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# Idaho Bureau of Laboratories Clinical Forum

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## Idaho Plague Update

Erin Peterson

Following the recent confirmation of *Yersinia pestis*, the causative agent of plague, in an Idaho ground squirrel, the LRN-B laboratory has been testing other samples for this outbreak. While the lab has done a few human plague rule-out tests (2), the majority of testing has been conducted on family pets and wildlife. The lab has seen three positives:

- One family dog from Boise tested positive via real-time PCR.

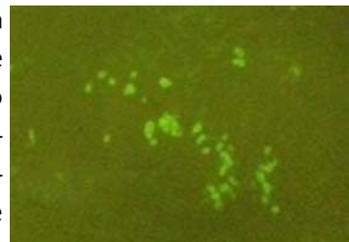


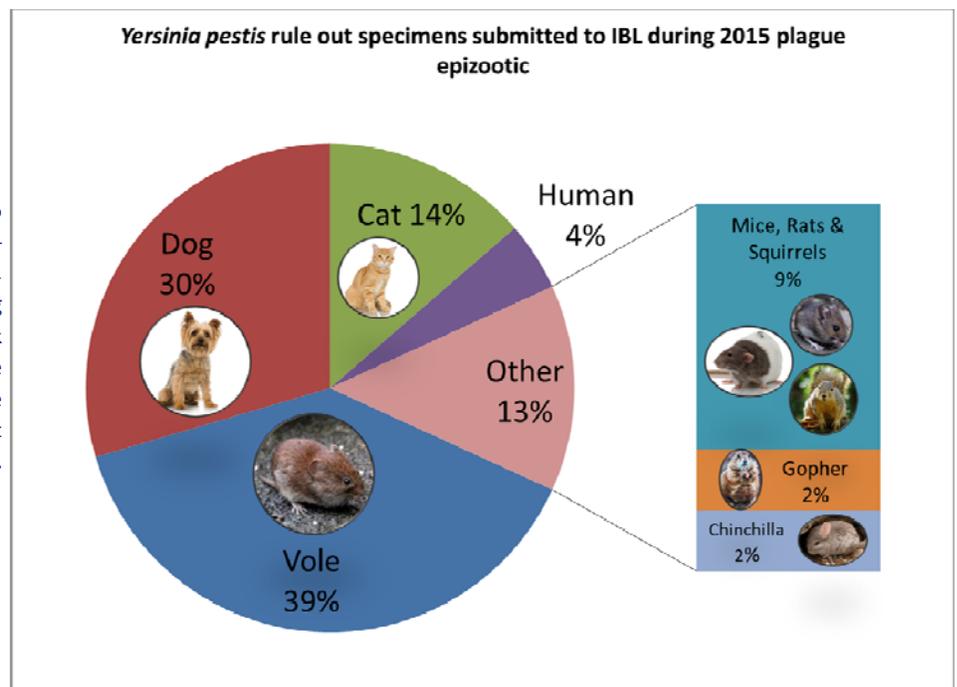
Figure 1. Polyclonal rabbit anti-*Y. pestis* F1 antigen antibody labeled with fluorescein isothiocyanate (FITC) reacts with the F1 antigen on the cell surface of *Yersinia pestis*. This smear was made from a *Yersinia pestis* positive control isolate.

- Two voles tested positive via Direct Fluorescent Antibody (DFA) microscopy in the Riddle, Idaho area and south of Caldwell.

Although no human cases have been identified, clinical laboratories are asked to be on alert for key indicators of *Yersinia pestis*.

(Continued on page 2)

Figure 2. Idaho Bureau of Laboratories tested 44 samples during the 2015 outbreak with 1 positive dog and 2 positive voles. The rest were negative.



Clinical Forum  
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# Idaho Plague Update

*continued from page 1*

## Yersinia pestis in the Lab

**Gram Stain Morphology:** Gram-negative, plump rods, 0.5 x 1-2 mm.

**Colony Morphology:** Slow growing, pinpoint (1-2 mm), gray-white to opaque colonies on BAP after 24 h; non-lactose fermenter on MAC/EMB; growing both at 25-28°C and at 35-37°C.

