

LPX-A Laboratory Preparedness Exercise

FINAL CRITIQUE

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2012 LPX-A FINAL CRITIQUE

Evaluation Criteria

The purpose of the LPX is to provide laboratories with an educational exercise that can be used to help prepare for the laboratory detection of pathogens of epidemiologic importance, including pathogens that can be used as biothreat agents. For this reason, the exercises are not graded and responses should be considered more in the light of intended responses rather than correct or incorrect.

LPX-01

This challenge was a simulated blood specimen from a Colorado rancher with painful right inguinal lymph node enlargement and recent onset of fever. The specimen contained *Yersinia pestis* in pure culture. The intended response for Sentinel Laboratories was either ***Yersinia pestis*, refer for confirmation, Yersinia sp., refer to rule out Yersinia pestis** or **Gram-negative bacillus, refer to rule out Yersinia pestis**; the intended response for LRN Reference Laboratories was ***Yersinia pestis*, confirmed**.

Table 1. Summary of the Participants' Identification of LPX-01, *Yersinia pestis*

| LPX-01 | Identification | Participants (1320) | |
|--------|--|---------------------|------|
| | | No. | % |
| | <i>Yersinia pestis</i> , confirmed | 55 | 4.2 |
| | <i>Yersinia pestis</i> , refer for confirmation | 127 | 9.6 |
| | <i>Yersinia sp.</i> , refer to rule out <i>Yersinia pestis</i> | 389 | 29.5 |
| | Gram-negative bacillus, refer to rule out <i>Yersinia pestis</i> | 628 | 47.6 |
| | Non-BT Culture | 72 | 5.5 |

Table 2. Summary of Participants' actions after identifying LPX-01, *Yersinia pestis*

| LPX-01 | What would be the next step that your laboratory would take in regards to the identification stated above? | Participants (1343) | |
|--------|--|---------------------|------|
| | | No. | % |
| | Contact the appropriate LRN Reference Lab and follow their instructions | 1195 | 89.0 |
| | Call the Centers for Disease Control and Prevention | 38 | 2.8 |
| | Refer the isolate to your normal commercial reference laboratory | 13 | 1.0 |
| | Refer the isolate to the Centers for Disease Control and Prevention | 8 | 0.6 |
| | No further action would be taken | 66 | 4.9 |
| | Other | 23 | 1.7 |

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. Specific local reporting or further network actions are performed at the discretion of the participant's LRN Reference Laboratory. In most instances, this is the state public health laboratory. LRN Sentinel Laboratories should not be calling or referring an isolate to the Centers for Disease Control as their next step. **The isolate from this challenge should have triggered a communication with the participant's LRN Reference Laboratory.**

