**OneLab Event Audio\_04.26.23**

**CHELSEA PARSONS:** Hello, everyone. Thank you so, so much for joining this OneLab network event, "Recognizing, Identifying and Reporting the Identification of Select Agents and Toxins." My name is Chelsea Parsons, and I'm a consultant with Guidehouse supporting CDC's OneLab initiative.

I have just a couple of notes for everyone before we dive into this session. If you're having any technical issues throughout the session, you can feel free to email our OneLab inbox. It's onelab@cdc.gov. That's onelab@cdc.gov.

If you have questions throughout the session regarding the session materials, you can pop those into the Q&A function. So in the bottom ribbon of your Zoom panel right now, you should see a Q&A. You can pop questions in there throughout the entire session.

We'll have a Q&A section at the very end of the event. Try to answer as many as we can. If we don't get to your questions today or if something pops up after the session you have a question about, feel free to email that OneLab inbox again. That's onelab@cdc.gov.

And if you submit a question to the Q&A we don't get to today, we'll try to get back to you with a response in a timely manner there. And so I want to note that we've posted the live captions link in the chat. But please be sure if you're going to use that to keep both the live captions link open as well as this Zoom session too. Just keep them up side by side.

All right. So let's get into our agenda for today. We are going to have some opening remarks from Dr. Victoria Olson of OLSS. Alicia Branch, our OneLab lead, will introduce us to today's presenters. We'll get into the main conversation, and then we'll close out with that Q&A.

We'll be providing some instructions to obtain PACE credits at the very end of the session and then talk to you a little bit about our upcoming events. So with that, I'll turn it over to Alicia Branch for our intro. Thanks, Alicia.

ALICIA BRANCH: Thank you, Chelsea. We at DLS want to say happy 48th Medical Laboratory Professionals Week, or Lab Week. Our theme for this year's Lab Week is the future's lab. We say thank you to all laboratory professionals on the call today.

We celebrate you this week and every day for protecting the future by skillfully adapting to meet today's evolving public health challenges and patient care through reliable diagnostics and prevention with resilience, innovation, and expertise. Please join us in celebrating Laboratory Week by participating in DLS lab week activities.

First, celebrate yourself for your outstanding contributions. And then always add in your exceptional lab-- any laboratory professional that's in your life. Use our digital tool kit to increase awareness of laboratory contributions by sharing social media hashtags, key messages, digital graphics with your colleagues. Also, you can download printable stickers, a coloring page, and a word search puzzle.

See the link in the chat for the DLS Lab Week tool kits. And at this time, it's my pleasure to introduce Dr. Victoria Olson, the deputy director here at CDC's Office of Laboratory Science and Safety, who will provide the opening remarks.

VICTORIA OLSON: Thank you so much, Alicia. And thank all of you for allowing me to join you here on your OneLab Network call. As Alicia was just mentioning, this week is Medical Laboratory Professional Week, or how we affectionately like to call it, Lab Week. Lab Week's purpose is to increase public awareness and appreciation of the clinical and public health laboratory community.

I'm personally honored to celebrate Lab Week with all of you today. I want to recognize that you do essential services to safeguard the public health. And it's not only during public health emergencies, even though that seems like it's been ongoing for years now, but it also is every day. Our laboratory scientists are our critical partners, and we value this collaboration immensely.

I want you to know that we are here to support you and have resources to help you meet the demands of your everyday duties. And that includes during those never-ending public health emergency responses. I am in awe of how much hard work and your continued dedication to strengthening diagnostic testing and improving patient care through the rapid detection of disease.

So I just wanted to take a few minutes to extend my appreciation and thank you for letting me have this time. I'd like to personally wish each of you a happy Lab Week and thank you again for your more remarkable contributions and continued commitment to improving public health. And now I'll turn things back over to Alicia. Thank you.

ALICIA BRANCH: Thank you, Dr. Olson, for joining us today and providing opening remarks. Before we present our speakers for today, we would like to provide the following disclaimer. I'm sorry. I actually changed the slide.