**Biochemicals:** Oxidase Negative, Indole Negative, Urease Negative and Catalase Positive

*Specimen is blood, sputum, or lymph node aspirate*

*Yersinia pestis* is notoriously misidentified on most automated systems (see *Automated Systems on High Consequence Organisms* on page 4). Misidentifications may include *Y. pseudotuberculosis*, *Shigella*, *Salmonella*, *Acinetobacter* and *Pseudomonas spp.*

Contact the LRN-B laboratory at 208-334-0515 with questions pertaining to this outbreak.

## Biosafety Bubble

### Aerosolization Potential in the Lab

Do your lab procedures generate aerosols? Odds are, they probably do. In fact, aerosol production is frequently associated with pipetting, mixing with a pipette or vortex mixer, and use of blenders, centrifugation, and sonicators. Keep these tips in mind to minimize the aerosolization risk in your lab:

**#1 General tips:** Wear gloves, eye protection, and a lab coat when working in the laboratory to protect against aerosolization. Work in a biological safety cabinet where prudent and applicable.

**#2 Pipetting:** Touch pipette tips to the inside of the well or tube before pressing the delivery plunger. Splashing and contamination occurs when the pipetting stream is directed into the middle of the well. Pipet mixing should be conducted with the tip of the pipet below the surface of the liquid. Avoid using the blow-out function of mechanical pipets unless absolutely necessary.

**#3 ELISA plate washers:** Large-particle aerosols can deposit infectious agents on immediate surfaces with finer aerosols traveling greater distances to be inhaled. Handle ELISA plates with gloves, disinfect plate washers and surrounding areas daily, and place aerosol containment covers over plate washers when possible.

**#4 Centrifugation:** Follow manufacturer instructions for care and use of centrifuges, clean centrifuges after each shift and immediately after a spill, and use aerosol containments and gasketed safety cups.

**#5 Sonication:** Use the lowest effective power setting, cover bath sonicators while in use, conduct organism lysis and homogenization procedures in closed containers, and change bath fluids frequently.

# Web Based Training for CLIA Requirements

Amanda J. Bruesch, M.S. and Wendy Loumeau

In 2014, the Idaho Bureau of Laboratories (IBL) conducted a survey to assess the needs of the Idaho Sentinel Laboratory Network. This assessment indicated that an online training regarding Clinical Laboratory Improvement Amendments (CLIA) regulations, proficiency testing (PT) requirements, and laboratory quality improvement were of interest to clinical labs (Figure 1). To respond to this need, IBL developed an online training module focused on CLIA and state PT requirements. It provides an on-demand resource to learn about the PT requirements needed to maintain a valid accreditation program in Idaho and offers guidance for clinical laboratory staff to troubleshoot failed PT events and develop corrective action plans.

The objectives for this module were as follows:

1. Classify CLIA certificate levels and describe the associated PT requirements for each level
2. Differentiate between PT requirements for regulated and non-regulated analytes
3. Describe strategies for successfully troubleshooting failed proficiency tests
4. List required elements for constructing an acceptable plan of correction in Idaho

This pilot module was available during April 2015 and dis-

tributed to individuals within Idaho who perform clinical diagnostic testing. Pre- and post-test results were collected and assessed (Figure 2), and feedback was collected; a revised training will be housed on the IBL website training page ([www.statelab.idaho.gov](http://www.statelab.idaho.gov)) in the coming months.

