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CHELSEA PARSONS: Good afternoon, everyone, or wherever you are, morning, evening. Thank you so much for joining us today. We're going to go ahead and get started. We have a lot to cover today. So this one lab session of "Biosafety Practices and Reporting Occupational Exposures for Select Agents and Toxins." My name is Chelsea Parsons, and I'm a consultant with Guidehouse, supporting CDC's OneLab Initiative.

I just have a couple of notes about the webinar before we dive in. If you're having any technical issues throughout today, please feel free to email our OneLab inbox, and it's onelab@cdc.gov. That's onelab@cdc.gov. If you have any questions throughout the session, we ask you to put those in the Q&A function. So in your bottom ribbon of your Zoom panel, you'll see Q&A. You can drop questions in there throughout the entire session whenever you have one.

We'll do a Q&A session at the very end of the presentation, and we'll try to review and answer as many questions as we can get to. If you don't get your question answered today, you can always email our OneLab inbox, and we'll try our best to answer any that we missed, following up with some emails. So right now, we'll post the link to the live captions in the chat. If you need to access live captions today, we just ask that you open that link, and you also keep this Zoom chat open as well.

All right, let's look at our agenda for today. So we are going to start with an introduction to today's presenters, led by our OneLab network lead Alicia Branch. And then we'll get into the meat of the conversation, led by our two fabulous presenters. And we'll close out with that Q&A that I mentioned. So I'm going to go ahead and turn it over to Alicia.

ALICIA BRANCH: Thanks, Chelsea. Before we get into the main presentation, I'd like to take a moment to state to disclaimers and then introduce today's presenters. Slide decks may contain presentation material from panelists not affiliated with CDC. In addition, presentation content from external panelists may not necessarily reflect CDC's position on topics covered. Next slide, please.

CDC, and planners, and our presenters wish to disclose they have no financial interest or other relationships with manufacturers of commercial products, suppliers, commercial services, or commercial supporters. Next slide.

Now I am excited to introduce our first speaker for today, is Dr. Tarsha Harris. Before joining Division of Select Agents and Toxins at CDC, Dr. Tarsha Harris researched immunology and microbial pathogens. Early in her career, she performed molecular diagnostic testing in clinical lab settings. She has been at DSAT for almost six years as a microbiologist, and currently serves as a microbiologist and APHIS/CDC Form 3 Technical Advisor. Next slide.

Our next presenter is Mr. Michael Perry. Michael Perry is the Associate Director of the Biodefense Laboratory at the New York State Department of Health in Wadsworth Center. Michael has been at the New York Department of Health since 2009. Michael is a member of the Association of Public Health Laboratories, better known as APHL, Biosafety Security Committee, the APHL Public Health Preparedness and Response Committee, and the APHL Laboratory Operations Workgroup chair.

Michael is committed to public health services, as evident by his work through CDC's global health security agenda, in which he has helped develop and deliver training on biological safety cabinet maintenance, operation, implementing a risk assessment program, and implementing a respiratory protection program in many African countries.

Our presenters for today is Dr. Tarsha Harris and Mr. Michael Perry.

TARSHA HARRIS: Thank you so much for that introduction, Alicia. Hello, everyone. For today's presentation, I'll be reviewing several aspects of the APHIS/CDC Form 3 Report, including the regulations associated with submitting the report, as well as providing some helpful information on its completion. Next slide, please.

So let's begin with a brief overview of the Federal Select Agent Program, commonly referred to as FSAP, which regulates the possession, use, and transfer of biological select agents and toxins, or BSAT, that have the potential to pose a severe threat to public, animal, or plant health, or to animal or plant products. FSAP is managed jointly by CDC's Division of Select Agents and Toxins and APHIS Division of Agricultural Select Agents and Toxins.

In the United States, there are two major types of entities regulated by the Federal Select Agent Program-- registered and non-registered entities. Registered entities are those laboratories approved to work with select agents and toxins through registration with FSAP. As of the end of 2021, there were 233 registered entities. Entity types include academic, federal government, non-federal government, commercial, as well as private companies.

The second major type of entity is non-registered entities. And this applies to the majority of the labs in the United States. These entities are not approved to work with known select agents and toxins, but many may perform diagnostic testing to identify select agents and toxins. Examples include hospitals, clinics, food testing, and veterinary laboratories, many of which may be on the call today. So for more information about the Federal Select Agent Program you can visit the website that will be dropped in the chat box, selectagents.gov. Next slide, please.

So now we discuss the purpose of the regulations, or should I say the purpose and the regulations associated with the APHIS/CDC Form 3, as well as provides some interesting statistics and reporting specifics for the APHIS/CDC Form 3 Report. Next slide, please.