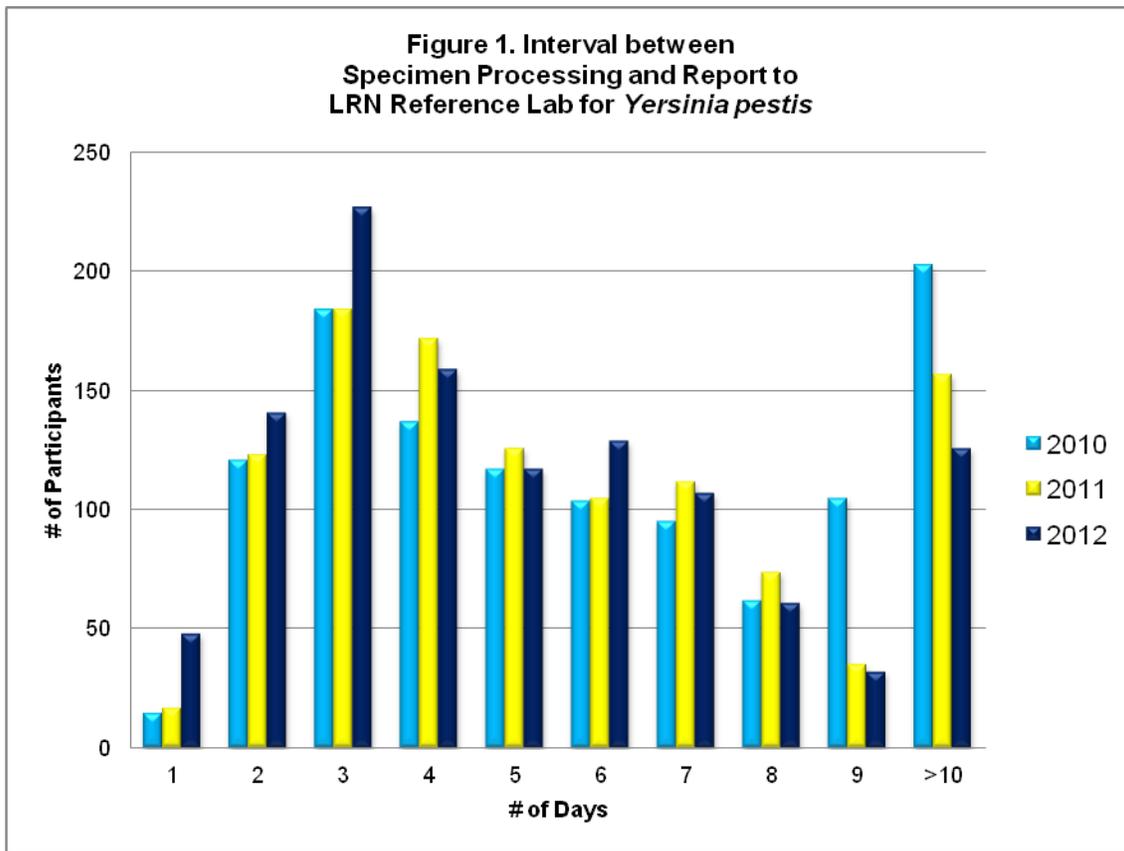


Figure 1 shows the interval between specimen set-up and notification of an LRN Reference Laboratory. Under optimal conditions, the isolate’s growth characteristics, Gram stain and testing from appropriate Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases should be completed within 48 to 72 hours after the initial specimen set-up. LRN Reference Laboratory notification, therefore, should occur in this time frame. From the data collected it is difficult to determine if reference lab notification was initiated as soon as the isolate was determined to be a suspect bioterrorism agent or if the reference lab was notified after testing on all isolates in the exercise was completed. Isolates from this exercise should be treated as patient isolates and **reporting to the LRN Reference Laboratory should be performed in real-time.**

Discussion LPX-01

This challenge contained *Y. pestis* in pure culture. One of the Intended Responses was given by 94% of participants; 6% of participants reported Non-BT Culture.

Y. pestis is a cause of naturally occurring zoonotic infections. The spectrum of disease caused by *Y. pestis* infections is collectively known as plague. Wild rodents are the most common reservoirs for this agent; human infection is caused by handling infected animals or being bitten by rodent fleas infected by feeding on an infected rodent. The epidemiology of human infection mirrors the distribution of the infected rodent population; in the United States, most infections are acquired in the Southwestern States.

Y. pestis has the potential for development as a bioterrorism agent, so notification of Public Health authorities and further epidemiologic investigation is needed as soon as possible after possible isolation of *Y. pestis* from culture. Initial evaluation of suspect isolates using the Sentinel Laboratory Guideline will facilitate timely reporting to Public Health authorities and minimize the potential for laboratory acquired infection with *Y. pestis*. A detailed description of the clinical and laboratory features of *Y. pestis* can be viewed at:

<http://www.asm.org/images/pdf/Clinical/Protocols/ypestis06-11-10.pdf>

Key Tests to rule-out *Yersinia pestis*: The growth of this challenge isolate on TSA sheep blood agar and Gram stain morphology should eliminate *Francisella* sp. and *Brucella* sp. from consideration without need for the specific screening tests for these agents. Gram stain, colony morphology and inconsistent growth on MacConkey agar may make *Burkholderia* species less likely. In this challenge, *Burkholderia* species are ruled out by the negative oxidase reaction.