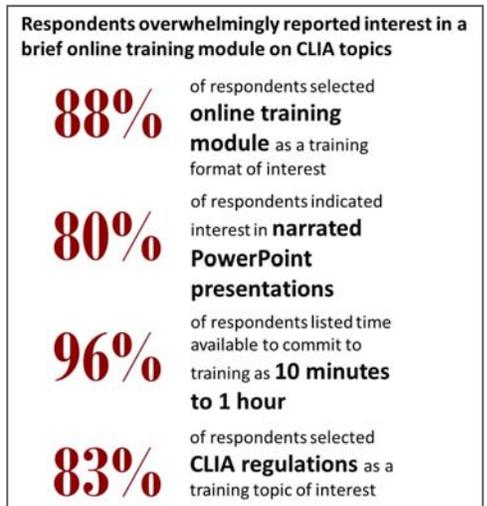


Figure 1. Summary of responses from the 2014 Sentinel Laboratory Needs Assessment.

This project demonstrated that concise, focused training modules can impact learner ability to interpret complex regulatory requirements and apply them to laboratory-specific situations. The use of beta-testing for a training web-based module prior to deployment resulted in substantial edits and improvements in the efficacy and quality of the final product. Contact Amanda Bruesch for more information at [bruescha@dhw.idaho.gov](mailto:bruescha@dhw.idaho.gov).

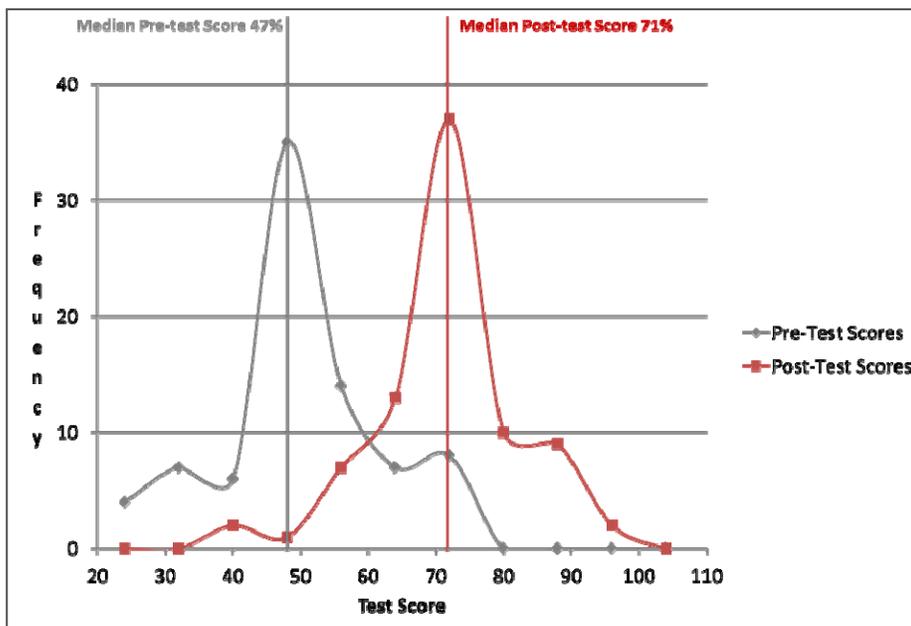


Figure 2. Frequency distribution of pre- and post-test scores show a substantial shift following observation of the training module.

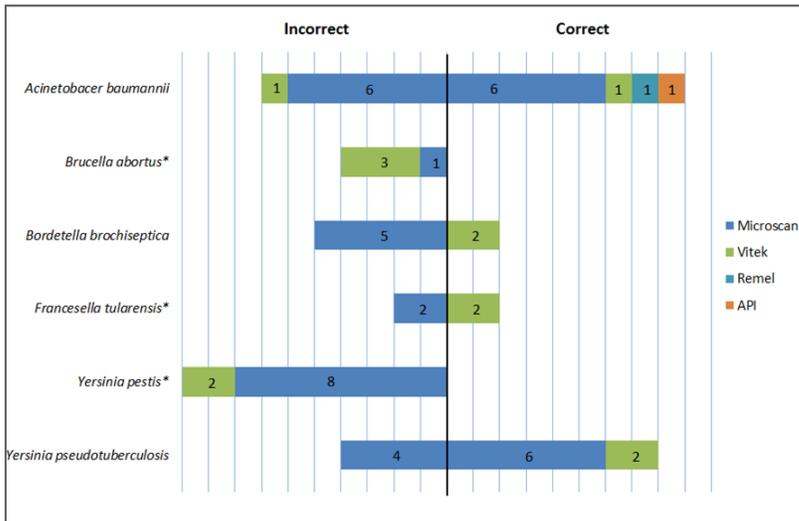
# Automated Systems on High Consequence Organisms

