

Michigan 2013 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 potential BT agents to public health authorities.

The LPX survey consists of organism identification (rule out) plus a notification component to test communications between 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 Sentinel 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 2013 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 Laboratory Response Network (LRN) Sentinel Laboratories on the 2013 LPX-B survey panel in aggregate and compares participating Michigan laboratory responses to those of participating labs throughout the country.

The **2013 LPX-B** survey contained the following samples:

LPX-04	<i>Yersinia enterocolitica</i> , in pure culture
LPX-05	<i>Yersinia pestis</i> , in pure culture
LPX-06	<i>Yersinia pseudotuberculosis</i> , in pure culture

Correct Result Reporting LPX-B		N = 38
Sample Number	% of MI Labs with Intended Response	
LPX-04	92.1% (35/38)	
LPX-05	78.9% (30/38)	
LPX-06	84.2% (32/38)	

2013 LPX-B-04

This challenge was a simulated blood specimen from a 50-year-old female with hemochromatosis presenting with fever, chills, and 102°F temperature.

CULTURE CHARACTERISTICS AT 24 HOURS:



5% SHEEP BLOOD AGAR:

Good growth at 24 hours: shiny, circular, raised, with entire edges.



CHOCOLATE AGAR:

Good growth at 24 hours shiny, circular, raised, with entire edges.



MACCONKEY AGAR:

No growth at 24 hours

CULTURE CHARACTERISTICS AT 48 HOURS:



5% SHEEP BLOOD AGAR:

Good growth at 24 hours, larger



CHOCOLATE AGAR:

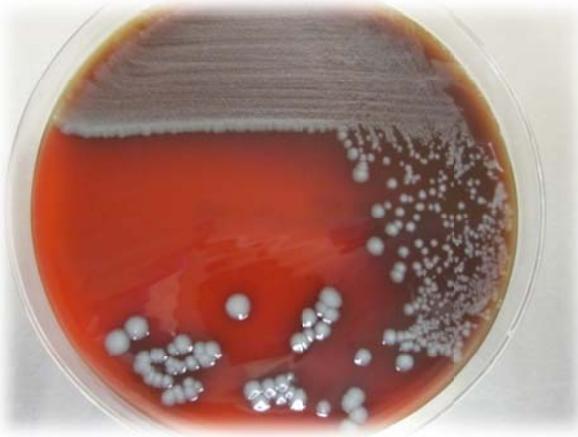
Good growth at 24 hours, larger



MACCONKEY AGAR:

Scant growth of lactose non-fermenting colonies at 48 hours

CULTURE CHARACTERISTICS AT 72 HOURS:



5% SHEEP BLOOD AGAR:

Circular, shiny, flat, 1-2mm. diameter colonies, gray with entire edges at 72 hours



CHOCOLATE AGAR

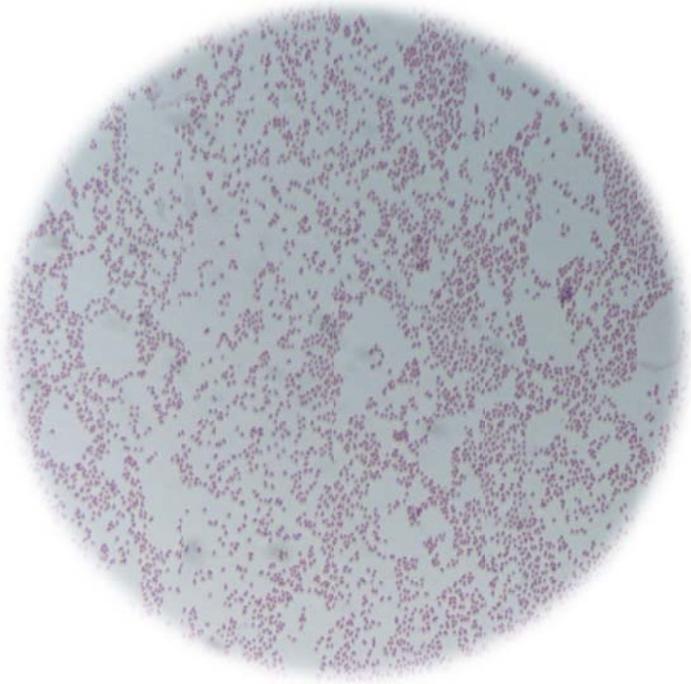
Circular, shiny, flat, 1-2mm. diameter colonies, gray with entire edges at 72 hours



