

Michigan 2014 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 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 surveys. 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 2014 LPX-A survey panel in aggregate and compares participating Michigan laboratory responses to those of participating labs throughout the country.

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

- LPX-01 *Bacillus anthracis*, in pure culture
- LPX-02 *Eikenella corrodens*, in pure culture
- LPX-03 *Haemophilus influenzae*, in pure culture

| Correct Result Reporting LPX-A | | N = 37 |
|--------------------------------|-------------------------------------|--------|
| Sample Number | % of MI Labs with Intended Response | |
| LPX-01 | 94.6% (35/37) | |
| LPX-02 | 100% (37/37) | |
| LPX-03 | 94.6% (35/37) | |

2014 LPX-A-01

This challenge was a simulated wound culture from a 50 year old archaeologist from Turkey.

CULTURE CHARACTERISTICS AT 24 HOURS:



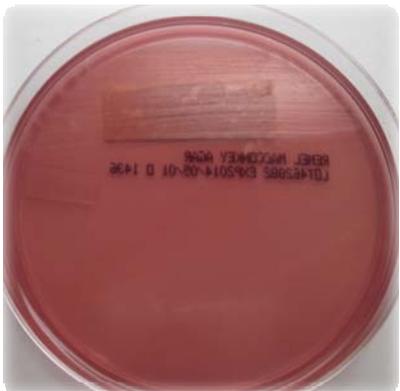
5% SHEEP BLOOD AGAR:

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:

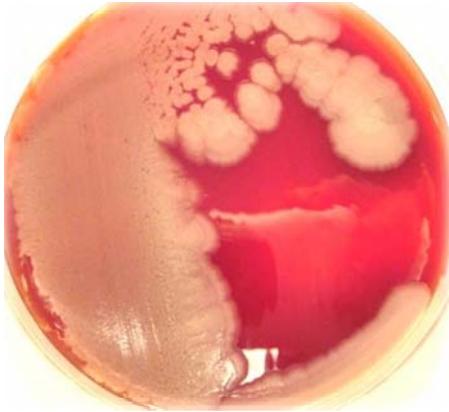
Colony morphology is the same as the 5% sheep blood agar plate at 48 hours.



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 Clinical Laboratory 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 biological safety cabinet (BSC) and was positive, which supports a potential identification of *Bacillus anthracis*. (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.)

BETA-HEMOLYSIS:

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

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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-01

SUMMARY:

2014 LPX-A-01 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 Level Clinical 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-01 as *Bacillus anthracis* in pure culture.

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2014 LPX-A-02

This challenge was a simulated hand wound from a 40 year old with a history of fighting.

CULTURE CHARACTERISTICS AT 24 HOURS:



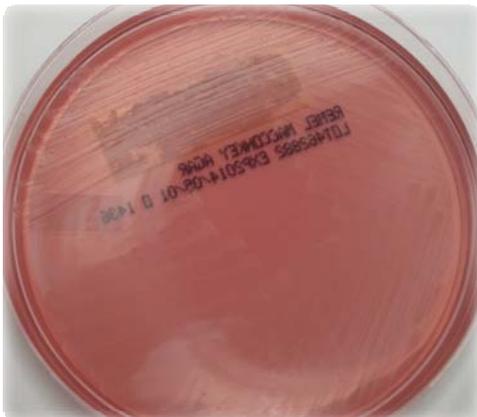
5% SHEEP BLOOD AGAR:

Pinpoint colonies at 24 hours



CHOCOLATE AGAR:

Pinpoint colonies at 24 hours.



MACCONKEY AGAR:

No growth at 24 hours.

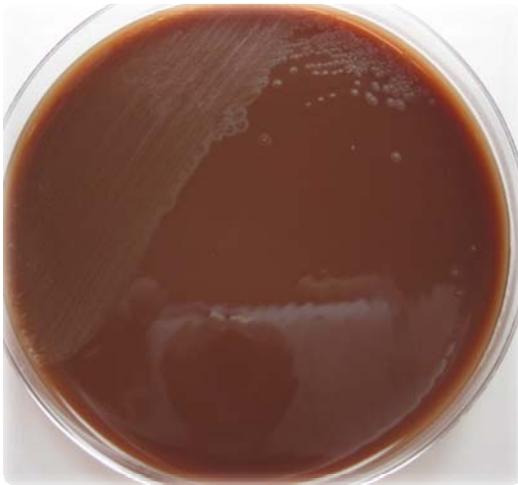
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CULTURE CHARACTERISTICS AT 48 HOURS:



5% SHEEP BLOOD AGAR:

Small, non-hemolytic, off-white, and opaque colonies with an erose edge and a raised center at 48 hours.



