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The Florida Lab Link

"A Biological and Chemical Preparedness Newsletter"

SAFETY AND ACCURACY OF MALDI-TOF MS FOR BIOTHREAT AGENTS

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Sentinel clinical laboratories are increasingly implementing matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) as a rapid and cost-effective method for bacterial pathogen identification. Many studies have been performed to determine the accuracy of MALDI-TOF MS for identifying commonly encountered organisms. However, the literature is lacking in terms of accurately identifying highly pathogenic organisms with this technology. Additionally, highly pathogenic organisms can present exposure hazards to laboratory personnel during sample preparation and analysis. Biosafety procedures are available to render organisms nonviable prior to testing however, not all instrument manufacturers provide these inactivation protocols. In the United States, many of these organisms are regulated by the Federal Select Agent Program so sentinel clinical laboratories do not have access to these potential bioterrorism threat (BT) agents to perform validation studies themselves.

In mid-2016, through a collaboration with the Association of Public Health Laboratories, the following eight state and city public health laboratories embarked on a study to determine the accuracy of the mass spectrometer manufacturers' databases and methods for rendering samples nonviable: Michigan Department of Health and

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Human Services, New York State Department of Health, New York City Department of Health and Mental Hygiene, Minnesota Department of Health, Iowa State Hygienic Laboratory, North Carolina Department of Health and Human Services, Texas Department of State Health Services, and our Bureau of Public Health Laboratories in Jacksonville. We published our findings in the December 2017 issue of the Journal of Clinical Microbiology.[1] This article is a summary of the publication; data are reproduced with the kind permission of the lead author, Dr. Jim Rudrik, Michigan Department of Health and Human Services. The full, open access paper can be downloaded from https://doi.org/10.1128/JCM.01023-17.

The initial phase of this multi-center study evaluated the ability of three MALDI-TOF MS sample preparation techniques to render highly pathogenic select agents nonviable prior to removal of the organisms from a biological safety cabinet. The three methods were direct colony, on-plate formic acid extraction, and ethanol/ formic acid tube extraction. The organisms tested were *Bacillus anthracis* Sterne strain, *Brucella abortus* strain 19, *Burkholderia thailandensis*, *Clostridium botulinum* types A, B, and E, *Clostridium perfringens*, *Francisella tularensis* subspecies *holarctica* LVS, and *Yersinia pestis* A1122.

Eighty-nine percent of samples contained viable organisms after drying the suspensions. This suggests that drying alone is insufficient to render most samples nonviable in the time frame associated with routine sample preparation. Exposure to air for an extended period may have contributed to the decreased viability of the *Clostridium* spp. After addition of the α-cyano-4-hydroxycinnamic acid (HCCA) matrix per the manufacturer's protocol, viability was reduced from the 89% to 18%. Formic acid, used for the on-plate formic acid extraction method, had no additional effect and viability remained at 18% after overlaying the HCCA matrix. Viability was further reduced to 11% when organisms were exposed to the ethanol/formic acid reagents used in the tube extraction method. Final filtration through a 0.1 µm pore-size filter rendered all samples nonviable. Due to these and similar findings elsewhere, the American Society for Microbiology document "Sentinel Level Clinical Laboratory Protocols for Suspected Biological Threat Agents and Emerging Infectious Diseases"[2] recommends that laboratories using MALDI-TOF MS for identification of suspect BT agents should use the tube extraction method followed by filtration through a ≤0.2 µm pore-size filter before removing the sample from the biological safety cabinet.

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SAFETY AND ACCURACY OF MALDI-TOF MS FOR BIOTHREAT AGENTS (CONTINUED)

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For the identification accuracy phase of the study, six laboratories tested extracts on a Bruker MALDI Biotyper equipped with one or more of the *in vitro* diagnostic (IVD), research-use only (RUO), and Security-Relevant (SR) software libraries. Three laboratories tested extracts on the bioMérieux VITEK MS system equipped with the IVD and RUO software libraries. An identification result was considered acceptable to the genus and species levels if the sample score was ≥2.0 for the Biotyper or ≥60% for the VITEK MS.

