

Michigan 2013 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 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-A survey panel in aggregate and compares participating Michigan laboratory responses to those of participating labs throughout the country.

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

LPX-01	<i>Francisella tularensis</i> , the live vaccine strain (LVS)
LPX-02	<i>Legionella pneumophila</i>
LPX-03	<i>Bacillus anthracis</i> , attenuated strain

Correct Result Reporting LPX-A		N = 37
Sample Number	% of MI Labs with Intended Response	
LPX-01	89.2% (33/37)	
LPX-02	94.6% (35/37)	
LPX-03	81.1% (30/37)	

Laboratory Analysis

2013 LPX-A-01

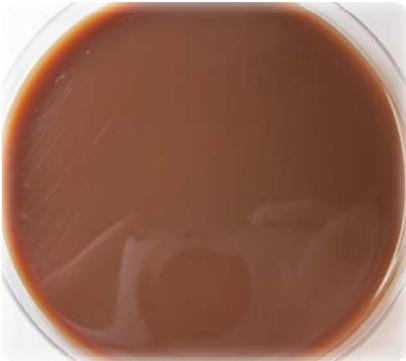
This challenge was a simulated bronchoalveolar lavage specimen from a 46-year old alcoholic homeless male from Arizona with fever, pleuritic pain, and pulmonary infiltrates.

CULTURE CHARACTERISTICS AT 24 HOURS:



5% SHEEP BLOOD AGAR:

Poor/scant growth at 24 hours



CHOCOLATE AGAR:

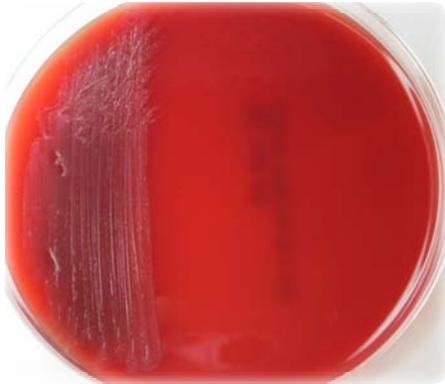
Poor/scant growth at 24 hours



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:

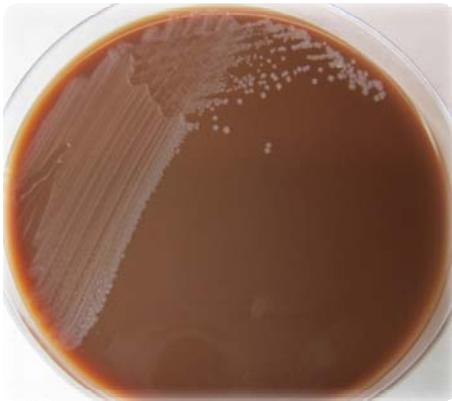
No growth at 48 hours

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:

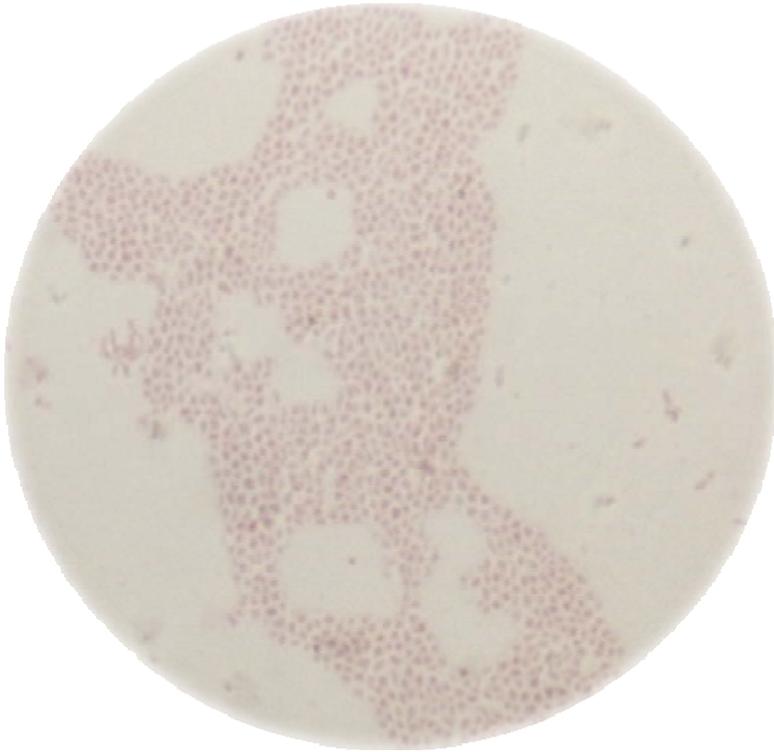
Shiny, flat, 2-3 mm. diameter colonies, white to gray-white, opaque, with entire edges at 72 hours



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 stain 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 will rule 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*.