Culture: Isolates of *Y. pestis* grow well on sheep blood and chocolate agar, as reported by almost all participants. The optimal growth temperature is 28-30°C, but slower growth is seen at 35-37°C incubation; isolation does not require an increased carbon dioxide atmosphere. On SBA, good growth usually requires 48 hours incubation, yielding 1-2mm white to yellowish colonies without significant hemolysis (as reported by 98% of participants). With prolonged incubation, characteristic colonies may be seen, demonstrating an irregular “fried egg” or shiny “hammered copper” surface. Growth on MacConkey agar usually requires extended incubation; small lactose-negative colonies are seen. Of 1018 participants that reported growth on MacConkey, **10% reported lactose-positive** reactions.

Gram stain: Cells of *Y. pestis* show avidly staining, plump gram-negative bacilli/coccobacilli (1-2 μ x 0.5 μ); cells commonly demonstrate bipolar staining, which may be confused with spore formation. Bipolar staining, however, is best demonstrated using special staining, like Wright-Giemsa, performed on patient specimens. Gram-negative bacilli or coccobacilli were reported by virtually all participants.

Catalase: *Y. pestis* isolates are catalase positive, as reported by 89% of participants. Less than 1% of participants reported a negative reaction for catalase, but **10% of participants reported Not Performed** for this Key Reaction.

Indole: *Y. pestis* isolates are indole negative, as reported by 81% of participants. Less than 1% of participants reported a positive indole reaction while **14% did not perform testing** for this Key Reaction.

Oxidase: *Yersinia* species, including *Y. pestis*, are oxidase negative, as reported by 95% of participants. **2% of participants reported a positive oxidase reaction and 3% did not perform oxidase testing.**

Urea: *Y. pestis* isolates are urease negative, as reported by 72% of participants. Less than 1% of participants reported a positive reaction and **16% did not perform urea testing.**

Other Tests: Automated systems do not consistently identify *Yersinia pestis* isolates correctly, and are not recommended for identification of *Yersinia pestis* or other biothreat agents. Common mis-identifications include: *Shigella* sp, H2S-negative *Salmonella* sp., *Acinetobacter* sp., and *Y. pseudotuberculosis*. Use of automated systems may also increase the risk for laboratory acquired infection with this agent.

NOTE: The accuracy of the Guidelines for detecting or ruling out potential BT agents depends on accurate performance of all tests included in the algorithms. Participants who did not achieve the expected results for these key tests of the Sentinel Level Clinical Microbiology Laboratory Guideline for *Yersinia pestis* should review their laboratory protocols and QC records for the specific tests failed.

Participants that did not perform Key Tests outlined in this Guideline should review their protocols for the application of appropriate Sentinel Laboratory Guidelines for potential BT agents.

See the 2012 LPX-A PSR for a complete summary of participant responses for growth characteristics and results of screening tests.

LPX-02

This challenge was a simulated wound specimen from a housing contractor with a slow-healing ankle wound acquired while working on an excavation site in California. This challenge contained *Bacillus megaterium* with *Staphylococcus epidermidis* added as a contaminant. One of the Intended responses was given by virtually all participants, but the responses, as discussed below, suggest that some participants may have failed to recognize the *Bacillus* species in the culture.