MACCONKEY AGAR:

Good growth of non-lactose fermenting colonies at 72 hours

GRAM STAIN: (of 24 hour blood agar)



This Gram stain shows coccobacilli that are smaller than typical *Yersinia pestis*.

LABORATORY TESTING:

CATALASE (3% HYDROGEN PEROXIDE) TEST:

A catalase test was performed in the BSC and was positive, with a large 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.



Oxidase
positive
control



Oxidase
negative
control



Oxidase
negative
LPX-B-04

Further tests following the ASM Sentinel Lab procedures for *Yersinia pestis* were performed. These included urea, motility at 25-28°, and indole.

UREA TEST:

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



Urease
positive
control



Urease
negative
control



Urease
positive
LPX-B-04

25° MOTILITY:

A semi solid motility medium was inoculated and incubated at 25°C to detect motility. This isolate was positive, indicated by the diffuse (cloudy) growth where the media was stab inoculated. *Yersinia pestis* would be negative for this test, further ruling out the possibility of this agent.



Positive control



Negative control



LPX-B-04 Positive

INDOLE:

A positive result is shown by the presence of a red or red-violet color in the surface layer of the broth. *Yersinia pestis* would be indole negative, further ruling out that possibility.



Positive Control



Negative Control



LPX-B-04 Positive



GROWTH ON 5% SHEEP BLOOD AGAR
at 25°

(at 48 hours)

LPX-B-04 SUMMARY:

2013 LPX-B-04 was oxidase negative, catalase positive, urease positive, motile at 25°C, but not at 35°C, indole positive, and grew better at 25°C on 5% sheep blood agar than at 35°C.

The positive tests for motility, urea, and indole ruled out the possibility of *Yersinia pestis*, based on the ASM Sentinel Laboratory Guidelines. These reactions would be consistent with an identification of *Yersinia enterocolitica*, although not considered an agent of bioterrorism, it is still an important human pathogen.

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

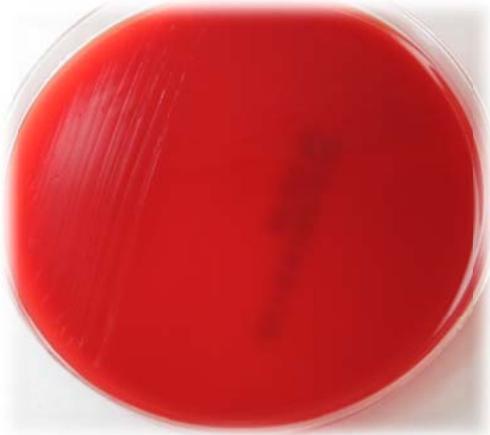
Participants in the LPX exercises were not required to contact the LRN Reference Laboratory on this isolate.

The CAP Laboratory Preparedness Exercise Final Critique identified LPX-B-04 as *Yersinia enterocolitica*.

2013 LPX-B-05

This challenge was a simulated blood specimen from a 57-year-old female from south Utah presenting with fever and chills after cleaning out an old barn.