Wendy Loumeau and Robert Voermans

The American Society for Microbiology Sentinel Laboratory Guidelines caution against using automated systems for identification of high consequence organisms (e.g. *Bacillus anthracis*, *Yersinia pestis*, and *Francisella tularensis*) in the clinical laboratory setting. Concerns about this practice include database accuracy, poor reactivity for some agents, and potential exposure from agent aerosolization.

To evaluate these concerns, the Idaho Bureau of Laboratories (IBL) has been monitoring performance data from automated systems use on Sentinel Laboratory Preparedness Surveys. This is done by comparing the isolate sent by IBL with the agent identified by sentinel laboratory’s automated systems. Figure 1 shows results from selected 2014 and 2015 surveys. The graphic displays the performance of several different automated systems and their ability to correctly identify biological threat (BT) and fastidious bacterial species. Overall, the accuracy rate for these species was about 40%. When testing the BT agents *Brucella abortus*, *Francisella tularensis*, and *Yersinia pestis*, automated systems correctly identified the sample only twice (11%). Misidentification of BT agents can lead to serious treatment complications for patients (see Case Studies sidebar).

Figure 1. Automated systems are often inconsistent in the identification of select agents including *Brucella* spp., *Francisella tularensis*, and *Yersinia pestis*. \*Biological threat agent



Thankfully, the prevalence of BT agents in clinical samples is very low in Idaho, but recently, ground squirrels, voles, and a dog have tested positive for *Yersinia pestis*, the causative agent of plague, in the Treasure Valley<sup>2</sup> (see *Idaho Plague Update* on page 1). IBL is reminding Idaho clinical labs to be on alert for samples that may be indicative of *Yersinia pestis*. This includes an increased awareness on the limitations of automated systems in identifying *Yersinia pestis* and other BT agents.

If a BT agent is suspected in a clinical sample, do not use a commercial identification system. Instead, call IBL’s LRN-B laboratory at 208-334-0515 for guidance and sample referral.

References on page 6.

## Case Studies

Cases 1-3 present published examples of automated system misidentifications where the isolate was later identified as a BT agent. It is essential for clinical labs to identify inconsistencies between automated system results and the isolate.

**Case #1:** In August 2008, a 35 year old man from Switzerland who had recently returned from Thailand was admitted to a hospital in Switzerland with a head abscess. Culture material from the abscess revealed gram negative, oxidase positive rods, and growth on sheep blood agar at 48 hours<sup>3</sup>.

Misidentification: *Burkholderia cepacia* (99% confidence)

Actual Culprit: *Burkholderia pseudomallei*

**Case #2:** A 35 year old man was admitted to the intensive care unit in critical condition with sepsis syndrome. The aerobic blood culture specimens were positive after 48 hours of incubation<sup>4</sup>.

Misidentification: *Bergeyella zoohelcum* (64% probability)

Actual culprit: *Brucella melitensis*

**Case #3:** Two patients, aged 17 and 42 years, experienced high fever and multiple bilateral inguinal buboes. Blood samples were collected from one patient, isolating a gram-negative rod with bipolar staining<sup>5</sup>.