So let's start with the purpose of the APHIS/CDC Form 3. The name of the form is "Report of a Select Agent or Toxin Release, Loss, or Theft." And its purpose is as the name implies, to notify either APHIS or CDC that your entity has had a theft, loss, or release, including an occupational exposure of a select agent or toxin. Note that your entity is not required to report to both APHIS and CDC, just one or the other. The purpose is also to inform APHIS and/or CDC of any potential threat to public health or safety, including animals, plants, and animal and plant products. Reporting includes all select agents and toxins on the FSAP list. This list can be found at the link that will be provided in the chat. Next slide, please.

So in addition to satisfying the regulatory requirements and being in compliance, there are additional benefits of reporting a theft, loss, or release of a select agent or toxin. The Form 3 team often provides contact information to engage with CDC and other subject matter experts who are available to provide guidance on approaches for managing medical treatments and/or surveillance to individuals following a potential occupational exposure or to provide information about other general questions you may have regarding specific BSAT.

In addition, through the Form 3 team's incident review process, once we receive a Form 3, we often engage with the entity to discuss several aspects, including biosafety, security, training, incident response, and other practices. And we do this to assist in identifying any gaps that may have contributed to the incident. These discussions aim to mitigate the risks of similar incidents in the future. Next slide, please.

For a brief overview of the Code of Federal Regulations, CFR, that are specific for the select agent and toxin regulations, I've listed the three regulations here, Title VII, Part 331, Title IX, Part 121, and Title 42, Part 73. We obviously won't have enough time to go into the specifics of these regulations for today's presentation, but you can learn more by visiting our website, which, again, is linked in the chat. We'll be providing you guys with lots of links today so please do note those. So today we'll focus on the section of the regulations that specifically apply to reporting the theft, loss, or release of a select agent or toxin. And this is Section 19. This section is Section 19, all three of the regulations listed here. Next slide, please.

So Section 19 of the regulations is divided into two major subsections. Section 19(a) defines the requirements for reporting a theft, loss, or release, as you can see here. Section 19(b) covers the requirements for reporting a release that I'll go over on the next slide. These two subsections both require immediate notification to APHIS or CDC upon the discovery of the incident and have some of the same information requirements when reporting. However, one major difference is that a theft or loss of a select agent and toxin also requires that the entity report the loss or theft to a federal, state, or local law enforcement agency.

Note that for an immediate notification-- we get this question quite often-- within 24 hours of discovering the incident is considered an acceptable time frame for us. Whether a theft, loss, or release, the APHIS/CDC Form 3 always requires a completed form to be submitted within seven calendar days following the discovery of the incident. Next slide, please.

So for the remainder of the presentation, we'll focus on BSAT releases and occupational exposures, as these reports are most relevant for non-registered entities. Subsection 19(b) reporting requirements relate to a release of a select agent or toxin, specifically a release resulting in occupational exposure, such as a manipulation of specimen or samples later identified as select agent outside of primary containment or a release of a select agent or toxin outside of containment whether an occupational exposure occurred or not. In addition to immediately notifying APHIS or CDC and submitting a completed Form 3-- I'm sorry, yes, Form 3, to either agency within seven days, the entity may notify other public health-- public health authorities and other necessary authorities as needed, depending on the details associated with the incident.

For example, when an individual exposed to the select agent or toxin becomes symptomatic, public health authorities in your area should be consulted. We are including a link to the website that lists the public health officials per state and territory. So now that I've gone over some of the basics of the regulations, I'll go into a few statistics related to reported releases in the next few slides. Next slide, please.

So this slide shows some statistics associated with a APHIS/CDC Form 3 Reports, obviously. On the left, the bar graph shows the number of releases reported from registered and non-registered entities over a three-year time frame, from 2020 through 2022. You can see that generally we have more reports from non-registered entities than registered entities. And we'll talk a bit more about these types of reports shortly. In 2020, we had a total of 158 releases. And this total number increased in 2021 to 177 total releases, which was also accompanied by an increase in non-registered entity reports compared to 2020.

Most recently, in 2022, there was a slight decrease in the total number from 2021, with a total of 170 reports. We see that the number of registered entities reporting decreased from 71 to 63. However, the number of non-registered entities reporting stayed almost the same, with 107, up from 106 from the previous year. The pie chart on the right shows the numbers of-- numbers and percentages of first time versus repeat submitters last year, in 2022. As you can see, the smaller portion, about 43%, of reports came from first-time submitters, while 57% were submitted by entities that have previously reported.

It's important to remember that while non-registered entities include those that are not registered for possession of BSAT, these are largely the entities that are identifying select agents and toxins in specimens for diagnosis, verification, or proficiency testing. Non-registered entities are exempt from the requirements of the select agent and toxin regulations provided that they meet the exemption requirements. This includes reporting to the Federal Select Agent Program any identification of select agent and toxins for a clinical or diagnostic sample, including environmental samples, and reporting to the Select Agent Program any theft, loss, or release of the identified select agent or toxin. Next slide, please.