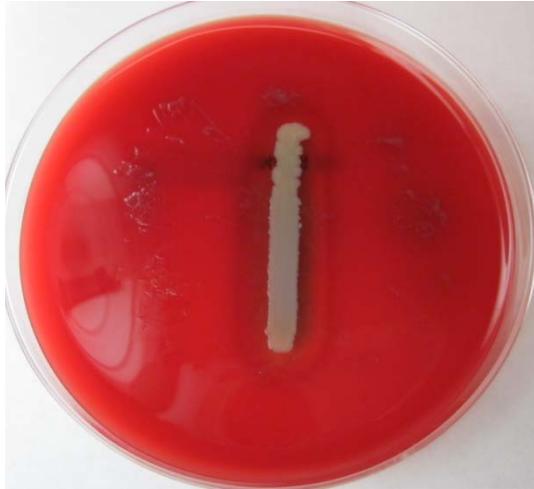
Urease
Positive
Control



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

LPX-A-01 SUMMARY:

2013 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).

2013 LPX-A-02

This challenge was a simulated bronchoalveolar lavage specimen from an 86-year-old male from Southern California with cough, 102°F temperature, and patchy pulmonary infiltrates with multi-focal abscesses.

CULTURE CHARACTERISTICS AT 24 HOURS:



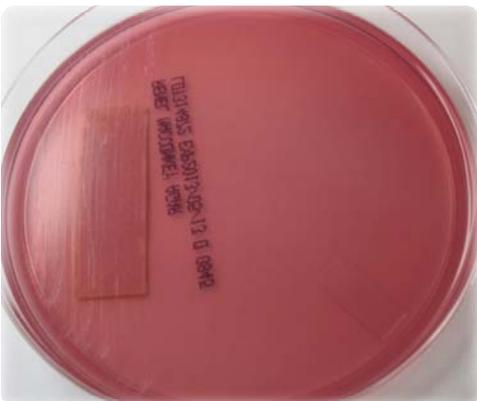
5% SHEEP BLOOD AGAR:

No growth at 24 hours



CHOCOLATE AGAR:

No growth at 24 hours



MACCONKEY AGAR:

No growth at 24 hours

CULTURE CHARACTERISTICS AT 48 HOURS:



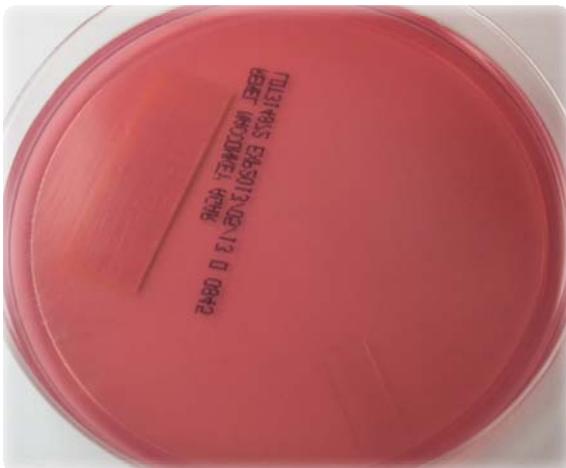
5% SHEEP BLOOD AGAR:

No growth at 48 hours



CHOCOLATE AGAR:

Scant growth (?) at 48 hours



MACCONKEY AGAR:

No growth at 48 hours

CULTURE CHARACTERISTICS AT 72 HOURS:



5% SHEEP BLOOD AGAR:

No growth at 72 hours



CHOCOLATE AGAR:

