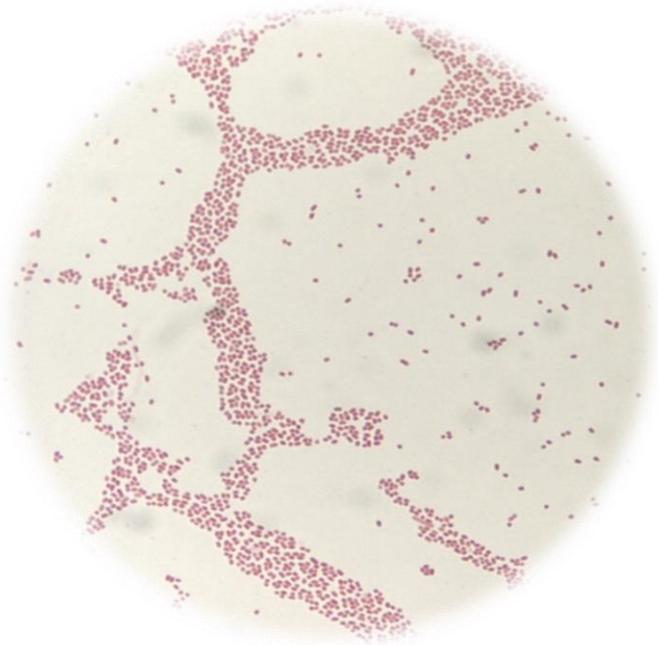
CHOCOLATE AGAR:

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-A-02

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*.



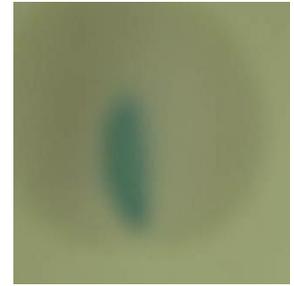
Spot indole positive

Control



Spot indole negative

control



Spot indole positive

LPX-A-02

UREA TEST:

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



control

control

LPX-A-02

MOTILITY TEST: (performed at 25°C)

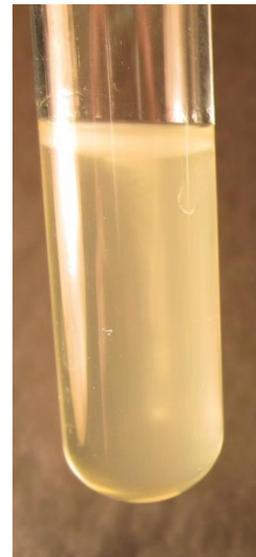
A semi-solid medium was used to detect motility and was positive, indicated by noticeable growth diffusion in the medium surrounding the stab line. This provides yet another test to rule out *Yersinia pestis*.



Motility positive
control



Motility negative
control



Motility positive
LPX-A-02

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:

2015 LPX-A-02 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.

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

Participants in the LPX exercises were not required to contact their LRN Reference Laboratory on this isolate.

The CAP Laboratory Preparedness Exercise Final Critique identified LPX-A-02 as *Yersinia enterocolytica* in pure culture. The Final critique emphasizes that clinical microbiology laboratories should be able to maintain competence in the identification of *Yersinia enterocolytica*, since it is considered an important human pathogen.

2015 LPX-A-03

This challenge was a simulated wound specimen from a 40 year old farmer with shortness of breath after returning from the Middle East.

CULTURE CHARACTERISTICS AT 24 HOURS:



5% SHEEP BLOOD AGAR:

Good growth at 24 hours. Colonies are 2-5 mm in diameter and round with irregular/erose edges and a “ground-glass” appearance. The colonies are gray, opaque, and non-hemolytic. When touched with a loop, the colonies have a tenacious consistency similar to beaten egg whites.



CHOCOLATE AGAR:

Good growth at 24 hours. Colony morphology is the same as the 5% sheep blood agar.



MACCONKEY AGAR:

No growth at 24 hours.

CULTURE CHARACTERISTICS AT 48 HOURS:



5% SHEEP BLOOD AGAR:

Colony characteristics same as 24 hour growth but obviously larger at 48 hours.



CHOCOLATE AGAR:

Colony morphology is the same as the 5% sheep blood agar plate at 48 hours.



MACCONKEY AGAR:

No growth at 48 hours.

CULTURE CHARACTERISTICS AT 72 HOURS:



5% SHEEP BLOOD AGAR:

Colony characteristics same as 24 hour and 48 hour growth but even larger at 72 hours.



CHOCOLATE AGAR:

Colony morphology is the same as the 5% sheep blood agar plate at 72 hours.



MACCONKEY AGAR:

No growth at 72 hours.

GRAM STAIN: (of 24 hour 5% sheep blood agar)



Large, broad width (1.5 to 2 μm) straight chaining Gram-positive bacillus with an occasional oval, central to subterminal spore which does not swell the vegetative cell.

This Gram stain is typical of a *Bacillus* species. Further testing following the ASM Sentinel Level Guidelines are needed to rule-in or rule-out *Bacillus anthracis*.

