



## NEW TECHNOLOGY UPDATE FOR SENTINEL LABS: MALDI-TOF SAMPLE PREPARATION SAFETY, LRN RULE-OUT & REFER APP

Leah Kloss\*

Advances in diagnostic technology are revolutionizing clinical microbiology for the sentinel laboratory. Matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) mass spectrometry is a versatile method used to analyze the composition of biomolecules and other organic macromolecules<sup>1</sup>. It involves the analysis of biomolecules due to the absorption of laser energy, desorption and ionization of proteins, and the time for ions to reach a detector. The analysis produces a spectrum of intensity versus mass-to-charge ration allowing users to match against known spectra of microorganisms. To prepare specimens/samples are prepared for MALDI-TOF analysis using either the direct method, extended method or tube extraction method.

It is currently estimated that 10%-15% of clinical sentinel laboratories use MALDI-TOF for clinical diagnosis. As the use of MALDI-TOF within sentinel laboratories becomes more prevalent, new questions are arising about laboratorian safety and risk reduction while preparing samples for analysis.

The Association of Public Health Laboratories (APHL) recently released a report that discussed the findings of a study designed to evaluate whether the three preparation techniques kill isolates before analysis by MALDI-TOF. If not, there is potential for aerosol production and subsequent lab exposures. A summary follows.

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### Special points of interest:

- *Sentinel Laboratory Guidelines updated September 4, 2015*
- *Wash Your Hands to help prevent infection*
- *Flu Season is here, will you get vaccinated?*
- *Meet the Staff—Betty Wheeler, Biodefense trainer from the Jacksonville Laboratory*

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(Find the complete report at <https://www.aphlweb.org/cmt/phprcmt/sot/MALDITOF/MALDI-TOF%20Safety%20Study%20Final%20Report%2006-30-2015.pdf>).<sup>2</sup>

The study examined samples of potential bioterrorism agents prepared by the three methods used to prepare isolates according to MALDI-TOF protocols: direct method, extended method, and tube extraction method. In the direct method, colonies from growth plates are applied directly to the MALDI-TOF target as a smear and are covered with  $\alpha$ -cyano-4-hydroxycinnamic acid (HCCA/CHCA) matrix. In the extended method, colonies are applied to the target as a smear and are covered with 70% formic acid before the HCCA/CHCA matrix. The tube extraction method extracts organisms by ethanol, formic acid, and acetonitrile before smearing and then overlays the HCCA/CHCA matrix.



The agents studied were *Bacillus anthracis* Sterne, *Brucella abortus* Strain 19, *Burkholderia thailandensis* ATCC 70038, *Clostridium botulinum* toxin producing strains (toxins A, B, and E), *Clostridium perfringens* WAL-14572, *Francisella tularensis* subspecies *holarctica* LVS, and *Yersinia pestis* A1122. After preparing the samples according to the three methods under study, growth was assessed at 24 hours, 48 hours, 7 days, and, for *B. abortus*, 21 days.

The study found that some viable organisms were present after preparation of samples using the direct and extended methods, and that viability did not seem to be related to the number of organisms present in the original suspension. No viable organisms were found following the tube extraction.

Therefore, the report recommends that sentinel labs using MALDI-TOF should utilize the tube extraction method for sample preparation of highly pathogenic organisms. Furthermore, the report recommends performing sample preparation in a Biological Safety Level (BSL) 3 environment or in a biosafety cabinet using BSL 3 practices; and, additionally, filtering samples from the tube extraction through a 0.1  $\mu$ m filter before preparing the MALDI target.

CDC has produced an application for iPad (available at the Apple App Store as a free download) that provides a new way for sentinel laboratories to access reference information.<sup>3</sup>

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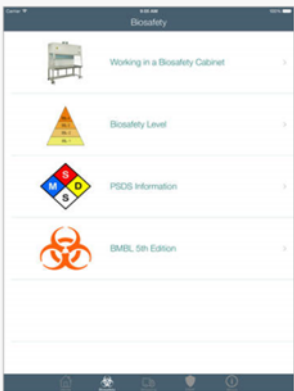
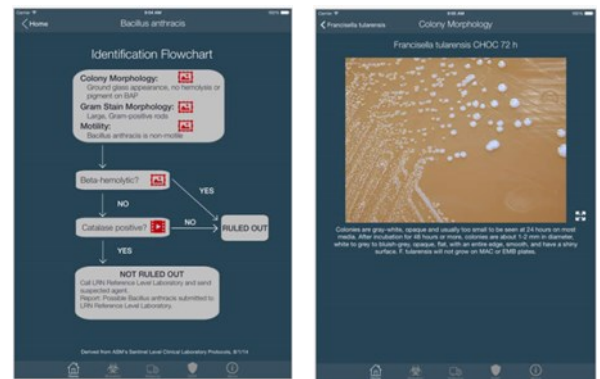


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The "Laboratory Response Network Rule-Out and Refer" app provides rule-out flowcharts for *Bacillus anthracis*, *Brucella spp.*, *Burkholderia mallei* and *pseudomallei*, *Yersinia pestis*, and *Francisella tularensis*. Related information and links about the select agent program, Category A and B packaging and shipping, and biosafety are included.