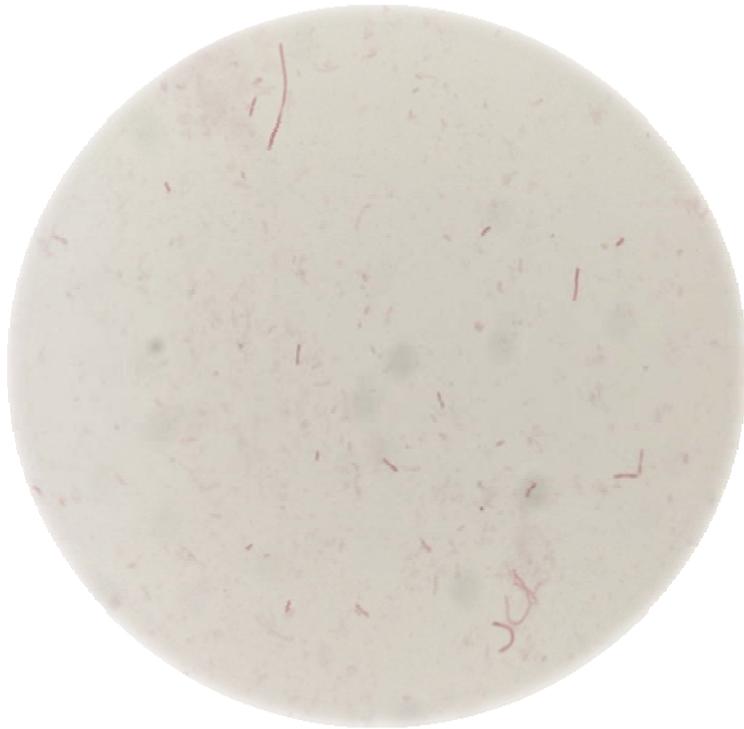
Scant to pinpoint growth at 72 hours



MACCONKEY AGAR:

No growth at 72 hours

GRAM STAIN: (of 72 hour chocolate agar)



Medium to long (10-25 μ m) Gram-negative rods

This gram stain and the poor culture growth characteristics suggest an organism not consistent with any select agent.

Due to the poor growth on the initial set up, a BCYE (buffered charcoal yeast extract) plate was added. This media contains a cysteine supplement.

CULTURE CHARACTERISTICS ON BCYE AGAR AT 72 HOURS:



Good growth with glistening, flat, opalescent, 1-3 mm. diameter colonies at 72 hours

LABORATORY TESTING:

Growth on BCYE with no growth on 5% sheep blood agar is one of the most useful presumptive clues that an isolate could be a *Legionella* species. These growth characteristics could also apply to *Francisella tularensis*. However, the size of the Gram-negative bacillus from the culture plate is not consistent with a *Francisella* species.

This isolate was weakly catalase positive and weakly oxidase positive, both supporting a potential identification of *Legionella* species.

LPX-A-02 SUMMARY:

2013 LPX-A-02 was weakly catalase positive, weakly oxidase positive, grew well on BCYE but not on 5% sheep blood agar, and Gram-stained as medium to long, straight, gram-negative rods. These combined results rule out the possibility of a select agent based on the ASM Sentinel Laboratory Guidelines.

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-A-02 as *Legionella pneumophila*.

2013 LPX-A-03

This challenge was a simulated bronchoalveolar lavage specimen from a 37-year-old West Texas male who suddenly develops fever, cough, and pulmonary infiltrates 3 days after rounding up cattle on a dusty prairie.

CULTURE CHARACTERISTICS AT 24 HOURS:



5% SHEEP BLOOD AGAR:

Non-hemolytic, gray, opaque colonies, 2-5 mm in diameter, with irregular edges and projections, and a ground-glass appearance, and of a tenacious and sticky consistency at 24 hours.



CHOCOLATE AGAR:

As blood agar 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:

As blood agar at 48 hours



MACCONKEY AGAR:

No growth 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:

As blood agar at 72 hours



MACCONKEY AGAR:

No growth at 72 hours

GRAM STAIN: (of 24 hour blood agar plate)



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

This Gram stain is typical of a *Bacillus* species. Further testing following the ASM Sentinel 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 motility 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

LPX-A 03 SUMMARY:

2013 LPX-A-03 was a catalase positive, non-motile, non-hemolytic broad Gram-positive bacillus. In conjunction with the colony morphology and growth characteristics, as well as the clinical diagnosis, these results 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*, attenuated strain.

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-01: <i>Francisella tularensis</i>		
Submitted Answers	Michigan Participants	All Participants
§ Suspect <i>Francisella tularensis</i> , refer for confirmation	9/37 24.3%	376/1333 28.2%
§ <i>Francisella</i> sp., refer to rule out <i>Francisella tularensis</i>	2/37 5.4%	110/1333 8.3%
§ Gram-negative bacillus/coccobacillus, Refer to rule out <i>Francisella tularensis</i>	22/37 59.4%	667/1333 50%
Non-BT Culture	2/37 5.4%	59/1333 4.4%

§ Acceptable response for Sentinel Laboratories

LPX-02: <i>Legionella pneumophila</i>		
Submitted Answers	Michigan Participants	All Participants
§ Non-BT Culture	35/37 94.6%	1179/1295 91%