LABORATORY TESTING:

CATALASE (3% HYDROGEN PEROXIDE) TEST:

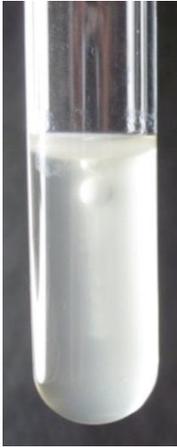
A catalase test was performed in the BSC and was positive, which supports a potential identification of *Bacillus anthracis*.

BETA-HEMOLYSIS:

This isolate is non-hemolytic on 5% sheep blood agar, which supports a potential identification of *Bacillus anthracis*.

MOTILITY TEST:

A semi-solid medium was used to detect motility and was negative, indicated by no diffusion from the stab line, with growth accentuated along the stab line only and the surrounding medium remaining clear. This supports a potential identification of *Bacillus anthracis*,



Motility
positive
control



Motility
negative
control



Motility
negative
LPX-A-03

SUMMARY:

2015 LPX-A-03 was a catalase positive, non-motile, non-hemolytic broad Gram-positive bacillus. These results combined with the colony morphology and growth characteristics, as well as the clinical diagnosis, cannot rule out the possibility of *Bacillus anthracis* based on the ASM Sentinel Laboratory Guidelines.

The intended response for Sentinel Laboratories was “Suspect *Bacillus anthracis*, refer for confirmation,” “*Bacillus* species, refer to rule-out *Bacillus anthracis*,” or “Gram-positive bacillus, refer to rule-out *Bacillus anthracis*.”

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-A-03 as *Bacillus anthracis* in pure culture.

Important Information

Testing performed on these isolates utilized the Sentinel Level Clinical Laboratory Guidelines and the accompanying LRN Sentinel Level Testing Protocols. The American Society for Microbiology (ASM), in partnership with the Association of Public Health Laboratories (APHL), and the Centers for Disease Control and Prevention (CDC), serves as the lead agency for maintaining the protocols and making them available to the Sentinel Level Clinical Laboratory community. These guidelines have been developed to promote uniform and standardized testing among clinical laboratories.

The guidelines are compliant with the Clinical Laboratory Standards Institute (CLSI) format based on current information and recommendations of the APHL Sentinel Laboratory Partnerships and Outreach Subcommittee.

Please refer to the following link to review and update your testing procedures:

<http://www.asm.org/index.php/guidelines/sentinel-guidelines>.

Aggregate Performance Summary:

Michigan Laboratory Results Compared to All Participants

LPX-01 <i>Francisella tularensis</i>		
Submitted Answers	Michigan Participants	All Participants
* <i>Francisella tularensis</i> , confirmed	0/35 0.0%	64/1370 4.7%
*Suspect <i>Francisella tularensis</i> , refer for confirmation	12/35 34.2%	451/1370 32.9%
* <i>Francisella</i> sp., refer to rule out <i>Francisella tularensis</i>	1/35 2.9%	126/1370 9.2%
*Gram-negative bacillus/ coccobacillus, refer to rule out <i>Franciseall tularensis</i>	21/35 60.0%	689/1370 50.3%
Non-BT Culture	1/35 2.9%	34/1370 2.5%

*Acceptable responses for Sentinel Laboratories

LPX-02 <i>Yersinia enterocolitica</i>		
Submitted Answers	Michigan Participants	All Participants
* Non-BT Culture	33/33 100.0%	1232/1332 92.5%
* <i>Yersinia</i> sp., refer to rule out <i>Yersinia pestis</i>	0/33 0.0%	58/1332 4.3%
*Gram-negative bacillus, refer to rule out <i>Yersinia pestis</i>	0/33 0.0%	26/13332 2.0%

*Acceptable responses for Sentinel Laboratories

LPX-03 <i>Bacillus anthracis</i>		
Submitted Answers	Michigan Participants	All Participants
*Equivocal for <i>Bacillus anthracis</i>	0/35 0.0%	57/1372 4.2%
*Suspect <i>Bacillus anthracis</i> , refer for confirmation	5/35 14.3%	344/1372 25.1%
* <i>Bacillus</i> sp., refer to rule out <i>Bacillus anthracis</i>	18/35 51.4%	635/1372 46.3%
*Gram-positive bacillus, refer to rule out <i>Bacillus anthracis</i>	11/35 31.4%	278/1372 20.3%
Non-BT Culture	1/35 2.9%	67/1372 4.9%

*Acceptable responses for Sentinel Laboratories

Notification Drill

Michigan Laboratories Notifying their LRN Reference Laboratory N = 35			
Sample Number	Notification Required?	%MI Labs Indicating Notification to the LRN Reference Lab	% MI Labs Actually Notified the LRN Reference Lab
LPX-01	Yes	97.1%	68.6%
LPX-02	No	Not required	-
LPX-03	Yes	97.1%	71.4%

To test communications between LRN Sentinel Laboratories and LRN Reference Laboratories, participants in the LPX exercises are required to contact their LRN Reference Laboratory, if, after performing the established Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases on a challenge isolate, they are unable to rule out an agent of bioterrorism. For this exercise isolates LPX-01 and LPX-03 should have triggered a communication with the participant's assigned regional public health laboratories in Kalamazoo, Kent, Saginaw and Oakland Counties or with the MDHHS Bureau of Laboratories in Lansing.