CHOCOLATE AGAR:

Colony morphology is the same as the 5% sheep blood agar at 48 hours, except the colonies are more yellow.

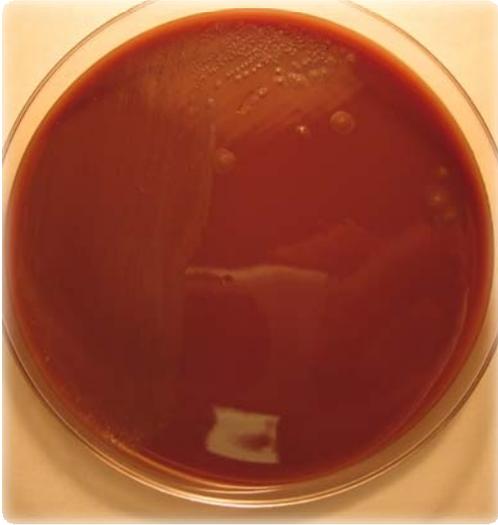


MACCONKEY AGAR:

No growth at 48 hours.

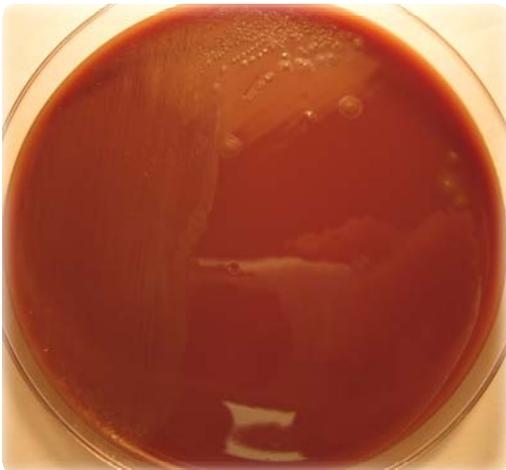
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CULTURE CHARACTERISTICS AT 72 HOURS:



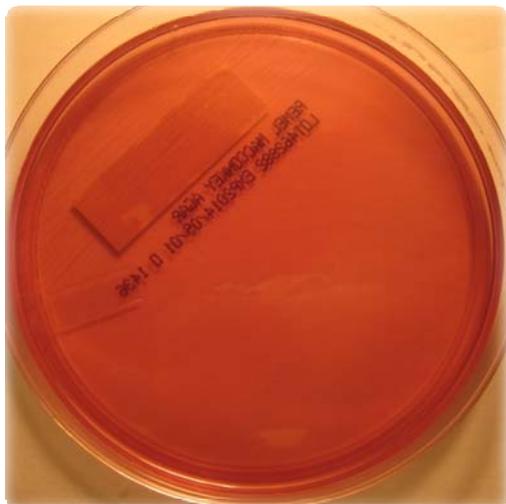
5% SHEEP BLOOD AGAR:

Colony morphology similar to 48 hour growth, but slightly larger at 72 hours.



CHOCOLATE AGAR:

Colony morphology similar to 48 hour growth, but slightly larger at 72 hours.



MACCONKEY AGAR:

No growth at 72 hours.

GRAM STAIN: (of 24 hour blood agar plate)



Short to medium length, medium width, Gram-negative bacilli, occurring in singles and pairs.

Further testing is required to rule-in or rule-out the Gram-negative biological threat agents. (This Gram stain does not fit the classic morphology of a *Brucella* species or *Francisella tularensis*.)

LABORATORY TESTING:

CATALASE (3% HYDROGEN PEROXIDE) TEST:

A catalase test was performed in the BSC on this organism and was negative, with no bubbles being 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.)

This negative catalase test helps rule out *Burkholderia mallei* and *Burkholderia pseudomallei* as well as *Yersinia pestis*. It also further supports the rule-out of *Brucella* species. *Francisella tularensis*, however, while usually positive, could be weakly positive or even negative.

OXIDASE TEST:

An oxidase test was performed and it was oxidase positive. This result further helps to rule-out *Yersinia pestis* and *Francisella tularensis*, as well as most *Burkholderia mallei* isolates.