CULTURE CHARACTERISTICS AT 24 HOURS:



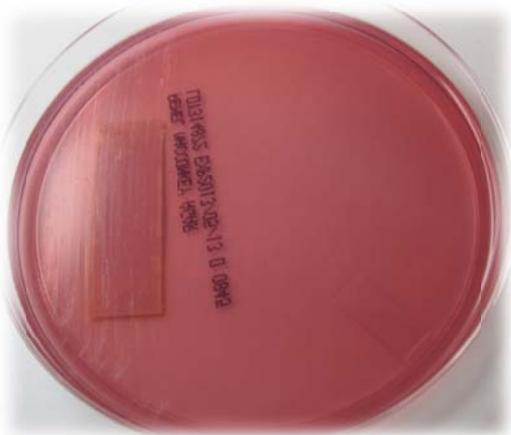
5% SHEEP BLOOD AGAR:

No growth at 24 hours



CHOCOLATE AGAR:

No growth at 24 hours



MACCONKEY AGAR:

No growth at 24 hours

CULTURE CHARACTERISTICS AT 48 HOURS:



5% SHEEP BLOOD AGAR:

Gray pinpoint growth at 48 hours



CHOCOLATE AGAR:

Gray/tan pinpoint growth at 48 hours



MACCONKEY AGAR:

No growth at 48 hours

CULTURE CHARACTERISTICS AT 72 HOURS:



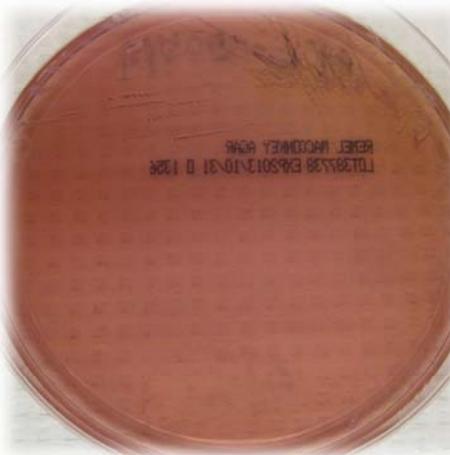
5% SHEEP BLOOD AGAR:

Good growth at 72 hours. Grey/tan colonies with no significant hemolysis. This growth pattern is consistent with *Yersinia pestis*.



CHOCOLATE AGAR:

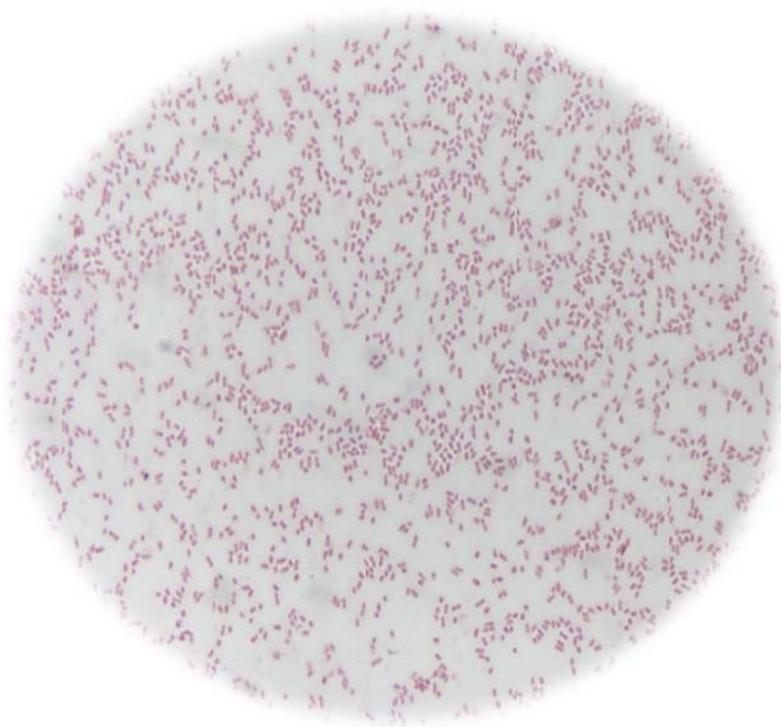
Good growth at 72 hours. Grey/tan circular, entire colonies. This growth pattern is consistent with *Yersinia pestis*.