§ Acceptable response for Sentinel Laboratories

LPX-03: <i>Bacillus anthracis</i>		
Submitted Answers	Michigan Participants	All Participants
§ Suspect <i>Bacillus anthracis</i> , refer for confirmation	6/37 16.2%	320/1337 24%
§ <i>Bacillus</i> sp., refer to rule out <i>Bacillus anthracis</i>	20/37 54%	633/1337 47.5%
§ Gram-positive bacillus, refer to rule out <i>Bacillus anthracis</i>	4/37 10.8%	282/1337 21.2%
Non-BT Culture	6/37 16.2%	62/1337 4.7%

§ Acceptable response for Sentinel Laboratories

Notification Drill Results

Notification Drill LPX-A			
Sample Number	Notification Required	% MI Labs Indicating Would Notify the LRN Ref Lab	% MI Labs Actually Notified the LRN Reference Lab
LPX-01	Yes	97% (36/37) ^	70% (26/37) ^
LPX-02	No	100% (2/2) #	0% (0/37) #
LPX-03	Yes	97% (36/37) ^	67.6% (25/37)

^ One laboratory suspected a BT agent, yet indicated they would refer it to a commercial laboratory.

Although notification of the LRN Reference Laboratory was not needed, all laboratories that could not rule out a BT agent in this sample indicated they would notify their LRN Reference Lab. However, no laboratory contacted their LRN Reference Lab.

Summary of Packaging and Shipping Participants

Of the 37 participating labs, only 14 (38%) labs submitted isolates to their LRN Reference Level Laboratory to evaluate their ability to properly package and ship. According to the CAP “Referring specimens to your LRN Reference Laboratory for purposes of this exercise will not violate the Clinical Laboratory Improvement Amendments (CLIA) Proficiency Testing rules.” The reasons the remaining labs did not submit isolates included:

- Additional expense for the shipping (FedEx)
- Inadvertently not requested to submit the isolate by the LRN Reference Level Laboratory
- Laboratory did not call to report the possible identification so there was no opportunity for the LRN Reference Level Lab to request the submission of the isolate
- No FedEx compliant software available to complete the DGF

Michigan Department of Community Health
Bureau of Laboratories

11/20/2013

Classification of the shipments varied by facility; 12/14 (86%) shipped as UN2814 Category A and 2/14 (14%) shipped as UN 3373 Category B. Either classification could be considered technically correct since the submitting lab has not confirmed the identification of the isolate as a Category A agent. It is up to the submitting laboratory (the shipper) to determine the classification. MDCH BOL suggests and advises to ship these as a UN2814 Category A infectious substance.

The submitted packages were evaluated to ensure compliance with the current shipping regulations. Adverse quality assurance events were documented for each shipment. Adverse quality assurance events are defined as “any deviation from established and written policies and procedures for an ongoing mechanism that monitors, assesses, and when indicated, corrects identified problems that could result in a negative or potentially negative outcome as stated in the Clinical Laboratory Improvement Amendments (CLIA) sec. 493.” Basically - did the packages meet the current shipping regulations. Very few of the packages received completely met the requirements of the US Hazardous Materials Regulations, but none of them were packaged in a manner that resulted in a risk to the safety of the package in transit. Most of the adverse quality assurance events were associated with the completion of the Declaration of Dangerous Goods form.

	UN2814 Category A	UN3373 Category B	Total
Isolates shipped to MDCH BOL	12 (86%)	2 (14%)	14
Isolates packaged and shipped without any adverse quality assurance events.	1/12 (8%)	1/2 (50%)	2/14 (14%)

Adverse Quality Assurance Event for Category A Shipments	Quantity*
Incomplete Declaration of Dangerous Goods Form	11/12 (92%)
Improper markings or labels on outer package	9/12 (75%)
Improper packaging used	2/12 (17%)
Missing/erroneous Itemized list of contents (test requisition)	2/12 (17%)

*Note: Total does not equal 100% since many isolate submissions had more than one quality assurance event per shipment.

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 = ~1343
Drill Type		% ∞
Internal (within your laboratory)		22.3
Internal (within your institution)		26.5
External (involving outside agencies)		30.8
Did not participate in BT drill in past two years		43.3

Michigan BT Drill Participation Over the Last Two Years LPX-A		N = 37
Drill Type		% ∞
Internal (within your laboratory)		13.5
Internal (within your institution)		24.3
External (involving outside agencies)		21.6
Did not participate in BT drill in past two years		54.0

∞ 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. 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.