Misidentification: *Acinetobacter lwoffii*, *Pseudomonas luteola*, *Yersinia pseudotuberculosis*

Actual culprit: *Yersinia pestis*

# Match the Pathogen to its Source

answers on page 6

1. \_\_\_\_\_ Beaver



2. \_\_\_\_\_ Cow



3. \_\_\_\_\_ Mouse



4. \_\_\_\_\_ Bat



5. \_\_\_\_\_ Pig



6. \_\_\_\_\_ Cat



7. \_\_\_\_\_ Mosquito



8. \_\_\_\_\_ Rabbit



9. \_\_\_\_\_ Tick



10. \_\_\_\_\_ Sprouts



11. \_\_\_\_\_ Rat



12. \_\_\_\_\_ Raw meat



## Pathogen List

- |  |                      |
|--|----------------------|
| a. <i>Yersinia pestis</i> (plague)           | g. Influenza         |
| b. Hantavirus                                | h. Toxoplasmosis     |
| c. Lyme Disease                              | i. <i>Salmonella</i> |
| d. <i>Francisella tularensis</i> (tularemia) | j. Rabies            |
| e. Giardia                                   | k. West Nile Virus   |
| f. <i>E. coli</i> O157:H7                    | l. Cryptosporidium   |

To be added

or removed

from the

Clinical Forum

email list:

[statelab@dhw.idaho.gov](mailto:statelab@dhw.idaho.gov)

## Solution to Match the Pathogen to its Source

- |               |  |
|---------------|--|
| 1. Beaver:    | E – Giardia                                      |
| 2. Cow:       | L – Cryptosporidium                              |
| 3. Mouse:     | B – Hantavirus                                   |
| 4. Bat:       | J – Rabies                                       |
| 5. Pig :      | G – Influenza                                    |
| 6. Cat:       | H – Toxoplasmosis                                |
| 7. Mosquito:  | K– West Nile Virus                               |
| 8. Rabbit:    | D – <i>Francisella tularensis</i><br>(tularemia) |
| 9. Tick:      | C – Lyme Disease                                 |
| 10. Sprouts : | I – <i>Salmonella</i>                            |
| 11. Rat:      | A – <i>Yersinia pestis</i> (plague)              |
| 12. Raw meat: | F - <i>E. coli</i> O157:H7                       |

## Upcoming Webinars

October 27, 2015; 11:00 am Mountain Time

“2015 Influenza Update”

October 29, 2015; 11:00 am Mountain Time

“Quality Management for the Medical Microbiology Laboratory”

Contact Wendy Loumeau at

[loumeauw@dhw.idaho.gov](mailto:loumeauw@dhw.idaho.gov) to access archived programs.

### Biosafety Bubble Reference

Centers for Disease Control and Prevention. Guidelines for Safe Work Practices in Human and Animal Medical Diagnostic Laboratories. Recommendations of a CDC-convened, Biosafety Blue Ribbon Panel. MMWR 2012;61 (Supplement).

### References for Automated Systems article

<sup>1</sup>American Society for Microbiology. (2015). Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases. Retrieved from <http://www.asm.org/index.php/guidelines/sentinel-guidelines>

<sup>2</sup>Idaho Department of Health and Welfare (2015). Treasure Valley ground squirrel tests positive for plague. Retrieved from <http://dhwblog.com/2015/05/22/treasure-valley-ground-squirrel-tests-positive-for-plague/>

<sup>3</sup>Weissert, C., Dollenmaier, G., Rafeiner, P., Riehm, J., & Schultze, D. (2009). *Burkholderia pseudomallei* Misidentified by Automated System. Emerging Infectious Diseases, 15(11). Retrieved from <http://wwwnc.cdc.gov/eid/article/15/11/pdfs/08-1719.pdf>

<sup>4</sup>Dash, N., Al-Zarouni, M., Rattan, A., & Panigrahi, D. (2012). Misidentification of *Brucella melitensis* as *Bergeyella zoohelcum* by MicroScan WalkAway®: A Case Report. *Medical Principles and Practice*, 21. Retrieved from <http://www.karger.com/Article/FullText/338391>

<sup>5</sup>Centers for Disease Control and Prevention. (2011). Two Cases of Human Plague—Oregon, 2010. *Morbidity and Mortality Weekly Report*, 8(7). Retrieved from <http://www.cdc.gov/mmwr/index2011.html>