Oxidase positive
control

Oxidase negative
control

Oxidase positive
LPX-A-02

SPOT INDOLE TEST:

A spot indole test was performed and was negative. This result does not rule-out *Burkholderia mallei* or *Burkholderia pseudomallei*.



Indole positive
control

Indole negative
control

Indole negative
LPX-A-02

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 result further rules-out *Burkholderia pseudomallei*.



Motility Negative LPX-A 02

GROWTH AT 42°:

A 5% sheep blood agar was inoculated and incubated at 42 degrees C. This isolate displayed pinpoint growth at 24 hours, which further rules out *Burkholderia mallei*.



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ARGININE DECARBOXYLASE TEST:

An arginine decarboxylase test was performed and was negative. This further rules out *Burkholderia mallei* and *Burkholderia pseudomallei*, which unlike other *Burkholderia* species, are positive.



Arginine
positive control



Arginine
negative control



Arginine
negative LPX-A-02

SUMMARY:

2014 LPX-A-02 was a catalase negative, oxidase positive, non-motile Gram-negative bacillus. The isolate was also spot indole negative, arginine negative, and grew at 42°C. These results in combination with the colony morphology and growth characteristics, rule-out the possibility of a select agent based on the ASM Sentinel Level Clinical Laboratory guidelines. Note: According to the ASM Sentinel Laboratory Guidelines, Polymyxin B, colistin, penicillin, and amoxicillin-clavulanate testing would also be indicated.

The intended response for Sentinel Laboratories was “Non-BT culture.”

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 *Eikenella corrodens* in pure culture.

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2014 LPX-A-03

This challenge was a simulated sputum culture from a two year old who had not been vaccinated due to the family's religious beliefs.

CULTURE CHARACTERISTICS AT 24 HOURS:

5% SHEEP BLOOD AGAR:

No growth at 24 hours.



CHOCOLATE AGAR:

Good growth with small (1-2 mm) tan, opaque colonies at 24 hours.



MACCONKEY AGAR:

No growth at 24 hours.



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CULTURE CHARACTERISTICS AT 48 HOURS:



5% SHEEP BLOOD AGAR:

No growth at 48 hours.



CHOCOLATE AGAR:

Tan, circular, and opaque colonies with entire and slightly erose edges at 48 hours.



MACCONKEY AGAR:

No growth at 48 hours.

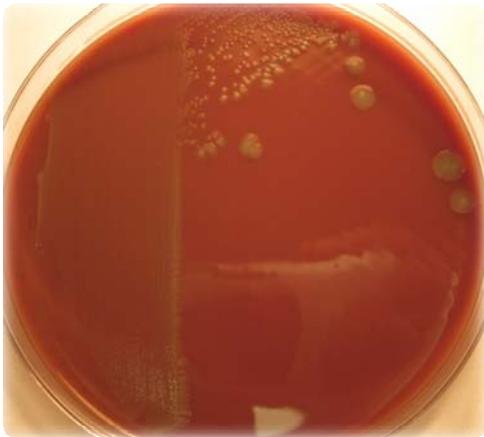
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CULTURE CHARACTERISTICS AT 72 HOURS:



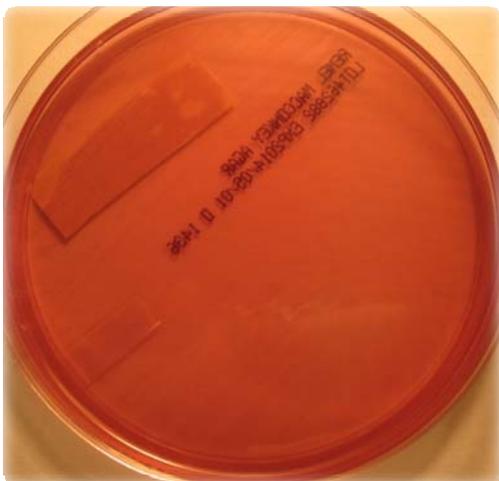
5% SHEEP BLOOD AGAR:

No growth at 72 hours.