Table 1. Summary of the Participants' Identification of LPX-02, *Bacillus megaterium*

| LPX-02 | Identification | Participants (1330) | |
|--------|---|---------------------|------|
| | | No. | % |
| | Non-BT Culture | 1150 | 86.5 |
| | <i>Bacillus</i> sp., refer to rule out <i>Bacillus anthracis</i> | 176 | 13.2 |
| | Gram-positive bacillus, refer to rule out <i>Bacillus anthracis</i> | 52 | 3.9 |

Table 2. Summary of Participants' actions after identifying LPX-02, *Bacillus megaterium*

| LPX-02 | What would be the next step that your laboratory would take in regards to the identification stated above? | Participants (1338) | |
|--------|--|---------------------|------|
| | | No. | % |
| | Contact the appropriate LRN Reference Lab and follow their instructions | 244 | 18.2 |
| | Call the Centers for Disease Control and Prevention | 1 | 0.1 |
| | Refer the isolate to your normal commercial reference laboratory | 18 | 1.4 |
| | No further action would be taken | 1058 | 79.1 |
| | Other | 17 | 1.3 |

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. Specific local reporting or further network actions are performed at the discretion of the participant's LRN Reference Laboratory. In most instances, this is the state public health laboratory. LRN Sentinel Laboratories should not be calling the Centers for Disease Control and Prevention as their next step. **The isolate from this challenge should not have triggered a communication with the participant's LRN Reference Laboratory.**

Discussion LPX-02

Participants should have recognized two different colony types. The *Staphylococcus* species is non-pigmented, non-hemolytic colony type showed gram-positive cocci; Coagulase or latex testing for *Staphylococcus aureus* was negative. These findings are consistent with a coagulase-negative *Staphylococcus*, presumably a contaminant, and should not raise suspicion for a potential bio-terror agent. Gram stain of the second colony type should have demonstrated large gram-positive bacilli.

Bacillus megaterium, the *Bacillus* species used for this challenge, has some characteristics similar to *B. anthracis*. *B. megaterium* is widely distributed in soil and has only rarely been associated with true infections. Growth of round or irregular, non-hemolytic colonies is seen at 24 to 48 hours. Bacterial cells are large: approximately 1.4 micrometers wide and 5 micrometers or greater in length.

Cutaneous anthrax is well described and occurs when vegetative bacteria or spores bacteria enter the skin at the site of a cut or abrasion. A papulo-vesicle lesion forms at the site, which ruptures to form a painless ulcer. Necrosis at the base of the ulcer results in formation of the typical black appearance. Systemic spread of infection is a life-threatening complication of cutaneous anthrax.

Key Tests to rule out anthrax: Though the colony morphology of the *Bacillus* species distributed for this challenge does not share the typical characteristics of *B. anthracis*, isolation of a non-hemolytic *Bacillus* species from a wound could be consistent with cutaneous *Bacillus anthracis* infection, which should be ruled-out by further testing according to the Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases.

Culture: The *Bacillus* colony type in this challenge showed white to off-white, creamy colonies with irregular shape on sheep blood agar. Colonies were 2-5 mm in diameter after 18 to 24 hours incubation. *B. anthracis* also forms 2-5 mm colonies on SBA after overnight incubation, but colonies are irregularly round with irregular edges and possible comma-shaped projections; colonies have a ground-glass appearance and may have a tenacious consistency. Growth was also consistently reported from chocolate, but not MacConkey agar.

Hemolysis on SBA: Both *B. anthracis* and *B. megaterium*, the *Bacillus* species used in this challenge, are non-hemolytic on SBA. Though beta-hemolysis on SBA rules-out *B. anthracis*, it is not specific: there are other non-hemolytic *Bacillus* species. The majority of participants reported no hemolysis on SBA, but 9% reported beta-hemolysis, while 3% reported that the hemolytic reaction was Not Applicable for this Key Reaction.

Gram Stain: *B. anthracis* shows large gram-positive bacilli (1-1.5 μ x 3-5 μ). Gram-positive organisms were reported by 98% of participants, but **60% of participants reported cocci**, and only 44% of participants reported gram-positive bacilli. These findings suggest that some participants were unable to detect both organisms in this mixed culture, and may have reported results based on evaluation of the coagulase-negative *Staphylococcus* only. *B. anthracis* forms oval, central to sub-terminal spores that do not cause swelling of the bacterial cell, as does *B. megaterium*, the species used in this challenge. The presence of spores was noted by 5% of participants.