So these data show statistics for Form 3 reports to FSAP specifically from non-registered entities. The numbers of reported incidents by severity and year from non-registered entities is shown in the bar graph. Note that these severity designations are determined by the entity and not FSAP. And they are captured upon submission of the Form 3 report on the report itself. You can see that the number of high and moderate severity incidents increased from eight to 16 and from 11 to 17 respectively over the two year time span.

Low severity incident numbers decreased which is-- low severity incidents-- I apologize-- decreased and negligible incidents increase. The increase in negligible incident increase is what we would like to see for that trend. The other items are trending opposite of what we would hope for, but it will be interesting to see if these trends remain the same in the coming year. It's important to note that determining the incident severity for release reports will be specific for the entity, wherein several factors would need to be considered.

The Biosafety in Microbiological and Biomedical Laboratories publication, or BMBL, as we probably all refer to it as, Sixth Edition, as many call it, is a good resource for more information on performing risk assessments for the work you are conducting at your entity, which may offer insight on assessing incidents, and more importantly, mitigating the risks of incident occurrence. BMBL Sixth Edition provides information and guidance on the risk assessment and management process throughout, but specifically outlines this process in Section 2 of the publication, which, again the link has been included in the chat. The pie chart on the right shows the general types of non-registered entities that submitted reports last year, in 2022.

While we generally receive most of our reports from clinical or hospital laboratories performing diagnostics work on human samples and specimen on the benchtop, about 10% of last year's reports were submitted by veterinary health clinics, health facilities generally associated with diagnostic activities and necropsy procedures outside primary containment. We also had a single report from a plant lab for work on a benchtop prior to select agent identification. Next slide, please.

So the line graph here shows the number of occupational exposures reported over the past three years. As you can see, these values have increased over the three-year time frame. The primary source of releases reported by non-registered entities are associated with laboratories manipulating or handling open samples or specimens outside of primary containment. Furthermore, if personnel are not donning adequate PPE to prevent an occupational exposure, to include respiratory protection, this constitutes a potential occupational exposure. So based on internal data, I will mention that most-- that the most common occupational exposure incidents reported to FSAP are benchtop exposures associated with three main tasks or assays.

One is opening culture plates to observe for growth or to pick colonies. Two is slide preparation for MALDI-TOF or other automated systems, or manual assays prior to sample inactivation or fixation. And the last one is performing necropsy on BSAT affected animals prior to identification and outside of primary containment. We often note that performing these tasks in primary containment equipment, such as a biosafety cabinet, would likely lead to a drastic reduction in release or potential occupational exposure incidents for non-registered entities.

We are also aware that there may be limitations in using-- excuse me-- in using primary containment devices, such as biosafety cabinets, as with performing necropsies or other procedures on larger animals. In these instances, it's important to perform entity-specific risk assessments, as mentioned on the previous slide, to mitigate the risk associated with the work performed, such as through the use of enhanced PPE or other administrative approaches. Next slide, please.

OK, so now we'll move on to the Form 3 itself to provide what we think is helpful information to be aware of when completing a Form 3. There are often specific items that require additional clarification from the entity after the form is submitted. So we're highlighting a few of these for your awareness. Question B2 requests the date of immediate notification. As a reminder, immediate notification is required for all APHIS/CDC Form 3 reports.

Question B2 is often misinterpreted by entities who provide the date of the internal notifications, rather than the date DSAT or DASAT was notified. We do require the date that FSAT was notified. Question B6 requests the strain designation of BSAT. So you should provide the strain designation that was identified, if known. Alternatively, please do enter unknown for that block. Leaving the response blank requires additional follow up and confirmation from the submitter to ensure that the block was not left blank unintentionally. Next slide, please.

Often entities enter a question before as a general department lab, for example, a microbiology laboratory. We will request more specific information for this block to ensure that this information is captured appropriately. So please do include more specific information, such as the building and room number, or room name, where the incident occurred. For question B10, we ask about the biosafety level where the incident occurred. If the incident occurred outside of a laboratory space, such as a barn or other area related to veterinary work, the other option in free text can be used to designate this information. Next slide, please.

Recall that APHIS/CDC Form 4 for the identification of BSAT that was covered during the April 1 lab network meeting or webinar. Since most of the Form 3 reports received from non-registered entities are associated with diagnostic identification activities, question B12 is an important question that allows us to link the associated samples, diagnostic activities, and the required APHIS/CDC forms. We recognize that there may be a delay in generating this ID in some instances. So note that an entity should not wait for the ID to be generated to submit the Form 3. The Form 3 and Form 4 team will work together to ensure that this block is updated on the form as necessary on the entity's behalf as needed. Next slide, please.

So question C3 requests the types of PPE worn at the time of the incident. The type of PPE worn by all individuals involved in the incident should be selected here. Clarification for this block is one of the most commonly requested due to the entity's designating surgical masks as respiratory protection. Note that surgical mask should be entered as other and not as a form of respiratory protection for the reasons highlighted here on the slide. Next slide, please.

