Michigan 2009 CAP LPX-A and LPX-B Survey Analysis

Introduction

One purpose of the CAP LPX survey is to provide laboratories with an educational exercise that can be used to help prepare for the detection of pathogens of epidemiologic importance, including pathogens that can be used as biothreat agents. Another purpose of the LPX is to prepare participant laboratories for effective and efficient communication of critical information related to potential BT agents. This report summarizes the results of the Michigan Laboratory Response Network (LRN) Sentinel Laboratories on the 2009 LPX-A and LPX-B survey panels in aggregate and compares Michigan lab responses to those of participating labs throughout the country.

Performance Summary

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 performing the established Sentinel Laboratory Guidelines on a challenge isolate, they are unable to rule out an agent of bioterrorism. Both organism rule-out and notification are summarized below.

Approximately 40% of Michigan sentinel labs participated in the 2009 LPX-A and LPX-B surveys.

Correct Result Reporting LPX-A		N = 43
Sample Number	Organism Present	% of MI Labs with Intended Response
LPX-01	*Y. enterocolitica*	100% (43/43) #
LPX-02	*Y. pestis*	74.42% (32/43)
LPX-03	*B. bronchiseptica*	93.02% (40/43) #
LPX-04	*F. tularensis*	97.62% (41/42) ^

Correct Result Reporting LPX-B		N = 43
Sample Number	Organism Present	% of MI Labs with Intended Response
LPX-05	*B. anthracis*	88.37% (38/43)
LPX-06	*F. tularensis*	93.02% (40/43)
LPX-07	*P. multocida*	97.67% (42/43) #
LPX-08	*Y. pestis*	78.57% (33/42) ^

Correctly identified as Non-BT culture
^ One lab did not test on indicated site/source thus N=42

Notification Drill Results

Notification Drill LPX-A			N = 43
Sample Number	Notification Required	% MI Labs Indicating Would Notify the LRN Ref Lab	% MI Labs Actually Notified the LRN Reference Lab
LPX-01	No	18.6% (8/43) # ∇	18.60% (8/43) ∇
LPX-02	Yes	79.07% (34/43)	41.86% (18/43)
LPX-03	No	7.0% (3/43) ∇	9.3% (4/43) ∇
LPX-04	Yes	97.62% (41/42) ¤	47.62% (20/42) ¤

Notification Drill LPX-B			N = 43
Sample Number	Notification Required	% MI Labs Indicating Would Notify the LRN Ref Lab	% MI Labs Actually Notified the LRN Reference Lab
LPX-05	Yes	86.05% (37/43) *	53.49% (23/43)
LPX-06	Yes	95.35% (41/43) ^	53.49% (23/43)
LPX-07	No	2.33% (1/43)∇	2.33% (1/43) ∇
LPX-08	Yes	71.43% (30/42) ¤	47.62% (20/42) ¤

One laboratory suspecting *Y. pestis* did not indicate notification of the LRN Reference Laboratory, instead would have referred the sample to a commercial reference laboratory.
* One laboratory suspecting *B. anthracis* did not indicate notification of the LRN Reference Laboratory, instead would have referred the sample to a commercial reference laboratory.
^ One laboratory suspecting *F. tularensis* did not indicate notification of the LRN Reference Laboratory, instead would have referred the sample to a commercial reference laboratory.
∇ Although notification was not necessary in these cases, it is great that sentinel labs are willing and able to communicate with their LRN Reference Lab.
¤ One lab did not test on indicated site/source thus N=42

Analysis by Sample

The 2009 LPX-A survey contained the following samples:

LPX-01	*Yersinia enterocolitica*
LPX-02	*Yersinia pestis*
LPX-03	*Bordetella bronchiseptica*
LPX-04	*Francisella tularensis*

The 2009 LPX-B survey contained the following samples:

LPX-05	*Bacillus anthracis*
LPX-06	*Francisella tularensis*
LPX-07	*Pasteurella multocida*
LPX-08	*Yersinia pestis*

LPX-01: *Yersinia enterocolitica*		
Submitted Answers	Michigan Participants	All Participants
§ Non-BT culture	36/43 83.7%	1060/1347 79.1%
§ *Yersinia* sp., refer to rule out *Yersinia pestis*	6/43 14.0%	221/1347 16.5%
§ Gram-negative bacillus, refer to rule out *Yersinia pestis*	1/43 2.3%	30/1347 2.2%