CDC, our planners, and presenters wish to disclose that we have no financial interests or other relationships with manufacturers of commercial products, suppliers of commercial services, or supporters. Now we're excited to introduce today's presenters for our part one of a two-part OneLab event. Our first presenter is Miss Susanna Schmink, a microbiologist form 4 coordinator with CDC Division of Select Agents and Toxins, or DSAT. Before joining DSAT in 2021, she worked as a public health scientist at CDC and the Texas Department of Public Health.

Dr. John McQuiston will deliver the second half of today's presentation. John earned a BS from the State University New York Fredonia, an MS degree from Virginia Tech, specializing in molecular genetics, and a PhD in evolutionary genetics from Emory University. He is a team lead for the Special Bacteriology Reference Laboratory in the Bacterial Special Pathogens branch within the Division of High-consequence Pathogens and Pathology at CDC.

Our OneLab speakers for today, Miss Susanna Schmink and Dr. John McQuiston.

SUSANNA SCHMINK: Thank you, Alicia. Good afternoon. Today's presentation will cover a brief overview of the Federal Select Agent Program, commonly referred to as FSAP, regulations for submitting the APHIS/CDC form 4A and provide some helpful information on completing the form. Next slide, please.

FSAP is managed jointly by CDC Division of Select Agents and Toxins and Division of Agricultural Select Agents and Toxins at APHIS. In the United States, there are two types of entities for the Federal Select Agent Program, those registered and those not registered or non-registered entities. Excuse me.

Registered entities are laboratories approved to work with select agents and toxins through the registration with the Federal Select Agent Program. There are approximately 233 registered entities, which include academic, federal government, non-federal government, commercial for-profit, and private nonprofit companies. Non-registered entities are not approved to work with known select agents and toxins and make up the majority of labs in the United States. Examples include hospital, clinic, food testing, and veterinary laboratories.

More information about the Federal Select Agent Program can be found at our website, selectagents.gov, that is listed in the chat. Next slide, please. I'll cover the regulations and reporting specifics for the identification of a select agent or toxin. Next slide, please.

The APHIS/CDC form 4A is for reporting the identification of a select agent or toxin and is used to notify the Federal Select Agent Program of the identification of a select agent or toxin contained in a specimen presented for diagnosis, verification, or proficiency. Today, we'll concentrate on reporting select agent and toxin identifications on the APHIS/CDC form 4A sections A and B and C and D. A link to the form will be posted in the chat for your reference.

This form can be used for various sample types, including human, animal, environmental, food, and plant. The report can only be used to report one select agent or toxin. A different agent or toxin identification will have to be submitted on a separate form.

This form is used by both APHIS and CDC and can be submitted to either agency. Later in the presentation, I'll talk about disposition. Listed on this slide are the regulations. Next slide, please.

Listed on this slide are the regulations from the Code of Federal Regulations, or CFR, that are specific for the select agents and toxins. Title VII part 331 covers agricultural regulations. Title IX part 120 covers animal products, and title 42 part 73 covers public health. We won't have time to go into the specifics of them, but they can be found at selectagents.gov, or once the slides are posted, you can select the hyperlinks in the slide.

Sections of the regulation that specifically apply to non-registered entities that possess or use or transfer a select agent or toxin contained in a specimen presented for diagnosis or verification are Title VII part 331 section five, Title IX part 121 section 5 and 6, and title 42 part 73 sections 5 and 6. On the next slide, I'll go over those specific requirements. Next slide, please.

The regulations allow for non-registered entities to be exempt from registering with the Federal Select Agent Program and meeting the requirement of the related regulations, provided that they do the following-- report the identification on a completed and signed form 4A within seven calendar days after the identification to either APHIS or CDC but not both agencies, secure the select agent or toxin to prevent theft, loss, or release while in the entity's possession. Next slide, please.

In addition to these requirements, the regulations require that a select agent containing a select agent be transferred to a registered laboratory or destroyed on site by a method that will kill the select agent. Select agents and toxins as well as samples containing select agents and toxins should not be taken off site for destruction by a commercial hazardous waste vendor but instead destroyed on site. Some common methods of destruction include autoclaving, chemical treatment, and incineration. This we'll cover later in more detail in the presentation.