So as previous data showed, the number of occupational exposures has increased over the past three years. Question C4 captures information for releases that resulted in potential exposures. Note that block C4a requests the total number of individuals animals or plants exposed, and block C4b requests the number of individuals who were laboratory staff. This is important-- this is an important distinction to make when reporting exposures that may have occurred outside of the lab setting or may involve other personnel type that were in the laboratory at the time of the incident, for example, maintenance staff or other individuals.

Considerations for determining potential exposure should be based on the entity's internal risk assessments to include what and where manipulations occurred with samples, later identified as select agent and toxins, personnel who performed these tasks, PPE worn and other relevant factors as determined by the entity. We recommend that biosafety personnel and occupational health staff familiar with the entity and work conducted be involved in these discussions if possible. Next slide, please.

The biosafety personnel and occupational employee health staff should also be a resource to further determine any medical surveillance and/or treatment options appropriate for the individuals involved in the incident, as requested on the Form for questions C6 and C6a. As stated previously, FSAP often provides CDC subject matter expert contact information to also assist in the discussion if the entity needs. Note that the total number of personnel receiving any of the listed treatment should be included for question C6a. We are aware that individuals may receive a subset of the surveillance or treatment options and not all the items selected. Next slide, please.

Question C7b is important to consider when working to reduce the likelihood of recurrence. This question asks about the corrective actions initiated or implemented following the incident. We continuously encourage entities to perform internal investigations and work to develop and implement corrective actions. Listed here are commonly provided corrective actions, which could also give you ideas on appropriate approaches that should be taken. There's also an other option that enables entities to interactions not listed on the form. Next slide, please.

The final aspect of the forum, and perhaps the most important aspect, that I would cover is Appendix 1. This is the section that initially describes the incident based on the information provided. The Form 3 team may further engage the entity through a request for information document to iron out any unclear or missing details. So the more thorough the information provided, the better. So we encourage you to keep in mind the five Ws and H questions. Who was involved? The individuals that were directly involved in the incident should be mentioned here.

Note that you should not include names or other personal identifiable information. General terms should be used, such as laboratorian number one, supervisor number one, et cetera. You should also include what happened, when did it happen, where did it happen, why did it happen, and how did it happen. This how question may also be addressed in block C7a or b, which asks about the root cause and corrective actions. This section is also a good place for the entity to describe any other relevant details related to the incident. Next slide, please.

And finally, we want to round off the presentation with a few scenarios. We would like for you all to interact with us in this portion. We'll quickly read through these scenarios and present multiple choice answers for your response. Next slide, please.

All right, on a Friday afternoon your laboratory received a reference laboratory notification of a Burkholderia pseudomallei identification from the culture isolate your laboratory tried to identify a few days before. Two technicians performed subculturing and additional testing on the open bench at your laboratory. What should your laboratory do? A, nothing, because it's Friday afternoon and it can wait till Monday. B, call the laboratory to verify results because Burkholderia pseudomallei is not known in your area. C, immediately notify FSAP because the two technicians worked on the open bench with a select agent. Or D, I do not know. I'll give you a couple of minutes to respond-- well, a few seconds, rather.

OK, so the results, 86% of you smart individuals put the correct answer. You should immediately notify FSAP because the two technicians worked on the open bench with a select agent. Recall the requirement for immediate notification upon discovery of a TLR event. I'm saying TLR, that's our short for theft, loss, of release. I guess I should have mentioned that earlier. Next slide, please.

While performing diagnostic testing, laboratorian A opened a blood auger plate on the open bench and observed tiny colonies. The next day, laboratorian B took the plate to the biosafety cabinet to perform a gram staining and other assays. Laboratorian C later opened the plate on the open bench to collect a colony to spot a MALDI-TOF slide. The isolate was identified as select agent. All laboratorians wore a lab coat and gloves, and no others were present in the laboratory while that individual was working. Which laboratorian should be counted on the Form 3 as in occupational exposure to select agent?

Oh, so it looks like we have a tie between A and C or all laboratorians. Next slide. The correct response is laboratorians A and C because they open the plate or worked with the select agent outside of primary containment. Laboratorian B did work inside a biosafety cabinet so that would not be considered a release nor occupational exposure.

Last scenario question. Next slide, please. All right, So question for the group. Which do you think is the most frequently reported select agent by non-registered entities for incidents involving a release or occupational exposure?

OK. OK, of course, this is a guess. OK, most people say SARS-CoV. So the correct response-- next slide, please-- is actually Brucella melitensis. Interestingly, for 2022, Brucella species select agents represented about 50% of the Form 3 reports from non-registered entities, with about 30% of the total reports being associated with Brucella melitensis. So in other words, be aware of the Brucella, folks. Next slide.