Catalase: *Bacillus* species are catalase positive. A positive catalase reaction was reported by 88% of participants. **A negative catalase was reported by 2% of participants, while 10% of participants reported Not Performed.**

Motility: *B. anthracis* is non-motile, while *B. megaterium* is motile. Only 17% of participants reported positive motility for this challenge. Negative motility was reported by 24% and Not Performed by 50% of participants for this Key Reaction.

NOTE: The accuracy of the Guidelines for detecting or ruling out potential BT agents depends on isolation of suspect isolates from mixed cultures and accurate performance of all tests included in the algorithms. Participants who did not achieve the expected results for these key tests of the Sentinel Level Clinical Microbiology Laboratory Guideline for Anthrax should review their laboratory protocols and QC records for the specific tests failed.

Participants that failed to recognize the *Bacillus* species in this challenge should review their protocol for culture examination to ensure that mixed cultures are consistently identified.

Participants that did not perform Key Tests outlined in this Guideline should review their protocols for the application of appropriate Sentinel Laboratory Guidelines for potential BT agents.

See the 2012 LPX-A PSR for a complete summary of participant responses for growth characteristics and results of screening tests.

LPX-03

This challenge was a simulated bronchoalveolar lavage specimen from a weaver who uses native wools from the Mideast on open looms with rapid onset of shortness of breath and mediastinal widening on chest x-ray. This challenge contained *Bacillus anthracis* with a *viridans streptococcus* species added as a contaminant. One of the Intended Responses was reported by a majority of participants; however 15.5% of participants reported a Non-BT Culture.

Table 1. Summary of the Participants' Identification of LPX-03, *Bacillus anthracis*

| LPX-03 | Identification | Participants (1325) | |
|--------|---|---------------------|------|
| | | No. | % |
| | <i>Bacillus anthracis</i> , confirmed | 38 | 2.9 |
| | <i>Bacillus anthracis</i> , refer for confirmation | 78 | 5.9 |
| | <i>Bacillus</i> sp., refer to rule out <i>Bacillus anthracis</i> | 905 | 68.3 |
| | Gram-positive bacillus, refer to rule out <i>Bacillus anthracis</i> | 273 | 20.6 |
| | Non-BT Culture | 205 | 15.5 |

Table 2. Summary of Participants' actions after identifying LPX-03, *Bacillus anthracis*

| LPX-03 | What would be the next step that your laboratory would take in regards to the identification stated above? | Participants (1345) | |
|--------|--|---------------------|------|
| | | No. | % |
| | Contact the appropriate LRN Reference Lab and follow their instructions | 1222 | 90.9 |
| | Call the Centers for Disease Control and Prevention | 35 | 2.6 |
| | Refer the isolate to your normal commercial reference laboratory | 11 | 0.8 |
| | Refer the isolate to the Centers for Disease Control and Prevention | 14 | 1.0 |
| | No further action would be taken | 42 | 3.1 |
| | Other | 21 | 1.6 |

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. Specific local reporting or further network actions are performed at the discretion of the participant's LRN Reference Laboratory. In most instances, this is the state public health laboratory. LRN Sentinel Laboratories should not be calling the Centers for Disease Control as their next step. **The isolate from this challenge should have triggered a communication with the participant's LRN Reference Laboratory.**