On the app home screen image, you see the agents addressed. Clicking on each one takes you to a one-page rule-out flowchart with links to images of colony and Gram stain morphology for reference and video clips of recommended biochemical tests. Along the bottom of the home screen, there are icons for additional information under the headings of Biosafety, Shipping, DSAT, and About.

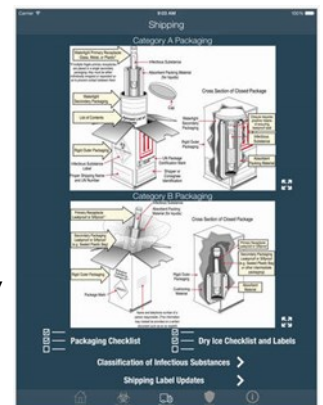


The "Biosafety" section contains a video on working in a biosafety cabinet, definitions of the four biosafety levels, product safety data sheets for the pathogens listed, and a link to the online version of Biosafety in Microbiological and Biomedical Laboratories, 5<sup>th</sup> edition.

"Shipping" has checklists for packaging and shipping Category A, B, GMO (genetically modified organisms), and Exempt specimens. "DSAT" (Division of Select Agents and Toxins) lists the Tier 1 Select Agents and Toxins and APHIS/CDC (Animal and Plant Health Inspection

Service/Centers for Disease Control and Prevention) Form 2 procedure, and "About" contains the app developer information, references, disclaimers, and privacy policy.

Note that the app is currently available only for iPads, not iPhones, to avoid conflicts with policies regarding phone use in the laboratory, so it will be most useful for those sentinel laboratories with dedicated iPads. An Android counterpart app is under development.



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***“not a  
replacement  
for training”***

The app is not a substitute for the LRN sentinel protocols nor is it a replacement for training in LRN rule-out methods. Instead, it should be thought of as a high-tech version of bench cards and cheat sheets. An introduction to the app and a demonstration is now included in the LRN Sentinel Laboratory Biodefense Classes provided on request and free of charge by the Bureau of Public Health Laboratories. Contact your nearest Biodefense Trainer (Betty Wheeler in Jacksonville ([betty.wheeler@flhealth.gov](mailto:betty.wheeler@flhealth.gov)) or Leah Kloss in Tampa ([leah.kloss@flhealth.gov](mailto:leah.kloss@flhealth.gov)) for more information or with questions.

*If your lab does not have iPads but you are interested in having non-paper access to similar information, either on a tablet or on a desktop computer, please contact Leah Kloss ([leah.kloss@flhealth.gov](mailto:leah.kloss@flhealth.gov)). She is developing a PDF file that could be used in a similar fashion and needs test users to provide feedback.*

## References.

1. Theory of MALDI-TOF Mass Spectrometry [https://www.youtube.com/watch?v=\\_PYFydliP4o](https://www.youtube.com/watch?v=_PYFydliP4o)
2. Association of Public Health Laboratories “Evaluation of Biothreat Agent Growth with Three Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI TOF) Mass Spectrometry Preparation Methods” June 30, 2015. <https://www.aphlweb.org/cmt/phprcmt/sot/MALDITOF/MALDI-TOF%20Safety%20Study%20Final%20Report%2006-30-2015.pdf>
3. Laboratory Response Network Rule-Out and Refer app <http://www.cdc.gov/mobile/applications/mobileframework/laboratory-response-network.html>

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**Editor - Betty Wheeler**

The American Society for Microbiology (ASM) Sentinel Level Clinical Laboratory Protocols For Suspected Biological Threat Agents And Emerging Infectious Diseases for *Bacillus anthracis*, *Brucella*, *Burkholderia*, *Coxiella burnetii*, *Francisella tularensis* and *Yersinia pestis* updates were released September 4, 2015. Please remember to update all of your laboratory's biodefense reference manuals.

***“updated  
September 4,  
2015”***

In coordination with the CDC and the Association of Public Health Laboratories (APHL), the ASM has updated protocols designed to offer Laboratory Response Network (LRN) Sentinel Level Clinical Laboratories standardized, practical methods and techniques to rule out microorganisms suspected as agents of bioterrorism, or to refer specimens to public health laboratories for confirmation.

The current edition is compliant with the Clinical Laboratory Standards Institute (CLSI) format based on current information and recommendations of the APHL Sentinel Laboratory Partnerships and Outreach Subcommittee. These protocols reflect the standard practices for specimen processing as well as agent specific guidance. In addition to promoting standardization and uniformity of testing, adherence to, and maintaining the highest level of safety practices is emphasized in the respective protocols. Updated guidelines can be found at the ASM website: <http://www.asm.org/index.php/issues/sentinel-laboratory-guidelines>.