And last slide. All right, so the slide lists the websites that you can use if you would like to learn more or to either contact the Division of Select Agents and Toxins, DSAT or DASAT rather. And that concludes my presentation. Thanks to everyone for listening and participating in our scenario questions. Now I'll turn it over to Michael Perry for his part of today's presentation. Michael, the Zoom floor's yours.

MICHAEL PERRY: So welcome, everyone, and thanks for joining. So today, I'm going to be talking about the biosafety practices for safely implementing MALDI-TOF MS in the laboratory.

So I have few things that I'd like to cover. So starting with a little bit of background on MALDI-TOF mass spectrometry, discussing the facility and safety considerations, example of high-risk pathogens, and then discussing a couple of clinical cases and best practices. By the end of today, I will have covered the following two objectives, identifying biosafety practices to minimize the release or exposure to select agents and = while using MALDI-TOF, and then recognizing a release or exposure of that while handling the select agent.

So mass spectrometry is really broken out into multiple components, the ion source, the mass analyzer, and the detector. MALDI is considered the ionization source, in which the ions are created in the sample as a result of pulse laser irradiation. Time of flight is the mass analyzer part in which uniform electromagnetic force is applied to all ions at the same time, causing them to accelerate down a flight tube. In this case, lighter ions travel faster, arriving at the detector first.

In this ionization method, samples are fixed in a crystalline matrix and then are bombarded by a laser. The sample molecule is vaporized into the vacuum while being ionized at the same time, without fragmenting or decomposing. A potential is applied that separates the ions by their mass to charge ratio and then determines that mass to charge ratio by the time it takes the ions to reach the detector. The particles will impinge upon the linear detector within a few nanoseconds after ionization. And then higher mass molecules arrive later than the lighter ones. The flight time measurements make it possible to determine molecule masses directly. And each peak in the spectrum corresponds to a specific mass of the particle along the time axis, starting when the ionization moment.

When using MALDI for identifying microorganisms recovered from patient specimens, general safety considerations include the potential for chemical and biological exposures. The things like direct contact with reagents used to prepare samples for analysis, including matrices and compounds used for microorganism inactivation or extraction, exposure to chemical fumes created during stock solution preparation, like target slide preparation and plate cleaning procedures, and again, inactivation or extraction procedures, examining or manipulating culture microorganisms, handling prepared slides or plates before or after MALDI-TOF MS analysis, and safe handling of primary patient specimens cultured microorganisms to prevent LAIs.

MALDI-TOF for microbial identification uses a phenolic acid matrix, so typically the CHCA, that is solubilized with organic solvents, such as acetonitrile or ethanol. Additional chemicals, such as formic acid or trifluoroacetic acid may also be used in protein extraction procedures, inactivation protocols, or to clean reusable target slides. But generally, small amounts of matrix and formic acid solution can be handled safely on a benchtop during target slide preparation and on target extraction, but you still want to do gloves, protective clothing, be in a well-ventilated room. If you're doing the tube extraction or inactivation procedures and some targeted cleaning, that may involve larger volumes and more hazardous chemicals, so working in something like a Class I, Type B1 or B2, or a chemical fume hood.

The higher risk of exposure occurs during handling, manipulation, and disposal of primary specimens and culture microorganisms before analysis. So direct transfer onto a MALDI-TOF target can be performed safely on the open benchtop using BSL2 practices in facilities, however, potentially more hazardous microorganisms should be manipulated using more stringent biosafety practices, including, at minimum, the use of a laminar flow, BSC, until the agent has been inactivated using a validated method or with the addition of a face shield. Matrix alone might not totally inactivate the microorganism because the inactivation will need to take into account the biomass thickness and whether the organic solution and matrix cover the spot entirely. So every lab should really adopt and verify the inactivation protocols that you use.

If you're not sure where to start with inactivation procedures or what has already been completed in the past, I have up here an article that was published in 2017. This covers the safety and accuracy of MALDI-TOF MS for high-risk pathogens. While this was a limited study, other publications show the same type of results. However, some strains were attenuated or surrogate strains. So wild types may act differently. It is recommended that if a high-risk agent is suspected, it needs to be run using this technology, that the tube extraction with filtration method be used. It's also important to know the limitations of the library that you're using and be cognizant of which, if any, BT agents are present in the database. It's advised to incorporate alternative libraries and the use of MicrobeNet to aid in identifications as well.

Despite enhanced biosafety education and improved lab practices, better engineering controls, and biocontainment equipment, LAIs continue to pose a risk to personnel working in medical and veterinary labs. If a high-risk infectious agent is suspected, labs should consult with appropriate reference labs first. Analysis of such isolates should be avoided, as culture manipulation may heighten the risk of environmental contamination as well as personnel exposure.