Both the transfer and destruction must take place within seven calendar days. We previously discussed your lab is required to report the select agent or toxin identification to APHIS or CDC. But you're also required to report the identification results to the specimen provider and your state or local authorities when required by local law. Let's move on to immediate notification, in which agents and toxins require immediate notification. Next slide, please.

The Federal Select Agent Program currently regulates 68 select agents and toxins. Please visit our website at selectagents.gov for the entire list. The identification of any of the tier-one select agents or toxins listed on this slide must be immediately reported by telephone, facsimile, or email to the Federal Select Agent Program.

Agents most frequently identified and reported to the Federal Select Agent Program are *botulinum* neurotoxins, *botulinum* neurotoxin-producing species of *Clostridium*, and *Francisella tularensi*s. Next slide, please. So let's talk a little bit about an immediate reporting and completing the form.

The date of notification on the form block B3 is the date the laboratory identifying the select agent or toxin notified either APHIS or CDC. This date is usually reported by the reference lab. It is captured only on the form 4A sections A and B, which is also known as part one of the form. This is not the date your laboratory immediately notified the sample provider or physician. Again, immediate reporting can be done by fax, phone, or email. Next slide, please.

Now let's discuss the date the sample provider was notified by the testing or reference laboratory. First, a sample provider is the person or facility that your testing or reference laboratory receives the sample from. There are times when there are more than one sample provider, such as when both the hospital and the state public health laboratory receives the sample from the same patient that you identified as containing a select agent or toxin. In this case, both sample providers would be listed. Please list all sample providers so we can follow up within to ensure appropriate disposal of any samples containing a select agent or toxin and to verify if any potential exposures occurred.

The date the sample provider was notified may be the date after the identification date. This is not the date of a presumptive or a preliminary identification. We recommend that notification to the sample provider be made as soon as possible after the final identification of a select agent or toxin.

When notifying the sample provider, please request they complete and sign a form 4A section C and D or part two. This helps us in obtaining the information on the form in quickly identifying possible exposure incidents. In the next slides, I'll provide some helpful information for a few questions on the form 4A section C and D. Next slide, please.

The date the sample provider is notified by the testing or reference laboratory is captured in block D2. On the form 4A section C and D, the date is often misunderstood and should be the date the reference laboratory provided the final identification of the select agent or toxin to your laboratory. This is not the date for any presumptive or preliminary identification. Since we'll already have sections A and B of the form 4A with the date the reference or testing laboratory has indicated as the date they notified the sample provider, this date should match that date.

If these dates do not match, we may contact either entity to verify the date that the information was provided. Next slide, please. The number of samples shipped-- this question is asking you to count the number of items your lab shipped to the reference lab. This would normally include counting each sample, plate, slant tube, et cetera for each patient, meaning the number would be three if you sent one blood culture, one slant, and one plate for patient x for the identification of *Brucella*.

If your laboratory sent multiple samples from different sources, only include the number of samples that were positive for the identification of the select agent. For example, if your laboratory sent 10 mosquito pool samples for Eastern Equine Encephalitis testing and only eight of the samples were positive, your laboratory would report eight in this box, even though 10 samples were shipped. Next slide, please.

The ZIP code information is the only patient-related information that we require on the form. This information should not be the ZIP code of the laboratory performing the testing or the facility that collected the sample. It should be the information specific to the patient when known.

If not known, the hospital or treatment facility ZIP code can be provided. Please do not provide any other patient-related or PII information such as name, date of birth, address, ages, et cetera. Also, please do not send additional documents containing information not related to the APHIS/CDC form 4A. Our system is not rated to contain PII information.

This ZIP code is important because it helps us understand where in the United States these infections in humans, animals, or the environment are occurring. We use this information to track and trend data over time. Next slide, please.

The sample disposition is important information to have and to understand how laboratories are taking appropriate safety measures to properly destroy select agents or toxins. This question is on both parts of the form. We need to know the disposition of the select agent or toxin, as well as know how the original samples containing the select agent or toxin were either transferred, destroyed, or retained.