§ Acceptable response

LPX-02: *Yersinia pestis*		
Submitted Answers	Michigan Participants	All Participants
§ *Yersinia pestis* confirmed	0/43 0%	53/1352 4%
§ *Yersinia pestis*, refer for confirmation	10/43 23.26%	272/1352 20.3%
§ *Yersinia* sp., refer to rule out *Y. pestis*	6/43 13.95%	271/1352 20.2%
§ Gram-negative bacillus, refer to rule out *Yersinia pestis*	16/43 37.21%	518/1352 38.7%
Non-BT culture	9/43 20.93%	156/1352 11.6%
Gram-negative bacillus, rule out *Burkholderia pseudomallei*	1/43 2.3%	-
Bacillus sp., rule out *B. anthracis*	1/43 2.3%	-

§ Acceptable response

LPX-03: *Bordetella bronchiseptica*		
Submitted Answers	Michigan Participants	All Participants
§ Non-BT culture	40/43 93.02%	1025/1342 76.7%
Gram-negative coccobacillus, refer to rule out *Brucella* sp.	2/43 4.65%	77/1342 5.8%
Gram-negative bacillus, refer to rule out *Burkholderia pseudomallei*	1/43 2.3%	159/1342 11.9%
Gram-negative coccobacillus, refer to rule out *Burkholderia mallei*	0/43 0%	19/1342 1.4%

§ Acceptable response

LPX-04: *Francisella tularensis*		
Submitted Answers	Michigan Participants	All Participants
§ *Francisella tularensis*, confirmed	0/42 0%	55/1342 4.1%
§ *Francisella tularensis*, refer for confirmation	7/42 16.7%	204/1342 15.3%
§ *Francisella* sp., refer to rule out *F. tularensis*	8/42 19.05%	171/1342 12.8%
§ Gram-negative coccobacillus, refer to rule out *F. tularensis*	26/42 61.9%	810/1342 60.8%
Non-BT culture	1/42 2.4%	48/1342 3.6%

§ Acceptable response

LPX-05: *Bacillus anthracis*		
Submitted Answers	Michigan Participants	All Participants
§ *Bacillus anthracis*, confirmed	0/43 0%	41/1354 3%
§ *Bacillus anthracis*, refer for confirmation	1/43 2.3%	89/1354 6.6%
§ *Bacillus* sp., refer to rule out *Bacillus anthracis*	32/43 74.4%	924/1354 68.2%
§ Gram-positive bacillus, refer to rule out *Bacillus anthracis*	5/43 11.6%	207/1354 15.3%
Non-BT culture	5/43 11.6%	131/1354 9.7%

§ Acceptable response

LPX-06: *Francisella tularensis*		
Submitted Answers	Michigan Participants	All Participants
§ *Francisella tularensis*, confirmed	0/43 0%	60/1353 4.4%
§ *Francisella tularensis*, refer for confirmation	3/43 6.98%	184/1353 13.6%
§ *Francisella s* sp., refer to rule out *Francisella tularensis*	6/43 13.9%	181/1353 13.4%
§ Gram-negative bacillus, refer to rule out *Francisella tularensis*	31/43 72.1%	782/1353 57.8%
GNCB, refer to rule out *Brucella* sp.	2/43 4.6%	NA
Non-BT culture	1/43 2.3%	68/1353 5.0%

§ Acceptable response

LPX-07: *Pasteurella multocida*		
Submitted Answers	Michigan Participants	All Participants
§ Non-BT culture	42/43 97.7%	1175/1354 86.8%
Gram-negative coccobacillus, refer to rule out *Brucella* sp.	0/43 0%	106/1354 7.8%
GNB/CB, refer to rule out *F. tularensis*	1/43 2.3%	NA

§ Acceptable response

LPX-8: *Yersinia pestis*		
Submitted Answers	Michigan Participants	All Participants
§ *Yersinia pestis*, confirmed	0/42 0%	55/1352 4.1%
§ *Yersinia pestis*, refer for confirmation	4/42 9.5%	210/1352 15.5%
§ *Yersinia* sp., refer to rule out *Yersinia pestis*	12/42 28.6%	316/1352 23.4%
§ Gram-negative bacillus, refer to rule out *Yersinia pestis*	17/42 40.5%	623/1352 46.1%
Non-BT culture	9/42 21.4%	112/1352 8.3%

§ Acceptable response

Discussion

LPX-01

This challenge contained *Yersinia entercolitica.* All of the 43 (100%) Michigan laboratories who participated in this survey responded with an acceptable response identifying this as either a Non-BT culture or recognizing the possibility of *Y. pestis* being present in this sample. This compares to 97.3% of all participating laboratories. It is important for laboratories to be able to differentiate this species from *Y. pestis*, as *Y. entercolitica* is an important human pathogen.