The only sample preparation process approved by bioMérieux for the VITEK MS is the direct method. Therefore, a preliminary study was performed comparing results from 50 clinical isolates that were tested by both the direct and tube extraction methods. Since the same identification was obtained 96% of the time it was determined that the tube extraction method from the Bruker user manual, followed by filtration, was also applicable to the VITEK MS and maintained the necessary biosafety practices for highly pathogenic BT agents.

Forty-six strains of *Bacillus anthracis*, *Yersinia pestis*, *Francisella tularensis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Clostridium botulinum*, *Brucella melitensis*, *Brucella abortus*, *Brucella suis*, and *Brucella canis* were extracted and distributed to participating laboratories for analysis. A total of 35 genetic and phenotypic near-neighbor non-BT isolates were also analyzed. Each extract was tested multiple times in each of the participating laboratories.

The Bruker IVD and RUO software did not correctly identify any of the BT agents. This was expected as these agents are not present in the software library. Most of these isolates were reported as "No reliable ID." However, an incorrect organism identification was reported for 11.9% of extracts by the IVD library and 16.2% by the RUO library. The IVD software misidentified 73.8% of the *Yersinia pestis* extracts as *Y. pseudotuberculosis*. The RUO software misidentified 8.3% of the *Bacillus anthracis* extracts as *B. cereus*; 81.5% of *Y. pestis* as *Y. pseudotuberculosis*; and *Burkholderia thailandensis* was incorrectly reported for 9.3% of *Burkholderia mallei* and 5.6% of *B. pseudomallei* extracted samples.

Of the BT agents tested, the Bruker SR library reported a correct identification for 365 of 697 (52.4%) extracts. No reliable identification was obtained for 38% of extracts. The remaining 9.6% of the results were incorrect identifications. Some extracts of *B. pseudomallei* were identified as *B. mallei* and vice versa. A total of 56 of 107 (52.3%) *Brucella* spp. were misidentified as *B. melitensis*; however, *B.*

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melitensis was the only species of the *Brucella* genus in the library. When reported to the genus level only, a total of 101 of the 107 (94.4%) extracts were identified as *Brucella* species, which could provide a valuable indication to the sentinel clinical laboratory.

The Bruker IVD software misidentified 1.4% of the 484 extracts from near-neighbor isolates; all 7 *Y. enterocolitica* were reported as *Y. pseudotuberculosis*. The RUO software misidentified 1.1% of the 625 extracts, with over half of the discrepancies accounted for by *B. thuringiensis* being identified as *B. cereus*. The SR software misidentified 10.7% of the 627 extracts tested. *B. thuringiensis* and *B. cereus* were misidentified as *B. anthracis*; *Y. pseudotuberculosis* and *Y. enterocolitica* were misidentified as *Y. pestis*; *B. thailandensis* was identified as either *B. mallei* or *B. pseudomallei*; and *B. melitensis* was reported in 12% of non-BT near neighbors of *Brucella*.

Like the Bruker Biotyper, the VITEK MS IVD library also did not perform well in identifying the 315 BT agent extracts tested, with most reported as "No identification." However, an incorrect organism identification was reported for 16.2% of the extracts. Unlike the Bruker Biotyper, several of the BT agents are present in the RUO library of the VITEK MS. However, only 11 of 333 (3.3%) extracts were correctly identified; all were *Francisella tularensis*. An additional 34 extracts of *F. tularensis* were reported as no identification. The RUO library misidentified 25 of the 333 (7.5%) extracts. For all the VITEK MS data, *Y. pseudotuberculosis* was the most frequently reported incorrect identification for the *Y. pestis* extracts; 60.7% and 33.3% misidentifications by the IVD and RUO software, respectively. The RUO software library did not differentiate any of the *Brucella* extracts to the species level; however, a correct identification to the genus level was achieved for 29 of 51 (56.9%) *Brucella* extracts. Like the Bruker SR library results, this could provide a valuable indication to the sentinel clinical laboratory.

The VITEK MS IVD software misidentified 7 of the 298 (2.3%) extracts from near-neighbor isolates. The RUO library reported incorrect identifications for 21 of 302 (7%) extracts from the near-neighbor isolates. Ten of 18 (55.6%) extracts of *Francisella novicida* were misidentified as *F. tularensis*.