That is retained only by entities registered with the Federal Select Agent Program. I'll repeat that again. Only entities registered with the Federal Select Agent Program can retain samples containing viable or active select agents or toxins.

If the select agent or toxin has been inactivated or made non-viable or non-toxic by an acceptable method, then the material can be retained by an entity not registered with the Federal Select Agent Program. An example would be a formalin-fixed, paraffin-embedded tissue sample identified as containing *Coxiella burnetii*. In this case, your laboratory would indicate destroyed on the form 4A and provide the method as paraffin-embedded, formalin-fixed tissue. Please call us or refer to our website for more information about samples containing a select agent or toxin and if your laboratory can retain these samples.

All isolates and samples containing a select agent or toxin that cannot be retained must be appropriately destroyed on site. The most common destruction methods include autoclaving. Using 10% bleach is another one. However, other methods include but are not limited to other chemicals, incineration, and irradiation. Please refer to the biosafety in microbiological and biomedical laboratories document, appendix I and K, for more information on the appropriate method to destroy the select agent or toxin.

If you would like an isolate or sample containing the select agent or toxin transferred, please first contact the laboratory that will receive the select agent or toxin material to ensure they are registered to receive that particular agent or toxin. Next slide, please. This slide is like the previous slide, except it is for section C and D.

It does not have an option to transfer but does include the selection option that the entire specimen was transferred to the reference laboratory. This is quite often the case for stool samples identified as containing *botulinum* neurotoxins. Again, retaining the select agent or toxin isolate or samples containing the select agent or toxin is not allowed for entities not registered with the Federal Select Agent Program. Next slide, please.

The final aspect of the form and perhaps the most important aspect I will cover is the question, were any samples containing a select agent or toxin handles outside of primary containment, which may have led to the unintentional release and/or exposure to the select agent or toxin? This is seen on the screen in question D9 or for sections C and D. However, this question is also on sections A and B, question B10.

If your response is yes for this question, an APHIS/CDC Form 3 is required. We hope to get a few yes responses. However, we have received many over the years, mostly related to working with or manipulating select agents or samples containing select agents on the laboratory open bench. Part two of this presentation next month will go over the APHIS/CDC Form 3 and related statistics. Next slide, please.

Now let's test your knowledge with a few scenarios. You should see a poll pop up on your screen so you can participate. Next slide, please.

Your hospital laboratory received two serum tubes and a wound swab from a patient that the doctor suspects has botulism. Your laboratory appropriately packages and ships the samples to the state health department laboratory. Three days later, state health department lab contacts you with a positive identification of *botulinum* neurotoxins for the wound swab.

Is your hospital laboratory required to submit an APHIS/CDC Form 4 section C and D? So we'll give you about 15 seconds to select your response. OK. So maybe we can wrap up that poll.

But go to the next slide, please. The correct response is yes because the select toxin was identified. Next slide, please.

From scenario A, your hospital laboratory received two serum tubes and a wound swab from a patient that the doctor suspects has *botulinum*. Your laboratory appropriately packages and ships the samples to the state health department laboratory. Three days laboratory, the state health department lab contacts you with a positive ID for *botulinum* neurotoxin from the wound swab.

What number of samples would you indicate for question D3 on the APHIS/CDC Form 4 section C and D? And we'll give you 15 seconds or so to respond to the poll. OK. We're wrapping up the poll.

Let's go on to the next slide. The correct response is C1 because only the wound swab was tested and positive for the select toxin. Next slide, please.

Your hospital laboratory identifies *Brucella abortus* RB51 in a patient's blood sample. Should your laboratory submit a completed APHIS/CDC Form 4A sections A and B? And we'll give you a couple of minutes to look at those responses and answer the poll.

OK. Looks like the poll closed, and so maybe we can go ahead and advance to the next slide. The most accurate response is C, no because *Brucella abortus* RB51 is not a select agent. We have seen Form 4's reporting misidentified select agents and toxins and non-select agents. So please be sure to let us know if the sample was sent to another laboratory for further identification. Also, be reminded that a presumptive identification does not require reporting with a Form 4A.