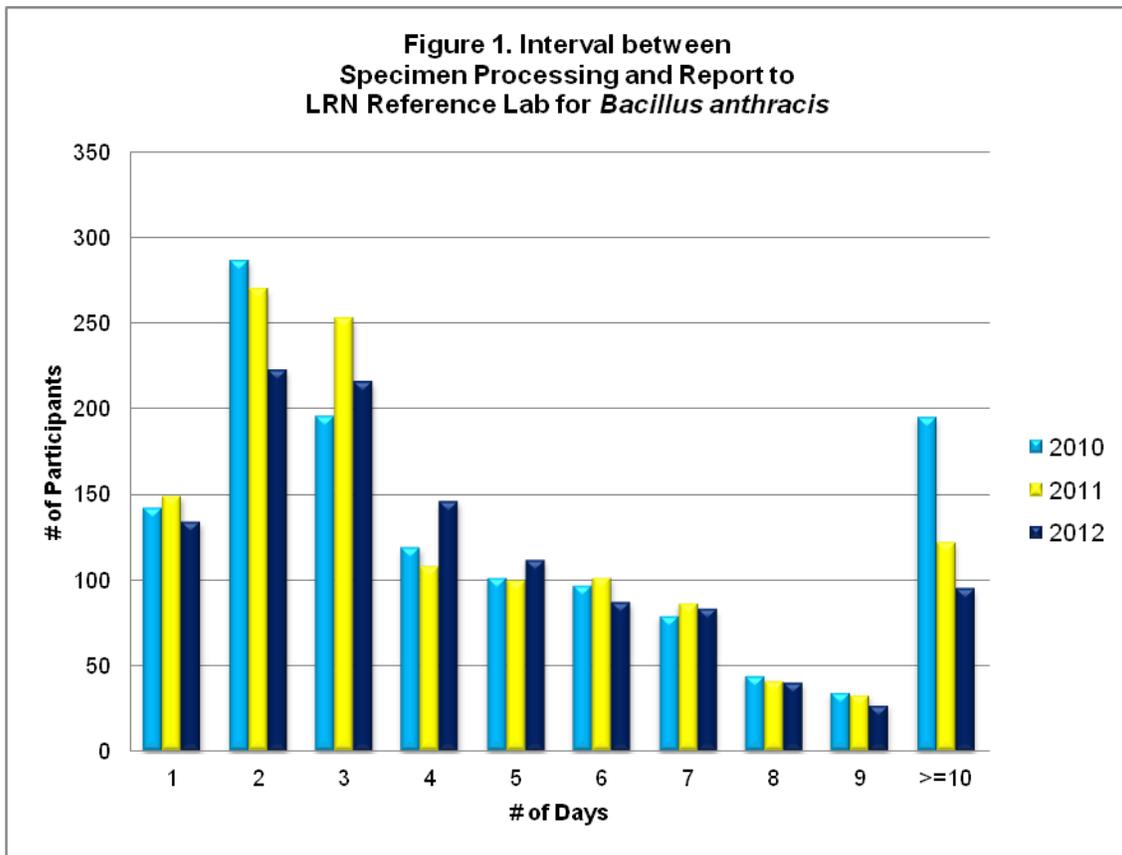


Figure 1 shows the interval between specimen set-up and notification of an LRN Reference Laboratory. Under optimal conditions, the isolate’s growth characteristics, Gram stain and testing from appropriate Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases should be completed within 48 to 72 hours after the initial specimen set-up. LRN Reference Laboratory notification, therefore, should occur in this time frame. From the data collected it is difficult to determine if reference lab notification was initiated as soon as the isolate was determined to be a suspect bioterrorism agent or if the reference lab was notified after testing on all isolates in the exercise was completed. Isolates from this exercise should be treated as patient isolates and **reporting to the LRN Reference Laboratory should be performed in real-time.**

Discussion LPX-03

Participants should have recognized two different colony types. An alpha-hemolytic colony type showed gram-positive cocci in chains; catalase testing was negative. These findings are consistent with a viridans *Streptococcus* species, presumably a contaminant, and should not raise suspicion for a potential bio-terror agent. Gram stain of the second colony type should have demonstrated large gram-positive bacilli.

Inhalational anthrax is well described and results from the inhalation of *B. anthracis* spores. The source of the spores may be from the environment, or materials contaminated by environmental sources, like native wools or skins from an endemic area. The spores may also be delivered intentionally as a bio-terror incident.