This study demonstrated that organisms could survive the most commonly used extraction techniques for MALDI-TOF MS: direct and on-plate formic acid sample preparation. Therefore, to reduce exposure risk to highly pathogenic agents,

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laboratories should implement risk assessments and follow the recommendations in the ASM Sentinel Level Clinical Laboratory Protocols[2] and use the tube extraction method followed by ≤0.2 µm pore-size filtration to remove all viable organisms. Rapid and accurate assays for the identification of highly pathogenic BT organisms are critical for patient management; infection prevention, including decreasing laboratory exposures; instituting appropriate public health interventions, and in an intentional release case, the initiation of a criminal investigation.

Some limitations of the available software libraries for MALDI-TOF MS identification systems and the need to include supplemental spectra to improve accuracy were also demonstrated. Additionally, those spectra would provide the most value to laboratory diagnosis if they were included in databases most commonly used by sentinel clinical laboratories.

Knowledge of these limitations can mitigate incorrect identifications. For example, information on common misidentifications for specific organisms can alert the laboratorian to perform additional tests when these results are obtained. This was seen when *Y. pestis* was repeatedly identified as *Y. pseudotuberculosis* on both MALDI-TOF MS platforms. Additionally, in this study the Gram stain result for several of the agents was discordant to the organism identified by the software libraries. Knowledge of bacterial taxonomy is also essential. When faced with the result of "*Bacillus cereus* group", it is critical to know that *Bacillus anthracis* is a member of the *B. cereus* group; this is not a misidentification, but specific knowledge is required to interpret the result and its potential implications.

MALDI-TOF MS can provide a rapid method for bacterial pathogen identification. However, due to the biosafety aspects and accuracy limitations identified in this study, when a highly pathogenic BT organism is indicated by phenotypic characteristics clinical laboratories should use the ASM Sentinel Level Clinical Laboratory Protocols prior to attempting identification with MALDI-TOF MS.

Reference

1. Rudrik JT, Soehnlen MK, Perry MJ, Sullivan MM, Reiter-Kintz W, Lee PA, Pettit D, Tran A, Swaney E. 2017. Safety and accuracy of matrix-assisted laser desorption ionization–time of flight mass spectrometry for identification of highly pathogenic organisms. J Clin Microbiol 55:3513–3529. https://doi.org/10.1128/JCM.01023-17.
2. American Society for Microbiology, Washington, DC, 2016. Sentinel Level Clinical Laboratory Protocols For Suspected Biological Threat Agents And Emerging Infectious Diseases. https://www.asm.org/index.php/guidelines/sentinel-guidelines

colLABorate

Laboratory Biosafety ColLABorate Community

Join Today!

Connecting Public Health Laboratory and Clinical Laboratory Communities

What is Laboratory Biosafety ColLABorate Community?

Given recent biosafety events and recognizing the need for a stronger network between public health and clinical laboratories, the Association of Public Health Laboratories (APHL) developed a new platform, Laboratory Biosafety ColLABorate. This platform will serve as a forum where participants can have discussions with peers, find and contact other community members and share resources pertaining to biosafety and biosecurity. Access to this community is free and does not require APHL membership.

Why Should I Join this Community?

Biosafety professionals from private clinical and governmental public health laboratories are essential in protecting the safety and security of their personnel, facilities and the general public. This platform will be a vital resource to facilitate the sharing of ideas, biosafety tools and other resources as well as to assist with answering biosafety-related questions (e.g.. biosafety practices, risk assessments and donning and doffing). Further, the platform will assist with strengthening the network of biosafety professionals domestically and globally.

Who is the Biosafety Community's Target Audience?

- **Biosafety Professionals from Governmental Public Health Laboratories**
- **Biosafety Professionals from Private Clinical Laboratories**
- **APHL Biosafety and Biosecurity Subject Matter Experts**

How Do I Join ColLABorate and the Biosafety Community Today?

1. Go to **aphl.org** and create an account by clicking the 'Create an Account' icon at the top right corner of the APHL home page – if you do not already have an account.
2. Contact APHL at biosafety@aphl.org with your name, institution and position title so APHL can determine eligibility.
3. If accepted, APHL will send an email acknowledging your acceptance into the Biosafety Community and share tools for use of the platform.

Where Do I Go to Access ColLABorate?