Once the sample is sent to the reference lab and confirmed as a select agent or toxin, that is when we should receive the Form 4A from both the reference laboratory and the laboratory submitting the sample for identification. That ends my presentation today, and I'll take questions at the end, or you may post your questions in the Q&A section. Thank you very much.

JOHN MCQUISTON: All right. Let me click this over to presentation view. OK. Hi, everyone.

I'm John McQuiston. I'm the second speaker for today, and today I'm going to be talking about MALDI-TOF and the limitations, misidentifications, and safety with regards to MALDI-TOF in general. Just as an overview, I'm going to talk a little bit about MALDI-TOF and taxonomy and why does taxonomy have to be so confusing, the safe handling of the MALDI-TOF specimens, and then database importance, a little few slides on MicrobeNet, and then some conclusions.

So bacterial identification has gone through phases over the years. And for many years we used biochemical and phenotypic tests for identifications. But they really are too slow and too expensive to continue using.

We also use PCR, but PCRs are generally very specific for a species or a strain level. We're starting to use genomics, and that's too slow, too expensive, and really too much expertise is needed, at least for now. We are moving in the right direction, I think, but MALDI-TOF kind of fits in that area that's just right.

MALDI-TOF is one of the fastest, easiest, and inexpensive specimen-to-result laboratory tests ever developed for thousands of bacterial and fungal species. It really can identify bacterial species in less than 10 minutes per sample for prep time. That's the full extraction method.

You can do hundreds of specimens per day with accurate results within minutes. It costs about $0.50 per isolate. That's excluding the equipment and staff, of course. And it's accurate to the genus and species level, and there are a few publications taking it down to the strain level in *E. coli* and some other species. And there's a new infrared technology that'll go to the strain level as well.

It also can identify some antimicrobial resistance markers. And even with all of this, though, it's not perfect. The biggest question I get every time I go and talk about MALDI-TOF is why can't I differentiate x species. This happens all the time. And many of the common species people have trouble differentiating in MALDI-TOF are really because of the limitations of the instrument and the technology.

It's really only accurate to the species level, at least for now, with a few recent exceptions. The results are really only as good as your database representation and quality. And then of course, the taxonomists get in the way.

Now, misidentifications have happened in all of the technologies we've used to try to identify species, including cross-reactivity and PCR, contamination, and genomics. Lateral gene transfers can cause confusion in molecular tests. And taxonomic and nomenclature issues and species relatedness really are what plague the MALDI technology.

One of probably the single most question I get asked about species relatedness is the *Shigella* and *E. coli.* And *Shigella* and *E. coli* on the MALDI-TOF will be misidentified. *Shigella* has come up as *E. coli*. *E. coli* has come up as *Shigella*.

And you can see here from this screenshot of MicrobeNet the results page shows that a *Shigella sonnei* came up as an *E. coli* as the detected species and matched the Bruker library. If we were to look at the top 10 hits for that strain, you can see it's a mix of *E. coli* and *Shigella* species and genera. And these are also mixed between the databases.

And this has been a long challenge for differentiating *E. coli* and *Shigella*. And *E. coli* and *Shigella* are actually taxonomic two different genera, according to taxonomists. But according to doing whole-genome sequencing and matching them up, and most people recognize this now that *E. coli* and *Shigella* species are closely related and genetically constitute the same species. This is why they can't be differentiated on a MALDI-TOF instrument.

Another problem we have is the *Brucella* and the *Ochrobactrum*. And this is leads directly to the select agent problem. Many *Ochrobactrum* have been misidentified-- or many *Brucellas* have been misidentified as *Ochrobactrum* on MALDI-TOF as well as VITEK and some other technologies. And that's because they are very closely related species and genus.