Bacillus anthracis has been used as a bioterrorism agent, so notification of Public Health authorities and further epidemiologic investigation is needed as soon as possible after clinical suspicion or isolation of *B. anthracis* from culture. Initial evaluation of suspect isolates using the Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases will facilitate timely reporting to Public Health authorities and minimize the potential for laboratory acquired infection with *B. anthracis*. A detailed description of the clinical and laboratory features of *B. anthracis* can be viewed at:

<http://www.asm.org/images/pdf/Clinical/Protocols/anthrax.pdf>

Key Tests to rule out anthrax:

Culture: *B. anthracis* forms 2-5 mm colonies on SBA after overnight incubation; colonies are irregularly round with irregular edges and comma-shaped projections; colonies have a ground-glass appearance and a tenacious consistency. Growth on SBA was reported by 98% of participants. Growth was also consistently reported from chocolate, but not MacConkey agar.

Hemolysis on SBA: *B. anthracis* isolates are non-hemolytic on SBA. Though beta-hemolysis on SBA rules-out *B. anthracis*, it is not specific: there are other non-hemolytic *Bacillus* species. Almost all participants (99%) reported no hemolysis on SBA.

Gram Stain: *B. anthracis* shows large gram-positive bacilli (1-1.5 μ x 3-5 μ). Gram-positive bacilli were reported by 99% of participants. *B. anthracis* forms oval, central to sub-terminal spores that do not cause swelling of the bacterial cell. The presence of spores was noted by 35% of participants.

Catalase: *Bacillus* species are catalase positive. A positive catalase reaction was reported by 90% of participants. **A negative catalase was reported by 2% of participants, while 8% of participants reported Not Performed.**

Motility: *B. anthracis* is non-motile. A negative motility was reported by 79% of participants. **3% of participants reported positive motility for this challenge; 9% of participants reported Not Performed for this Key Reaction.**

NOTE: The accuracy of the Guidelines for detecting or ruling out potential BT agents depends on isolation of suspect isolates from mixed cultures and accurate performance of all tests included in the algorithms. Participants who did not achieve the expected results for these key tests of the Sentinel Level Clinical Microbiology Laboratory Guideline for Anthrax should review their laboratory protocols and QC records for the specific tests failed.

Participants that did not perform Key Tests outlined in this Guideline should review their protocols for the application of appropriate Sentinel Laboratory Guidelines for potential BT agents.

See the 2012 LPX-A PSR for a complete summary of participant responses for growth characteristics and results of screening tests.

2012 LPX-A Summary

It is important that all laboratories which process blood, wound, lower respiratory and other critical specimens for bacterial isolation be able to recognize virulent organisms of epidemiologic importance early in the testing process. Initial suspicion is based on growth characteristics, colony morphology and Gram staining, as well as information about the patient's presenting illness. Testing for further characterization of isolates to rule-out agents of epidemiologic importance should follow protocols to minimize possible exposure of laboratory staff and to provide informative test results with a minimum turn-around-time. This approach will ensure that patients receive timely and appropriate care, laboratory staff is protected from possible laboratory acquired infection and public health officials are able to perform appropriate assessment and interventions to protect the community from spread of infection. The Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases provide detailed protocols for recognition of many relevant agents. The CAP Laboratory Preparedness Exercises are a tool for clinical microbiology laboratories to assess their ability to recognize and characterize potential BT agents and to communicate relevant findings to within the Laboratory Response Network.

The participants in the LPX-A 2012 exercise performed acceptably overall in terms of culture evaluation and communication.

LPX-01 was a challenge of *Yersinia pestis*. 94% reported one of the intended responses as a potential or confirmed BT agent. 6% of participants reported a Non-BT Culture. These laboratories should review their test results and application of the appropriate algorithm to rule-out *Y. pestis*. Specifically, review assessment of the growth rate and colony morphology of the challenge organism on SBA and MacConkey agar, and performance and results of oxidase, catalase, urease and indole tests.

The results of the established testing protocols do not rule-out *Yersinia pestis* infection, so this culture should trigger communication with the participant's LRN Reference Laboratory.