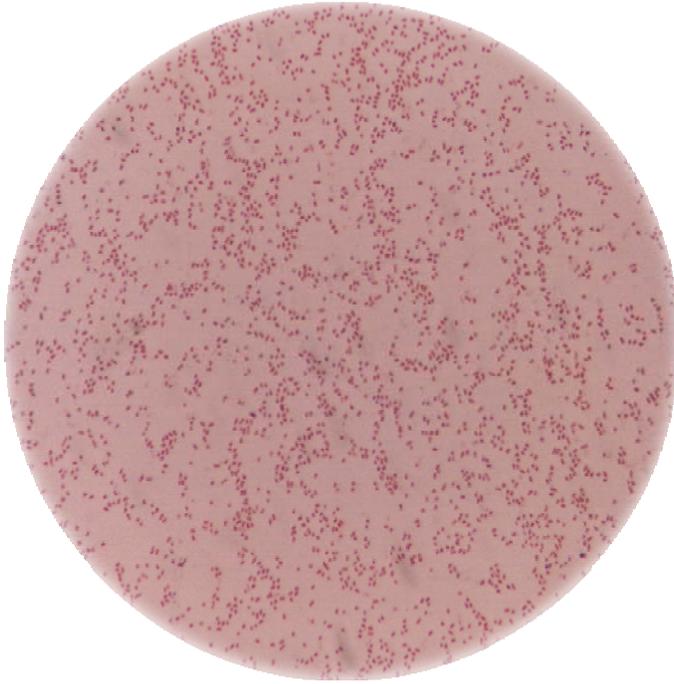
CHOCOLATE AGAR:

Colony morphology similar to chocolate agar at 48 hours but slightly larger at 72 hours.



MACCONKEY AGAR:

No growth at 72 hours.



GRAM STAIN: (of 48 hour chocolate agar)

Small, pleomorphic Gram-negative coccobacilli.

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

LABORATORY TESTING:

CATALASE (3% HYDROGEN PEROXIDE) TEST:

A catalase test was performed in the BSC and was positive, with the generation 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.)

A positive catalase test is observed with *Brucella* species and *Francisella tularensis* (although *F.tularensis* may be weak or even negative).

OXIDASE TEST:

An oxidase test was performed and was oxidase positive. This result rules out the possibility of *Francisella tularensis*.



Oxidase positive
control



Oxidase negative
control



Oxidase positive
LPX-A-03

BETA-LACTAMASE TEST:

A beta-lactamase test (nitrocefin-based) was performed and was beta-lactamase negative, as indicated by the lack of a pink to red color change on the disk. This result further rules out the possibility of *Francisella tularensis*.



Further testing following the ASM Sentinel Level Clinical Laboratory Guidelines for *Brucella* species were performed. This includes a urea and satellite test.

UREA TEST:

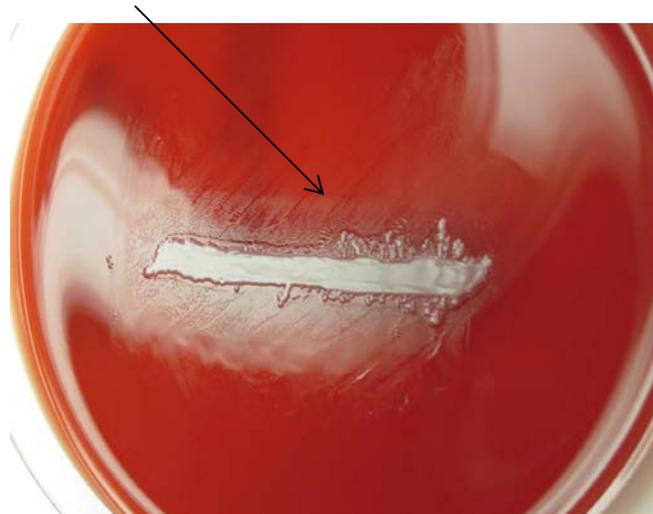
A urease test was performed and was urease positive, as indicated by the development of a pink color due to the shift in pH to alkaline.



This result continues to support the potential identification of a *Brucella* species.

SATELLITE TEST:

A satellite test was performed and was positive, as indicated by enhanced growth only along the *Staphylococcus aureus* ATCC 25923 streak line. This result rules out the possibility of a *Brucella* species. In conjunction with the observation that this isolate only grew on Chocolate agar, one would need to consider a *Haemophilus* species as the identification.



SUMMARY:

2014 LPX-A-03 was an oxidase positive, catalase positive, beta-lactamase negative, urease positive, and satellite test positive. Combined with the Gram stain, colony morphology and growth characteristics, as well as the clinical diagnosis, these results rule out the possibility of a select agent based on the ASM Sentinel Level Clinical Laboratory Guidelines.