And in 2020, Hordt et al published an analysis of 1,000 type strain genomes and determined that *Brucella* and *Ochrobactrum* should actually be in the same genus. So just recently, they have now added the *Ochrobactrums* to the *Brucella* genus. And we look at it as the new *Brucellas*, formerly known as *Ochrobactrum*, the old *Brucellas*, which also contain the select agents *Brucella* [INAUDIBLE] and others. So if you are seeing *Brucella* and *Ochrobactrum* names changing in the current databases, just be aware that *Ochrobactrum* has been renamed as *Brucella*.

Now, there's a great publication out from ASM called "*Brucella* and *Ochrobactrum* Taxonomic Updates for Laboratory." I highly recommend you read this if you deal with either of these, and most people probably will deal with *Ochrobactrum* at some point. And make sure that you understand what to do with the *Ochrobactrum* and *Brucellas*.

But even within the *Ochrobactrum* and *Brucella*, there's arguments. Taxonomists love to argue about what's a species and what's not a species. And you can see this with *Brucella lupini*, which is formerly *Ochrobactrum lupini*, where this publication in 2019 said the species is a later heterotrophic synonym of *Ochrobactrum anthropi*, which is now *Brucella anthropi.*

And then a year later, Hordt et al said the species is not a later [INAUDIBLE] synonym of *Brucella anthropi*. So basically, just taxonomist arguing with each other. Another group that is difficult to identify on the MALDI-TOF is the *Burkholderias*.

*Burkholderia cepacia* and the *cepacia* complex can be very difficult to differentiate. And this is a case where you have a match to *Burkholderia cepacia* with the CDC library on MicrobeNet at a 2.5 score. And those of you who use MALDI-TOF know that anything above a 2.0 is generally considered a species identification and a good score. But here's a case where you have seven different identified species named above a 2.0 score.

And we see this problem with some of the different genera. And this is a consideration that I'll talk about in a second, but this is basically a red flag. If you see more than one species above a 2.0, you need to be aware that this could be a closely related complex. And you may not be able to differentiate these using this instrument.

So what are you supposed to do? And how do you solve the multi-misidentification problems? First, check that your database is up to date and includes the representatives of the select agents. This is very important. So if you don't have the select agents in your database, you could be misled as getting a high score from a different species.

Be wary when two species or two or more species are above a 2.0 score in the MALDI-TOF results and within 10%. So some labs use two different-- if two different species are greater than 10% apart above a 2.0, they'll call those different species. So for example, that last example, if it's 2.5 and your next one was 2.1 and they're both above a 2.0, they will confidently say that 2.5 was the correct species.

We don't do that. We prefer that everything is either above or below the 2.0 line. If we can't get that resolution, then we generally use other tests to determine this.

Be aware of the taxonomic synonyms in the bacterial species. Those are like the *Brucella* and *Ochrobactrum*, where they're changing the names. And you could potentially have a problem. And check with the LSPN, which is the list of prokaryotic names with standing and nomenclature. And the link is right here.

Your state public health lab, the CDC, or MicrobeNet, and you can email us at microbenet@cdc.gov if you have any questions. And we can either point you in the right direction, or we can hopefully answer your question. And then I highly recommend using MicrobeNet for the Bruker MALDI, as it's kept up to date.

It's combined with the Bruker releases and contains select agents as well as many other databases in our database collection. And then look out for your own safety. And you have to advocate for yourself.

The biggest risk of MALDI-TOF is open-culture exposure of an unknown. Since MALDI-TOF is so fast and so easily and readily available for those labs that are using it, it's easy to quickly want to spot something on the plate and throw it into MALDI-TOF without taking proper precautions. In our labs and hopefully in all the labs treat all the isolates as unknowns until identified, and all of the BSPB, Bacteria Special Pathogen Branch, bacterial culture work is performed in a BSC with full PPE on, as shown here.

Think of that biosafety cabinet space as bench space. And we really recommend whenever you're setting up the prep for the MALDI-TOF instrument you do this in a biological safety cabinet. And we highly recommend the full tube extraction protocol, which is 10 minutes, which is the best proven method for safety. And this is a publication by Jim Roderick in 2017.