One Michigan laboratory that identified the possibility of *Y. pestis* being present in this sample did not indicate they would contact their LRN Reference Lab but stated they would refer the sample to their commercial reference lab. Participants in LPX exercises are required to contact their LRN Reference Laboratory if, after performing the established Sentinel Laboratory Guidelines on a challenge isolate, they are unable to rule out an agent of bioterrorism.

LPX-02

This challenge contained *Yersinia pestis.* 32/43 (74.4%) of Michigan labs participating in this survey responded with an acceptable response indicating the likelihood of *Y. pestis* being present in the sample. This compares to 82% of all participating labs. One Michigan lab indicated the possibility the sample contained *B. anthracis* and another indicated the possibility of *Burkholderia pseudomallei* being present.

All Michigan labs detecting the possibility of *Y. pestis* in this sample indicated they would contact their LRN Reference Lab. Only those Michigan laboratories indicating this was a Non-BT culture said they would not notify their LRN Reference Laboratory. However, it is critical to note that not all labs indicating they would notify their LRN Reference Laboratory did so. The isolate from this challenge should have triggered a communication with the participant's LRN Reference Laboratory.

It is recommended that participant laboratories who did not perform, or observed aberrant reactions for this isolate, review LRN Sentinel Laboratory Guidelines and their own laboratory procedures. Also, note that automated systems may identify *Y. pestis* poorly. When an agent of bioterrorism is suspected, it is recommended to NOT use automated systems.

LPX-03

This challenge contained *Bordetella bronchiseptica.* 40/43 (93%) of participating Michigan labs correctly reported this as a non-BT culture. Two participating Michigan labs identified the possibility of *Brucella* in this challenge and another indicated the possibility of *Burkholderia pseudomallei*. This compares to 77% of all participating labs

who identified this sample as a non-BT culture; 6% indicating the possibility of *Brucella*; and 13.5% indicating the possibility of *Burkholderia* sp.

When bioterrorism agents have been ruled out, LRN Reference Lab notification is not required. None of the Michigan labs participating in this challenge who correctly reported this as a Non-BT culture reported that they would have notified the LRN Reference Lab concerning this sample. The three participating Michigan laboratories indicating the possibility of *Brucella* or *Burkholderia* did correctly indicate they would notify their LRN Reference Laboratory.

In this challenge, *B. bronchiseptica* was used as a mimic of *Brucella* sp. because both are catalase, oxidase and nitrate positive and both are rapid urease positive. Nationally, 35% of laboratories did not perform a urease test; 32% of participating Michigan laboratories did not. While *B. bronchiseptica* can be identified by some automated systems and commercial identification kits, *Brucella* species cannot. Nationally, 77.7% of participants inoculated this organism into a commercial kit or instrument; 79% of participating Michigan laboratories did so. This is not recommended until the likelihood of a *Brucella* species or other agent of bioterrorism has been ruled out.

LPX-04

This challenge contained *Francisella tularensis.* 41/42 (97.6%) of participating Michigan labs responded with an acceptable response indicating the ability to detect a probable *F. tularensis* in the sample. This compares to 92.4% of all nationally participating labs. One participating Michigan laboratory reported this as a non-BT culture.

Gram stain results and growth characteristics should cause suspicion for *F. tularensis* and trigger communication with the LRN Reference Lab. All participating Michigan laboratories that identified this isolate as possible *Francisella* species correctly indicated the need to notify their LRN Reference Laboratory. It is intended that isolates from these exercises be treated as patient isolates and reported to the LRN Reference Laboratory in real-time, as soon as the isolate is determined to be a suspect bioterrorism agent, without waiting until all tests are completed. It is difficult to tell from the data if notification was initiated as soon as a BT agent was suspected.

Francisella tularensis is occasionally isolated from clinical specimens in endemic regions in the United States. Identification of this organism in clinical microbiology labs is accomplished via bacterial culture. Identification may be performed using standard algorithms, but clinical laboratories must be aware of the risk of laboratory-acquired infection. Commercial identification systems should not be used due to the risk of aerosolization and cannot be relied upon for accurate identification. Despite this, nearly one-third of all participants and 35.7% of Michigan participants attempted to identify this isolate using a commercial system.