Visit **collaborate.aphl.org**. You will be prompted to enter your APHL account username and password. Once entered, you will now have access to your ColLABorate platform. Please bookmark this page for future reference.

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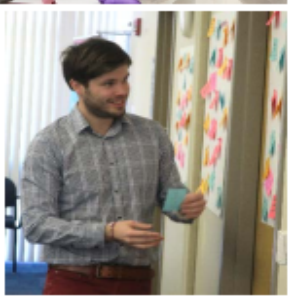

2018 Division 6.2 Infectious Substances Packaging and Shipping Training

The Bureau of Public Health Laboratories (BPHL) is again sponsoring 2018 Division 6.2 Infectious Substances Packaging and Shipping Training for our Sentinel Laboratory partners, including hospital and health department laboratory personnel as well as non-sentinel laboratory personnel as space is available. This training program is funded through the Public Health Emergency Preparedness Cooperative Agreement and is available to those who are responsible for packaging and shipping infectious substances and diagnostic specimens.

Infectious Substances Packaging and Shipping Training is required every two years to maintain certification. Please note there is a new vendor for the training this year. CargoPak, Corp. has been contracted to conduct 20 live classes throughout the state, and there is no charge for this training sponsored by the BPHL. **All classes are scheduled from 9:00 am to 4:30 pm local time but may run longer.**

Available classes with dates and locations are listed in FL TRAIN. To register, please log in to your FL TRAIN account https://fl.train.org/Desktopshell.aspx. If you do not currently have a FL TRAIN account, click the box "Create Account" and complete the required information. Once registered as a TRAIN user, the course can be found by typing in the search box either "FDOH 2018 Division 6.2 Infectious Substances Packaging and Shipping" or the course identification number 1075674. Click on the registration tab. If you would like to receive CEUs, select credit type or select "none" if you don't have a clinical or nursing license. Then select the session you wish to attend by clicking on the "Get Approval" button. You will receive an email notification once you are approved only if you elected to receive emails from TRAIN and confirm your email address. We can assist you with registration if needed.

If you have questions regarding this training please contact Betty Wheeler at 904-791-1568 or betty.wheeler@flhealth.gov or Leah Kloss at 813-233-2278 or leah.kloss@flhealth.gov.

Do you have questions about packaging and shipping regulations that are not easily answered?

http://www.aphlblog.org/2018/02/questions-packaging-shipping-regulations-not-easily-answered/

By Patricia Payne, president, JBM Associates, Inc.; consultant, APHL

Biosafety risk assessment and lab biosafety training

The Bureau of Public Health Laboratories biosafety outreach officers (BOOs) are currently offering a course in biosafety risk assessment and laboratory biosafety to clinical laboratory institutions. The training consists of two sessions that are approximately one hour each and offered on-site at no charge to the facility. The first session discusses biosafety risk assessment and the second session focuses on biosafety in the clinical laboratory.

Biosafety risk assessment is a systematic process of evaluating the potential risks involved in a laboratory procedure and determining the measures needed to manage any gaps or risks identified. The BOOs have created standard operating procedures and resource documents to assist clinical hospital laboratories in biosafety risk assessment and laboratory biosafety. This session will train clinical laboratory personnel how to use these documents to perform risk assessments in their laboratory.

The second session is for anyone who works in the laboratory or is responsible for a safe working environment. Topics include general laboratory biosafety, the use of biological safety cabinets (BSCs), choosing correct personal protective equipment, proper use and removal of gloves, and spill cleanup. This training awards Florida clinical laboratory and nursing continuing education credits.

For more information or to schedule training, contact Ed Kopp at 813-233-2260 (Edgar.Kopp@flhealth.gov) or Lylah Seaton at 904-791-1569 (lylah.seaton@flhealth.gov).

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Editor - Betty Wheeler

Chemical Threat (CT) Preparedness Training

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 when clinical specimens are 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 Bureau of Public Health Laboratories Directory for contact information).

Laboratory Response Network (LRN) Training—Biological Defense

The Bureau of Public Health Laboratories 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.

The training syllabus includes: an overview of the LRN; Biosafety risk assessment and biosafety for the clinical laboratory; 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; and an introduction to the CDC Select Agent Program.

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) to schedule a class for your facility.

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