MACCONKEY AGAR:

Scant growth of non-lactose fermenting colonies at 72 hours.

GRAM STAIN: (of 72 hour blood agar)



Small to medium gram-negative rods observed with some bipolar staining.

This gram stain and the growth characteristics of this isolate strongly suggest an identification of *Yersinia pestis*. To confirm this, several other conventional biochemicals were set up, including indole, urea, and a motility slant incubated at 25°C.

LABORATORY TESTING:

CATALASE (3% HYDROGEN PEROXIDE) TEST:

A catalase test was performed in the BSC and was positive, with a large 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. A negative oxidase supports a potential identification of *Yersinia pestis*.



Oxidase
positive
control



Oxidase
negative
control



Oxidase
negative
LPX-B-5

UREA TEST:

The test for urease was performed and was urease negative. This result is consistent with an identification of *Yersinia pestis*.



Urease positive control



Urease negative control



Urease negative LPX-B-05

INDOLE TEST:

A positive result is shown by the presence of a red or red-violet color in the surface layer of the broth. *Yersinia pestis* is indole negative.



Positive Control



Negative Control



Negative LPX-B-05

LABORATORY TESTING:

Two semi-solid motility tubes were set up on this isolate and incubated at both 25°C and 35°C. Both tests were negative. *Yersinia pestis* would be non-motile at both of these temperatures. See photos on LPX-04 above.

LPX-B-05 SUMMARY:

LPX-B-05 was catalase positive, oxidase negative, urease negative, indole negative, motility negative at both 25°C and 35°C. Combined with the Gram stain, colony morphology and growth characteristics, as well as the clinical diagnosis, these results cannot rule out the possibility of *Yersinia pestis* based on the ASM Sentinel Laboratory Guidelines.

The intended response for Sentinel Laboratories was “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*.

2013 LPX-B-06

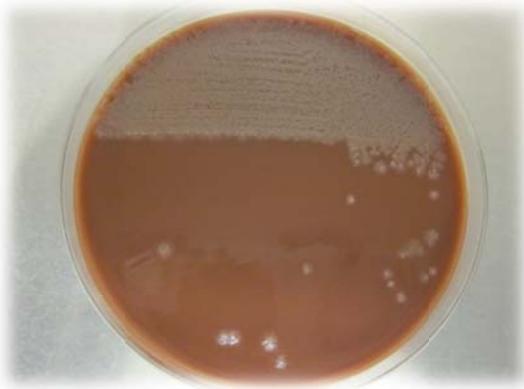
This challenge was a simulated blood specimen from a 47-year-old male with alcoholism and cirrhosis presenting with a 3-day history of fever and chills

CULTURE CHARACTERISTICS AT 24 HOURS:



5% SHEEP BLOOD AGAR:

Good growth of gray, circular, entire, glossy, non-hemolytic colonies at 24 hours.



CHOCOLATE AGAR:

Good growth of gray, circular, entire colonies at 24 hours.



MACCONKEY AGAR:

No growth at 24 hours

CULTURE CHARACTERISTICS AT 48 HOURS:



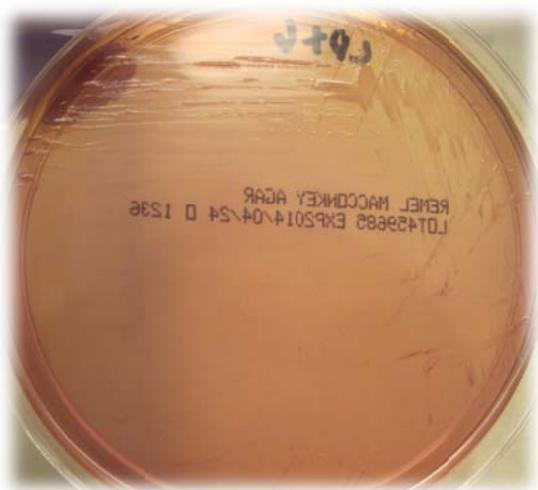
5% SHEEP BLOOD AGAR:

Similar colonial morphology as 24 hour growth but larger at 48 hours



CHOCOLATE AGAR:

Similar colonial morphology as 24 hour growth but larger at 48 hours



MACCONKEY AGAR:

Poor growth of non-lactose fermenting colonies at 48 hours.