His publication studied the safety and accuracy of MALDI-TOF with the highly pathogenic organisms. And what he found was he took *Bacillus anthracis*, *Burkholderia*, *Clostridia*, *Francisella*, *Yersinia*, and *Brucella*, and he compared direct colony spotting on the plate compared to the on-plate formic acid, meaning spotting on the plate and then adding formic acid and then a full tube extraction. And what he found was the survivability of the cells on the plate after the tube extraction killed all the cells for that species, whereas the direct colony as well as on-plate formic acid one still had surviving select agents.

So again, we recommend the full tube extraction. It literally is only 10 minutes. It's also the fastest protocol you'll still do in the lab even at the longest preparation method.

Now, databases are important. Keep your databases up to date, and that goes to, for example, the *Ochrobactrum Brucella*. The name changes are happening. In fact, the species do-- using genomics, there have been 1,800 species name changes in the last couple of years. And also check for accurate taxonomic curation of the strains that are added to a database. So if you're using an in-house database or if you're using a database that is from somebody else, make sure that what they added to that database is actually taxonomic correct because that can get really confusing.

As I said, the *Ochrobactrum* are now identified as *Brucella*. And most of the commercial databases, including Bruker and VITEK, have changed this to, for example, *Brucella anthropi*, formerly *Ochrobactrum* in parentheses or something along those lines. We're also changing that in MicrobeNet in order to let people know that this is actually an *Ochrobactrum*. And these can be distinguished.

The former *Ochrobactrum* from the former *Brucellas* or the old *Brucellas* can be distinguished by MALDI-TOF. So it's just the naming that's causing the confusion. And then misidentifications if the correct species data is not in the database. And we do see this, where you have a low score or something around 1.9-1.8, but the actual species is not in your database. You could be confused and thinking that the top result must be correct.

So make sure that your scores are acceptable to the species level, which we recommend the 2.0 level. And don't just pick the top result because that's where you can get into problems. So I'm going to give a brief overview of MicrobeNet and to close this out.

And for those of you who haven't seen MicrobeNet or aren't users, MicrobeNet is an online virtual reference laboratory. You can request an account on the main page. There is a tutorial to show you how to add files to it, and then you can sign in.

And this is the current dashboard where you can drag and drop your MALDI or FASTA files here to do either mass spec searching or blast against our 16S and ITS database. We also have a phenotypic module for biochemists. So if you want to build your own virtual biochemical test tube rack, you can do that as well.

The vast majority of our users use the MALDI-TOF module. And you get something that looks like this result. It's very similar to what you would see on the instrument when you're running the MALDI-TOF, except with a few key differences.

You can see the different databases that it matches, including MicrobeNet and our partners down in Buenos Aires, Argentina. Not only small brand has shared their database with us. And we host it on MicrobeNet. And they are the coordinating center for MicrobeNet Latin America, our partners down there.

You can also click on the links that are in bold, such as the species name, and get more information on the species, including contact information at CDC, the genus information, growth conditions, as well as culture images, and publications, and more. So just to conclude, your safety must come first. And you don't know what you're working with when you first get an isolate in the door.

Even if it says what you think it might be, there have been many times where we've gotten isolates in where lab thinks they've identified it. And it turns out to be something completely different. So please work in a biosafety cabinet.

Think of it more as bench space, and they are 1/25 the cost of a MALDI-TOF instrument. And they cost much less than a lab shutdown or staff comp time if you end up causing an exposure in the lab. And do the full extractions. It's only 10 minutes.

It's actually only five minutes longer than the faster method. So you're really only saving five minutes, and it's still the fastest method out there for bacterial identification. And every other method I can think of like PCR and anything else takes hours. And this is literally 10 minutes per isolate.

Taxonomy is always going to be a challenge. And you need to be aware of something that could be a select agent and know which two species are closely related, and they're above 2.0. This should be an alert of a possible taxonomic issue. When in doubt, use MicrobeNet. We keep our databases up to date all the time.

We have multiple databases. We're going to be growing those numbers over the years, adding more databases as well. We're adding the fungal databases. Other countries will be joining us too, so please join us.