In the event that an unsuspecting high-risk or select agent is inadvertently analyzed on a MALDI-TOF MS either with or without the inactivation step, the incident should be assessed immediately and reported to lab manager in accordance with the lab's biosafety and infection control procedures. Risk management steps should include determining which lab workers were potentially exposed to the agent without appropriate safety measures, potential need for post-exposure prophylaxis, as well as health monitoring, gathering any of those culture containing the hazardous agent, labeling them, sequestering them.

Prepare contaminated disposables for either onsite or offsite autoclaving, and thoroughly cleaning all the infected bench areas. It's also recommended that if your MALDI instrument contains an air intake or exhaust filter, that at minimum those should be replaced as well. And we also heard all about the Form 3 that you would want to include if there are those exposures.

Additionally, safety measures are needed because of limitations to databases. But depending on the database for MALDI systems, bio threat agents might not be included. So when you analyze and isolate not in the database, you either get something that's a no identification, or you might get another pathogen, or closely related species, but with a very low score. Additionally, the opposite can be true. You can use the information gathered to help direct your identification.

So, for instance, if you did not have the security database for the instrument, which is an additional database that needs to be purchased for the Bruker system, that contains high-risk pathogens and BP agents, then if you were to get an ID of something like Clostridium sporogenes or bacillus cereus, then you might consider that these could also be potentially Clostridium botulinum or Bacillus anthracis.

You're going to want to look at early indicators in your assessment, including gram stain biochemical results, any travel history of patients if it's available. Locally, for some of the near neighbors the systems will flag the results and tell you of other potential identifications. Such as with bacillus, there will be a flag saying that the isolate could potentially be bacillus anthracis. Here you can see the table. This is from Chapter 17 of the Clinical Microbiology Procedures Handbook, which lists some of the common MALDI-TOF mass spec misidentifications.

So you can see where many of these agents result in either no identification, and so bacteria misidentified by the MALDI-TOF MS can be cause of a more potentially hazardous situation in the lab than a result of no identification. So those misidentifications, they mean may lead the laboratory to perform either further testing and manipulations of the bacteria when work should have been halted. The APHL and ASM sentinel guides for ruling out select agent should always be followed prior to performing MALDI-TOF MS just to ensure that the appropriate safety levels in the laboratory are maintained.

I'm just going to talk a little bit about some clinical cases that we've seen. So back in 2015, New York state had four high-risk pathogen cases. They were all in a three-month period. The three cases resulted in 60 lab staff being exposed. Patients had traveled abroad where the disease was endemic and consumed unpasteurized milk and milk products. And then the isolates were also slow growing as well.

So, surprise, the exposure resulted in Brucella isolates being processed in the laboratory. Several of the exposures related to new instrumentation and cleaning the MALDI-TOF. The Public Health Laboratory Response Network worked with the labs to provide information on preventing lab acquired exposures and infections. The LRN looked to evaluate the MALDI-TOF for biosafety concerns, some of which is part of that paper that I referenced a few slides ago.

With this case, as I mentioned, there were exposures at three laboratories, including the New York State Department of Health. Really, the crazy part is that all of the exposures occurred as a result of one patient. The patient traveled from Mexico to the US, and then here at the New York State Department of Health, we received two isolates in our bacteriology laboratory. The first isolate was received from one hospital, in which the hospital ID'd the isolate as Haemophilus influenza. Once it was received here, it was subcultured on the bench as a normal Haemophilus and that are received in our laboratory. It was prepared and plated for MALDI on the benchtop. At this point, it really wasn't suspected to be Brucella.

And then a second isolate was received from a different hospital. The second isolate ID'd-- the second hospital the isolate as an unknown gram negative coccobacillus. In this case, it was worked up in the biosafety cabinet, but initial biochemicals did not rule in Brucella. This one was also plated for MALDI. The MALDI ID'd the isolate as Brucella, and at which point it was moved to our BSL-3 laboratory, where we were able to do confirmatory methods and actually confirmed it to be Brucella melitensis.

So here, there's a lot going on with this slide, but really, the takeaway is that this is the pre-exposure workflow. So after this event and reviewing the rule-out algorithms, we realized that the current algorithm was overly complex. And rather than concentrating on the gram stain and slow-glowing organisms, the algorithm did not really put the necessary weight on those criteria. It had it listed that expected reactions for all the select agents, not just Brucella and really didn't take into account key tests that should have been highlighted. This also indicated that we would need to spend some time retraining individuals on the algorithm.

So why do these exposures occur? A couple reasons-- the rule-out algorithm was way too complex, needed to be simplified. Some of the laboratories were using Bunsen burner's to sterilize metal inoculating loops, which obviously, are going to generate some aerosols. Additionally, the MALDI targets were spotted on an open bench without facial barriers. So if you've never seen a MALDI target, the circles where you spot the isolates are really small. I've seen folks spot all different ways, including holding the targets right up to their faces so they can see what they're doing. As you can imagine, this is far from ideal, as the laboratorian really has that plate as close as possible to their face as they potentially are generating aerosols.