CULTURE CHARACTERISTICS AT 72 HOURS:



5% SHEEP BLOOD AGAR:

Similar colonial morphology as 24 and 48 hours yet even larger at 72 hours



CHOCOLATE AGAR:

Similar colonial morphology as 24 and 48 hours yet even larger at 72 hours



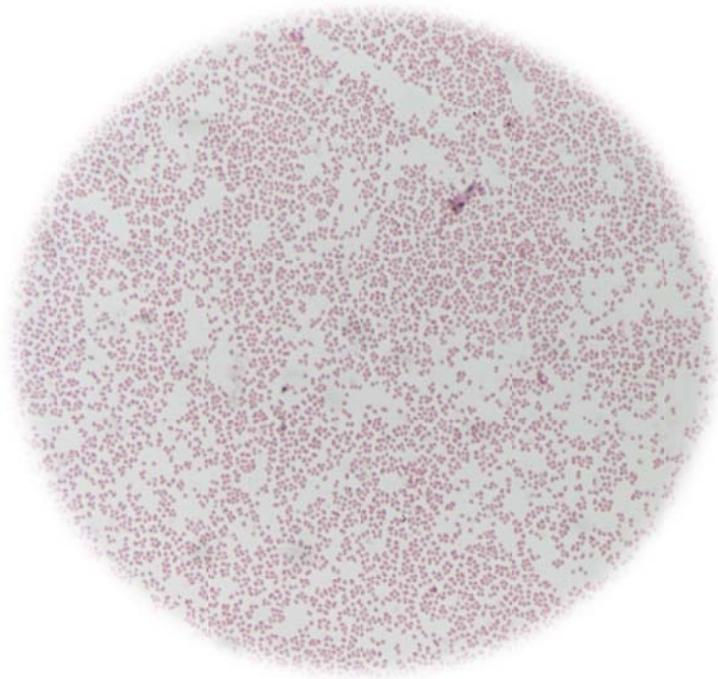
MACCONKEY AGAR:

Good growth of non-lactose fermenting colonies at 72 hours

GRAM STAIN: (of 24 hour blood agar plate)

The gram stain shows a small pleomorphic gram negative coccobacilli.

This Gram stain is typical of a *Yersinia* species, probably on the small side for a *Yersinia pestis*. Further testing following the ASM Sentinel Guidelines are needed to rule-in or rule-out *Yersinia pestis*.



LABORATORY TESTING:

CATALASE (3% HYDROGEN PEROXIDE) TEST:

A catalase test was performed in the BSC and was positive. (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. See photos on LPX-05 above.

5% SHEEP BLOOD AGAR at 25°:

This isolate grew slightly better on sheep blood agar at 25°C than at 35°C. This is typical for *Yersinia* spp. See photo on LPX-04 above.

25°C MOTILITY TEST:

A semi solid motility medium was inoculated and incubated at 25°C to detect motility. This isolate was positive, indicated by the diffuse (cloudy) growth where the media was stab inoculated. *Yersinia pestis* would be negative for this test, further ruling out the possibility of this agent.



Positive control



Negative control



LPX-B-06

UREASE TEST:

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



Positive Control



Negative Control



LPX-B-06

INDOLE TEST:

A positive result is shown by the presence of a red or red-violet color in the surface layer of the broth. *Yersinia pestis* is indole negative.



Positive Control



Negative Control



LPX-B-06

LPX-B-06 SUMMARY:

2013 LPX-B-06 was oxidase negative, catalase positive, urease positive, motile at 25°C, but not at 35°C, indole negative, and grew better at 25°C on 5% sheep blood agar than at 35°C.