The intended response for Sentinel Laboratories was “Non-BT Culture.”

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-03 as *Haemophilus influenzae* 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>Bacillus anthracis</i> | | |
|---|-----------------------|-------------------|
| Submitted Answers | Michigan Participants | All Participants |
| § <i>Bacillus anthracis</i> , confirmed | 0/37 0% | 41/1394 2.9% |
| § Suspect <i>Bacillus anthracis</i> , refer for confirmation | 6/37 16.2% | 328/1394 23.5% |
| § <i>Bacillus</i> sp., refer to rule out <i>B. anthracis</i> | 28/37 75.7% | 784/1394 56.2% |
| § Gram-positive bacillus, refer to rule out <i>Bacillus anthracis</i> | 1/37 2.7% | 197/1394 14.1% |
| Non-BT Culture | 2/37 5.4% | 57/1394 4.1% |

§ Acceptable response for Sentinel Laboratories

| LPX-02: <i>Eikenella corrodens</i> | | |
|------------------------------------|-----------------------|--------------------|
| Submitted Answers | Michigan Participants | All Participants |
| § Non-BT Culture | 37/37 100% | 1309/1383 94.6% |

§ Acceptable response for Sentinel Laboratories

| LPX-03: <i>Haemophilus influenzae</i> | | |
|--|-----------------------|--------------------|
| Submitted Answers | Michigan Participants | All Participants |
| § Non-BT Culture | 35/37 94.5% | 1276/1388 91.9% |
| Gram-negative bacillus, refer to rule out <i>F. tularensis</i> | 2/37 5.4% | - |

§ Acceptable response for Sentinel Laboratories

Notification Drill

| Michigan Laboratories Notifying Their LRN Ref Lab | | | | N = 37 |
|---|-----------------------|---|---|--------|
| Sample Number | Notification Required | % MI Labs Indicating Would Notify the LRN Reference Lab | % MI Labs Actually Notified the LRN Reference Lab | |
| LPX-01 | Yes | 91.7%^ | 78.4% | |
| LPX-02 | No | Not Required | - | |
| LPX-03 | No | 50% of labs unable to rule out a BT agent^ | - | |

^ One laboratory suspecting biothreat agents indicated they would forward these specimens to a commercial reference laboratory.

Notice: When unable to rule out an agent of bioterrorism in an LPX exercise, LRN Sentinel Laboratories are required to contact their LRN Reference Laboratory. Please remember to state “This is an exercise” when speaking with your LRN Reference Laboratory about an LPX sample.

Notice: It is necessary to actually notify your LRN Reference Laboratory when a BT agent cannot be ruled out. It is not sufficient just to indicate you would notify them.

Participation in Drills and Exercises

Drills and exercises provide an opportunity to determine preparedness and practice response. BT drills can be performed in multiple ways, paper-based table-top exercises, computer simulation, and/or operational drills.

| National BT Drill Participation Over the Last Two Years | | N = 1389 |
|---|--|----------|
| Drill Type | | % ∞ |
| Internal (within your laboratory) | | 23.8 |
| Internal (within your institution) | | 27.5 |
| External (involving outside agencies) | | 30.6 |
| Did not participate in BT drill in past two years | | 42.1 |

| Michigan BT Drill Participation Over the Last Two Years | | N = 37 |
|---|--|--------|
| Drill Type | | % ∞ |
| Internal (within your laboratory) | | 10.1 |
| Internal (within your institution) | | 24.3 |
| External (involving outside agencies) | | 37.8 |
| Did not participate in BT drill in past two years | | 43.2 |

∞ Does not total 100% as some laboratories participated in multiple types of drills.

Our concern still exists for the lack of participation in drills and exercises in Michigan laboratories. Forty-three percent of Michigan laboratories have not drilled within the past two years. If your laboratory wishes to discuss participation in a bioterrorism drill or exercise, please contact the Michigan Department of Community Health Bureau of Laboratories Bioterrorism Coordinator, Valerie Reed, via e-mail at ReedV@michigan.gov.

Thank you for participating in the CAP LPX Exercise. Over time, improvement has been made by participating laboratories in both the testing and notification components of these exercises providing Michigan with improved biothreat agent detection and preparedness status.

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