So this was the proposed changes to the algorithm based on the exposure. Again, it looks a little complicated, but the takeaway is really the use of the biosafety cabinet throughout the testing procedures until select agents are ruled out. You can see that all the work starts in the biosafety cabinet. Additionally, the change in the algorithm is to immediately send isolates to the biodefense laboratory that are slow growing and have suspicious gram stain. Again, previously, isolates were sent to the biodefense laboratory only after performing biochemicals. So this will give us a rapid detection of a select agent, such as Brucella.

Fast forward two years later, now we're into April of 2017, we had another large network micro lab involved in a Brucella case. The patient had a history of travel to Mexico. The blood culture bottles indicated positive after three days. The oxidase catalase were positive. Those were done on an open bench and not a BSC. There were small, faint colonies on blood culture plates after 24 hours. And the staff decided to run it on the MALDI, in which the targets were again prepared on the open bench. The MALDI gave a result of no identification. The gram stain was a gram negative. And the isolate was urease positive. So this laboratory was also set up as an open laboratory.

So you can imagine, there were many technicians which were considered exposure risks. Since this was the same hospital laboratory that had exposures two years prior to this, we thought maybe they weren't listening, or they had a high turnover of staff and weren't getting trained, or they were just too overwhelmed to try and separate out what was going on. But we really thought maybe they weren't listening to some of the recommendations on how to handle some of the high-risk pathogens.

So we ended up reviewing multiple things to consider in their workflows and algorithms with them. And then later in 2017, so the same year, the two sentinel labs who received the positive Brucella specimens that I discussed a little earlier, again had some-- received some more suspicious isolates. All their work this time, including gram staining, subculturing, was done in a biosafety cabinet. The blood culture bottles were positive. There was gram negative gram stain. No additional work was done. The lab secured any subplates and blood culture bottles.

And so we started to think, all right, how many people this time did they have exposed? Well, surprisingly, they did listen. There were no exposures. So we were very happy about that.

The risk assessment is really going to be important when it comes to analyzing specimens using MALDI, especially if high-risk pathogen is suspected. So things that need to be considered are the extraction method you're using. Are using a tube extraction or other methods? That initial spotting-- again, this includes where to spot, like in a biosafety cabinet, on a benchtop with a face shield. How do you spot? Away from your face, using a face shield. Again, what types of matrix applications are you doing? Are you covering the entire spot? Are you using-- where are you doing your target cleaning? Are you using disposable target? So how is the target being moved to the instrument or removed from the instrument?

Think about what are some of the things that should be considered in relation to sample preparation and decontamination. Though as part of the risk assessment there are a few things to consider as part of the sample preparation, but this is in no way all inclusive. Some of these were already discussed, but in short you want to review the culture handling steps when picking a colony. You want to-- which extractions or processing sample preparation methods are you going to use? Whether it be the direct method and on-plate formic acid or some type of ethanol and formic acid tube extraction.

Are you doing a filtration step? How's that matrix added? What is a standard inoculum used? How much is that biomass is on there? Because that does play a big role in the inactivation as well.

Similarly, these are things that should be considered in relation to decontamination. So what type of PPE are you using? What disinfectants? Are things being decontaminated outside of the instrument and on any space around it? Are you decontaminating the inside of the tray and the sample door? Obviously, do not attempt to disinfect or decontaminate the inside of the instrument without contacting the manufacturer, but you can call them to explain the incident, request their input for decontamination response.

Really, as we talked about, the extraction method for MALDI involves the lysis of the bacterial colonies by the chemicals to release the protein. So when working with highly pathogenic bacteria, such as select agents, pre-analytical extraction in a separate tube and chemicals prior to inoculation onto the target slide does provide the most protection against viable bacteria, although this method of extraction may not provide complete inactivation of select agents. So what biosafety set ups can help to prevent exposures? Venting your blood culture bottles in a BSC, using the BSC when working with unknowns. Reviewing those ASM protocols for ruling out and referring BT agents. Being in contact LRN labs. Limiting not only automated systems of MALDI, but all automated ID systems. And then implementing the use of benchtop shields and/or face shields.

And then lastly, what questions can labs ask when implementing just a new platform in general? Just keep in mind what is potentially causing errors. What are those errors self-generating steps? Are there potentials for spills and splashes or any type of contamination concerns, any type of facility concerns. What PPE would you use? How will you do the training? And then is there a way to do an inactivation method? And have do you verify those? So with that, I wrap up my portion.

ALICIA BRANCH: We'll wait till Tarsha comes back on camera and we'll take a few questions. OK. Let's see, let's take the first question. I'm not sure who wants to answer this. Can you please repeat the four common occupational exposures for NREs?