The positive tests for motility, and urea ruled out the possibility of *Yersinia pestis*, based on the ASM Sentinel Laboratory Guidelines. These reactions would be consistent with an identification of *Yersinia pseudotuberculosis*, although not considered an agent of bioterrorism, it is still an important human pathogen.

The intended response for Sentinel Laboratories was either “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 the LRN Reference Laboratory on this isolate.

The CAP Laboratory Preparedness Exercise Final Critique identified LPX-B-06 as *Yersinia pseudotuberculosis*.

Important Information

Testing performed on these isolates utilized the Sentinel Level Clinical Laboratory Guidelines and the accompanying LRN Sentinel Level Testing Protocols current at that time.

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 were updated since the CAP-2013 LPX-A was completed and 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-04: <i>Yersinia enterocolitica</i>		
Submitted Answers	Michigan Participants	All Participants
§ Non-BT Culture	35/38 92.1%	1195/1346 88.8%
§ <i>Yersinia</i> sp., refer to rule out <i>Y. pestis</i>	3/38 7.9%	77/1346 5.7%
§ Gram-negative bacillus, refer to rule out <i>Yersinia pestis</i>	-	39/1346 2.9%

§ Acceptable response for Sentinel Laboratories

LPX-05: <i>Yersinia pestis</i>		
Submitted Answers	Michigan Participants	All Participants
§ Suspect <i>Yersinia pestis</i> , refer for confirmation	7/38 18.4%	304/1343 22.6%
§ <i>Yersinia</i> sp., refer to rule out <i>Y. pestis</i>	8/38 21.1%	181/1343 13.5%
§ Gram-negative bacillus, refer to rule out <i>Yersinia pestis</i>	15/38 39.5%	527/1343 39.2%
Non-BT Culture	7/38 18.4%	-
Gram-negative bacillus/coccobacillus, refer to rule out <i>Francisella tularensis</i>	1/38 2.6%	-

§ Acceptable response for Sentinel Laboratories

Michigan Department of Community Health
Bureau of Laboratories

LPX-06: <i>Yersinia pseudotuberculosis</i>		
Submitted Answers	Michigan Participants	All Participants
§ Non-BT Culture	28/38 73.7%	821/1344 61.1%
§ <i>Yersinia</i> sp., refer to rule out <i>Y. pestis</i>	2/38 5.3%	121/1344 9.0%
§ Gram-negative bacillus, refer to rule out <i>Yersinia pestis</i>	2/38 5.3%	121/1344 9.0%
Gram-negative coccobacillus, refer to rule out <i>B. mallei</i>	3/38 7.9%	-
Suspect <i>B. mallei</i> , refer	2/38 5.3%	-
Suspect <i>F. tularensis</i> , refer	1/38 2.6%	

§ Acceptable response for Sentinel Laboratories

Notification Drill

Michigan Laboratories Notifying Their LRN Ref Lab			N = 38
Sample Number	Notification Required	% MI Labs Indicating Would Notify the LRN Reference Lab	% MI Labs Actually Notified the LRN Reference Lab
LPX-04	No	2.6% (1/38)	5.3% (2/38)
LPX-05	Yes	80.0% (30/38)	68.4% (26/38)
LPX-06	No	23.7% (9/38)	21.0% (8/38)

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 = 1355
Drill Type		% ∞
Internal (within your laboratory)		22.0
Internal (within your institution)		27.7
External (involving outside agencies)		30.3
Did not participate in BT drill in past two years		43.5

Michigan BT Drill Participation Over the Last Two Years LPX-B		N = 38
Drill Type		% ∞
Internal (within your laboratory)		10.5
Internal (within your institution)		29.0
External (involving outside agencies)		31.6
Did not participate in BT drill in past two years		44.7

∞ 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. Although more laboratories reported doing drills this year than last, there are still 44.7% of Michigan laboratories who 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.

Michigan Department of Community Health
Bureau of Laboratories