LPX-02 was a mixed wound culture of *Bacillus megaterium* and a coagulase-negative *Staphylococcus* species. Participant laboratories should have recognized both isolates; the *Bacillus* isolate should have been processed according to the Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases protocol for anthrax. Anthrax would be ruled-out by a positive motility test.

While almost all participants reported this challenge as a Non-BT Culture, results of Gram stain testing suggest that many laboratories may have based their report on evaluation of the coagulase-negative *Staphylococcus*. Because many agents of epidemiologic importance are slow growing and may be isolated from specimens with contaminating host flora, like cutaneous and lower respiratory specimens, careful examination of all media and evaluation of all colony types is essential. Participants who did not recognize the *Bacillus* species in this challenge should review their protocols for cultures and examination of media to ensure that all potential pathogens are detected.

LPX-03 was also a mixed BAL culture challenge including the vaccine-strain *Bacillus anthracis* and a viridans streptococcal species. Almost all participants (97%) recognized and evaluated the gram-positive bacillus in this challenge. 15% of participants reported Non-BT Culture. These laboratories should review their test results and application of the appropriate algorithm to rule-out anthrax. Specifically, review assessment of the growth rate, hemolysis and colony morphology of the challenge organism on SBA agar, and performance and results of catalase and motility tests.

The results of the established testing protocols do not rule-out anthrax, so this culture should trigger communication with the participant's LRN Reference Laboratory.

Additional Comments:

Mixed Cultures: Two specimens (LPX-02 and LPX-03) were mixed culture challenges. Because potential BT agents may be intentionally or naturally transmitted by the airborne or direct inoculation, specimens submitted for culture may be contaminated by the patient's normal flora. Though the *Bacillus* species in LPX-03 was recognized by almost all participants, it appears that a significant number of participants only recognized the staphylococcal contaminant in LPX-02. LPX challenge cultures should be examined for multiple isolates in the same manner that patient cultures are examined.

Communication with LRN Reference Laboratories: The histogram for the intervals between specimen processing and LRN Reference Laboratory contact are shown above for LPX-01 and LPX-03. Testing of isolates according to Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases should yield actionable results within 48 to 72 hours after initial specimen processing. These histograms show peaks in the distribution of reporting at two to three days. However, the reporting interval for a significant number of participants is greater than 4 days, with many reporting results at >10 days.

According to the LPX Kit Instructions: "To test appropriate communications between LRN Sentinel Laboratories and LRN Reference Laboratories, participants in this exercise are **required** to contact their LRN Reference Laboratory if indicated, after performing the established Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases on an isolate. At the discretion of the LRN Reference Laboratory, the participating LRN Sentinel Laboratory may then be asked to package and ship the isolate to their LRN Reference Laboratory or may otherwise be directed as to the next steps to follow." A number of LRN Reference Laboratories do not require their Sentinel Laboratories to contact them directly for LPX isolates. However, the date of initial processing and the date on which results of relevant testing from Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases are completed should be reported on the Result Form for each challenge in order that testing efficiency may be assessed by the LRN Reference Laboratories.

Participants should report the date of completion of relevant testing for each challenge culture individually and not report turn-around-times based on completion of testing for all challenge cultures (e.g., date of reporting final results to CAP).

Availability and performance of Key Tests in LRN Laboratories: There is some concern that laboratories no longer perform some of the Key Tests used in the Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases, such as urease and TSI/KIA.

For LPX-01, participants reported that indole testing and urease testing, Key Tests to rule-out *Y. pestis*, were Not Available in 4% and 12% of labs, respectively.

For LPX-02 and LPX-03, 9% of participants reported that motility testing was Not Available.

In addition, as discussed above, Key Tests from the applicable Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases were Not Performed. It is recommended that participants review test availability and application of relevant Sentinel Level Clinical Microbiology Laboratory Guidelines for Suspected Agents of Bioterrorism and Emerging Infectious Diseases with their LRN Reference Laboratories.

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