## WASH YOUR HANDS!!

Hand washing with soap removes germs from hands. This helps prevent infections because:

- People frequently touch their eyes, nose, and mouth without even realizing it. Germs can get into the body through the eyes, nose and mouth and make us sick.
- Germs from unwashed hands can get into foods and drinks while people prepare or consume them. Germs can multiply in some types of foods or drinks, under certain conditions, and make people sick.
- Germs from unwashed hands can be transferred to other objects, like handrails, table tops, or toys, and then transferred to another person's hands.



Removing germs through hand washing helps prevent diarrhea and respiratory infections and may even help prevent skin and eye infections.



The CT laboratory coordinators continue to reach out to the health and medical community by offering training for CT preparedness at hospitals and county health departments (CHDs). This training covers chemical terrorism awareness and the collection of clinical specimens after a chemical terrorism event. Hospital and CHD staff play an important role in the response to a chemical exposure event since clinical specimens will be collected for analysis. For your convenience and to increase participation, this training can be presented at your facility. Each course lasts approximately one hour with one 15-minute break between courses. Florida clinical laboratory and nursing continuing education credits will be offered. Training manuals, "hands on" exercise materials, and CT preparedness kits will be provided. This training is recommended for physicians, nurses, epidemiologists, emergency department personnel, phlebotomists, hospital and health department laboratory personnel, and others who may collect clinical specimens. Contact the CT laboratory coordinators in your region for more information (see the BPHL Directory on the back of this document for contact information).

## FLU SEASON IS HERE, WILL YOU GET VACCINATED?



How can you protect yourself and your loved ones from influenza? The flu vaccine is the first and most important step in protecting against this serious disease. The CDC recommends a yearly flu vaccine for everyone 6 months of age and older. While there are many different influenza viruses, the seasonal flu vaccine is designed to protect against the main viruses that research suggests will cause the most illness during the upcoming flu season. People should begin getting vaccinated as soon as the flu vaccine becomes available, ideally by October, to ensure that as many people as possible are protected before flu season begins. Remember, health care workers and laboratory personnel who work with flu specimens are at a higher risk of contracting the virus and should be vaccinated as early as possible.

For the most current information about influenza in Florida, please see Florida's weekly surveillance report, the Florida Flu Review, which can be found at: <http://www.floridahealth.gov/diseases-and-conditions/influenza/florida-influenza-weekly-surveillance.html>



Betty Wheeler, Biodefense Trainer  
Bureau of Public Health Laboratories—  
Jacksonville

Betty Wheeler is one of two biodefense trainers for the Florida Department of Health Bureau of Public Health Laboratories. She schedules and conducts LRN Sentinel Laboratory, Infectious Substances Packaging and Shipping, and First Responder Sample Collection trainings mainly in north and northwest Florida, but will travel throughout the state. In addition, she is the administrative assistant for the Bioterrorism Program Advisor, maintains the sentinel laboratory database, the BT/CT training database, tracks the CAP Laboratory Preparedness Exercise participants and compiles the results, conducts the sentinel laboratory communication drills, is a FL TRAIN administrator and course provider, and is the editor of the Lab Link newsletter. Ms. Wheeler may be contacted by phone at (904) 791-1568 or by email at [Betty.Wheeler@flhealth.gov](mailto:Betty.Wheeler@flhealth.gov).

## LABORATORY RESPONSE NETWORK (LRN) TRAINING— BIOLOGICAL DEFENSE

The BPHL is currently offering an LRN sentinel laboratory training course at no cost to you at your facility. This training follows the American Society for Microbiology (ASM) Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases. Scheduling the training at your facility is a relatively easy process. Determine when you would like to have the training and how many people will be attending. A time will be set up that is convenient for all. The training materials are provided, as well as the biodefense reference manuals for your laboratory.

*“at no cost to  
you at your  
facility”*

The training syllabus includes: 1) an overview of the LRN; 2) the ASM protocols for ruling out potential bioterrorism agents and how to refer a sample to the state LRN Public Health reference laboratory when a bioterrorism agent cannot be ruled out; 3) the role of the sentinel laboratory in responding to pandemic influenza; 4) a brief introduction to packaging and shipping of infectious substances; 5) an introduction to the CDC Select Agent Program; and 6) the College of American Pathologists Laboratory Preparedness Exercise (CAP LPX).

This class awards Florida clinical laboratory continuing education credits based on five hours of instruction. Please contact Betty Wheeler at (904) 791-1568 ([Betty.Wheeler@FLhealth.gov](mailto:Betty.Wheeler@FLhealth.gov)) to schedule a class for your facility.

**FLORIDA DEPARTMENT OF HEALTH  
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