If you have any questions, don't be afraid to email us at microbenet@cdc.gov. For those of you in Latin America, you can email our coordinating center down there at the email address shown here. And I'd just like to thank everybody that's working on MicrobeNet.

This is the current team working on MicrobeNet and all of our partners. There are many, many more people that have worked on MicrobeNet. MicrobeNet celebrated its 10-year anniversary this year in January. We've been live since 2013, and many people have worked on this over the years.

Our next big adventure is going to be whole-genome sequencing, which will be coming later this year. So thank you, and please feel free to contact us if you have any questions. At this point, I guess we can start taking questions.

ALICIA BRANCH: OK. Yeah. We'll take a few minutes to answer as many questions as possible. If we don't get to answer your question, we will do our best to respond via email.

If you have any questions after today, please email the OneLab inbox at onelab@cdc.gov. OK. Let's see.

First question-- when should APHIS/CDC Form 4 be submitted for suspected identification of a select agent?

SUSANNA SCHMINK: You said a suspected identification?

ALICIA BRANCH: Be submitted for suspected identification of a select agent.

SUSANNA SCHMINK: Well, usually, they don't if it's a suspect. We usually like to see that the sample be referred to a reference laboratory that can do a final identification for it. So that's what we prefer to have done.

ALICIA BRANCH: OK. If my laboratory is not immediately notified of the select identification, how soon does my laboratory need to submit the CDC Form 4?

SUSANNA SCHMINK: So could you repeat that question again?

ALICIA BRANCH: If my laboratory is not immediately notified of the select identification, how soon does my laboratory need to submit the form?

SUSANNA SCHMINK: Within seven days of the identification date is when it would be reported or should be reported to us.

ALICIA BRANCH: OK. And next question is, what happens if our laboratory does not submit the form?

SUSANNA SCHMINK: Well, if they don't submit the form, there's a number of things that could happen to them. We would eventually find out about it. And we would follow up with them to figure out what's going on and why they're not reporting. And there are possible penalties that could occur because of not submitting the form.

ALICIA BRANCH: OK, and let's see. The next question is, how many people or labs use MicrobeNet for the identification per year?

JOHN MCQUISTON: So MicrobeNet is worldwide. We have 97 countries involved with 3,700 users. And we actually get a hit about 155,000 times per year. So a lot of labs using MicrobeNet for identifications.

ALICIA BRANCH: I'll give you this one also, John. They want to know-- I'm hearing that some clinical laboratories are no longer doing Gram stains and just testing by MALDI-TOF. What are your thoughts on this practice?

[CHUCKLING]

JOHN MCQUISTON: So yeah, I would prefer that still some traditional microbiology is done. But most high-throughput labs, especially the big hospitals, are going straight to MALDI. And they have decreased using pretty much anything else.

We generally like to have at least two tests so that they match or that it makes sense. So I still recommend doing Gram staining or some other tests, that is you can confirm that what you've seen on the MALDI makes sense [INAUDIBLE].

ALICIA BRANCH: Last question-- do you know if ASM guidelines will change to allow MALDI extractions in place of manual methods?

JOHN MCQUISTON: I don't know of any guidelines along those lines.

ALICIA BRANCH: [CHUCKLES] OK. All right. Thank you so much, Susanna and John, for joining us today.

We'd like to let all the participants know that we're offering one PACE credit for today's webinar. To receive PACE credit, visit the link posted in the chat and use the passcode also in the chat, and complete the evaluate evaluation within two weeks. You will receive an email containing these instructions after today's event.

As a reminder, the slides with links will be posted to www.cdc.gov/onelab within the next two weeks. We will present part two of today's presentation entitled working-- I think this is the wrong title.

Let's see if we can get the correct title for this for part two. Which is second? Just noticed that. It's "Biosafety Practices in Reporting Occupational Exposures to Select Agents and Toxins." Again, this is part two of the next OneLab event, which will occur on Wednesday, May 31st at 1:00 PM.

Again, that's Wednesday, May 31st at 1:00 PM Eastern Standard Time. The registration should be getting posted in the chat as well. Again, I would like to say thank you for joining.

Happy Lab Week, and have a great rest of your day.