TARSHA HARRIS: That would probably be me. So I did mention this, but this is just based on internal data that we've collected over time. And it's kind of hard to bulk everything into specific categories, but I tried to do that. Because obviously, with the various entities, you have different iterations of the assays that they're performing. But generally-- and I have it noted here. So it would be opening culture plates to observe for growth or pig colonies. We see that quite often-- outside of primary containment. Everything would be outside of primary containment that I'm mentioning here, and without appropriate respiratory protection.

Then the other, as Michael mentioned, would be slide preparation. For us, it's slide preparation for MALDI-TOF or other automated systems, which also, as he pointed out, there are other steps in that process that can be considered a release or exposure as well. And then we have sample or specimen manipulation while conducting other types of manual assays prior to sample inactivation or fixation. And then for more geared towards veterinary labs would be performing necropsy on select agent infected animals, prior to their identification, of course, outside of primary containment.

ALICIA BRANCH: OK. The next question is while most of the biosafety and occupational trainings are offered by experts from microbiology departments, can you talk about the hazards from clinical chemistry labs?

MICHAEL PERRY: So really, this was more focusing on those bio threat agents. And so with that, it's going to be a lot of the work that's being done in the micro section of the laboratories. So some of the chemicals that you do run into, not so much from the clinical laboratory perspective, but it would really be the micro labs that are doing some of the Bruker systems and the bioMerieux, and that type of mass spec, versus looking at Mass spec from the clinical identifications.

ALICIA BRANCH: Does PCR workstation sufficient-- they must have meant is it sufficient-- workstation sufficient for MALDI target spotting?

MICHAEL PERRY: So with the workstations, you want to look at what type of workstation you use and what the flow is for that. So there's a lot of different types of workstations that are out there. And you want to see if that is for personal protection or if that is something that is related to actually product protection. So in that case, if it's a product protection, the air's going through the HEPA filter first and then blowing back at the user. So you just want to make sure that the hood that you're using is taking air from the outside in and then going through the HEPA filter, so that you actually have personnel protection.

ALICIA BRANCH: OK, this says, regarding the second presentation, if a lab had experienced exposures the second time instead of zero exposures, what would have-- what would be the course of action for your agency?

MICHAEL PERRY: So really, if they had exposure the second time, what we would do is-- and it's pretty much what we do in all cases, is we do hold a call with the laboratory. And we'll go through step by step all the procedures that they went through and try to identify what was the reason that this happened. Sometimes it is unavoidable. You can't expect clinical laboratories to put every single thing in a biosafety cabinet. That's just not possible. There's way too many specimens.

And I've heard that thrown out there before, like, well, this is the option, do it. But when you have thousands of samples coming in, and one biosafety cabinet, and two people working, it's just not an option. So we just try to at least go with them with the risk assessment to try to identify potentials where maybe in the future we can at least lower that risk. We are very lucky in New York state that every clinical laboratory has to have a risk assessment. Some are better than others. But then it also gives us the opportunity to go through the risk assessment with them to see are there areas of improvement.

ALICIA BRANCH: We can go to this one. I'm not sure if it's complete. Unless you are analyzing CSF on a chemistry analyzer-- so I'm not sure that they completed the question. Let's see, clinical chemistry laboratories work with blood specimens where hepatitis can be contracted too. But most of the labs don't process their specimens in biosafety cabinets. Can we still think of this?

MICHAEL PERRY: Yeah, so a lot of times what we try to suggest is even if you can't-- if you can't do a biosafety cabinet, at least getting some type of facial barrier just to give yourself some type of protection. Again, we know that it's not possible to put everything in the biosafety cabinet, but If you can at least get some type of facial barrier in there, that's what we would recommend.

ALICIA BRANCH: Is there a correlation between labs with high staff turnover rates and more exposures reported?

MICHAEL PERRY: So I could talk to this from what I've seen, and then maybe too Tarsha could do it from the Form 3 side, maybe if she has some information. But I know that we haven't seen it as much. It's really kind of all over the place. Sometimes, though, if it's a lab that we've worked with quite a bit in the past, and they've been really doing a really good job for a while, then occasionally we might see with some high staff turnover those exposures creep back up. But in general, I'd say it's just kind of all over the place.

TARSHA HARRIS: Well, unfortunately, we don't have access to correlate-- we don't have that other half of that information on entity turnover so we could not make a comment on that one, unfortunately.

ALICIA BRANCH: OK. That's all the questions we'll take for today. So if we didn't get to your question, we'll try to answer it, but via email. If you have any additional questions after today, please email the OneLab inbox at onelab@cdc.gov. And, again, thanks again, Tasha and Michael. We are offering one PACE credit for today's webinar. To receive PACE credit, visit the link in the passcode posted in the chat and complete the evaluation within two weeks. You will also receive an email containing these instructions if you miss the link and passcode.

As a reminder, the slides with the links will be posted to www.cdc.govform/onelab within the next two weeks. And thanks again and have a great rest of your day.

TARSHA HARRIS: Thanks, everyone.