LPX-05

This challenge contained the Sterne strain of *Bacillus anthracis* devoid of the plasmid pX02. 38/43 (88.4%) of participating Michigan labs correctly identified this challenge as *Bacillus anthracis, Bacillus* species or a gram-positive bacillus. This compares to 93% of all participating labs. Five (11.4%) Michigan labs incorrectly identified this specimen as a non-BT culture compared to 9.7% of all participating labs.

The isolate from this challenge should have triggered a communication with the LRN Reference Laboratory. Only one participating Michigan lab detecting this pathogen that could potentially be used as a biothreat agent did not indicate the need to notify the LRN Reference Laboratory. However, it is important to note that the number of labs who actually did notify the LRN Reference Lab was much lower than the number who indicated notification was necessary.

LPX-06

This challenge contained *Francisella tularensis,* the live vaccine strain (LVS). 40/43 (93%) of participating Michigan labs correctly identified this challenge as *Francisella tularensis, Francisella* species or a gram-negative bacillus/coccobacillus. This compares to 89% of all participating labs. Two (4.6%) Michigan labs incorrectly identified this specimen as a possible *Brucella* sp. and one (2.3%) as a non-BT culture.

The isolate from this challenge should have triggered a communication with the LRN Reference Laboratory. Two (4.6%) Michigan participating labs that detected this pathogen that could potentially be used as a biothreat agent did not indicate the need to notify the LRN Regional Laboratory but instead would have referred it to a commercial reference laboratory. Notification of the LRN Reference Laboratory is required if unable to rule out an agent of bioterrorism.

LPX-07

This challenge contained *Pasteurella multocida* in pure culture. 42/43 (98%) of participating Michigan labs correctly identified this challenge as a non-BT culture. This compares to 87% of all participating labs. One (2.3%) Michigan lab incorrectly identified this specimen as a GNB/CB and would refer to rule out *Francisella*.

When bioterrorism agents have been ruled out, LRN Reference Lab notification is not required. The one participating Michigan laboratory that suspected the isolate of containing *Francisella* did correctly indicate the need to notify the LRN Reference Laboratory.

LPX-08

This challenge contained a *Yersinia pestis* strain devoid of the 75 kb low calcium response (LCR) virulence plasmid. 33/42 (78.6%) of participating Michigan labs

correctly identified this challenge as *Yersinia pestis*, *Yersinia* species or a gram-negative bacillus. This compares to 89% of all participating labs. Nine (21%) Michigan labs incorrectly identified this specimen as a non-BT culture compared to 8.3% of all participating labs.

The isolate from this challenge should have triggered a communication with the LRN Reference Laboratory. LRN Sentinel Labs are required to notify their LRN reference laboratory when they are unable to rule out an agent of bioterrorism.

Overall Performance

Participants are recognizing that, for most Sentinel Laboratories, the proper identification process does not have to be a complete identification, but rather only to the point where suspicion suggests contact with the LRN Reference Laboratory. However, some labs are not properly applying the Sentinel Laboratory Guidelines to rule-out or refer potential agents of bioterrorism as indicated by labs still responding with "Non-BT agent" for isolates containing *B. anthracis, F. tularensis,* and *Y. pestis*.

Trends since 2007 suggest that most participating Sentinel Labs are developing good management practices including early recognition of possible BT agents and shortened reporting intervals to the appropriate LRN laboratory.

Automated detection systems are not recommended for use to identify potential BT agents. Trends suggest that these systems are being used less frequently for potential bioterrorism agents.

Even though laboratories indicated they would notify their LRN Reference Laboratory, only a small percentage of participating Michigan laboratories actually did complete this notification requirement. As soon as an isolate is suspected of containing an agent of bioterrorism, notification of the LRN Reference Laboratory is required. In the case of these LPX exercises, if, after performing the established Sentinel Laboratory Guidelines on a challenge isolate, labs are unable to exclude an agent of bioterrorism, they must call the LRN Reference Laboratory in real-time and report a possible agent of bioterrorism in the LPX sample.

Overall, participating Sentinel Laboratories are now more likely to correctly identify and report a potential agent of bioterrorism than in 2007 when live organisms where introduced into the LPX challenges.