Transcript

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Welcome
Sean Courtney, CDC Division of Laboratory Systems

Testing for Viral Vaccine-Preventable Diseases
Paul Rota, CDC Division of Viral Diseases

Update on Locally Acquired Malaria and Recommendations for Laboratories
Brian Raphael and Molly Freeman, CDC Division of Parasitic Diseases and Malaria

Sean Courtney: All right, we'll go ahead and get started today. So good afternoon, everybody. Thank you for joining our call today. My name is Sean Courtney. And I'm a Health Scientist in CDC's Division of Laboratory Systems.

On the screen is the agenda for today's call. But before we get started, I just want to cover a few announcements and some general housekeeping items. So as you've heard on previous calls, DLS is the CDC division that works closely with clinical and public health laboratories across the country to support laboratory emergency preparedness and response activities. And we've been hosting these calls since March of 2020.

DLS supports this work across four goal areas, quality, workforce and training, preparedness and response, and informatics and data science. As always, we'll be sharing the slides from today's call along with audio and transcript. And we'll post them online hopefully by the end of next week or within the next two weeks.

You can find them on CDC's Laboratory Outreach Communications Systems page at the link shown here. And so we want to hear from you. Our Training and Workforce Development Branch is interested in hearing more about the education and training gaps that you're currently experiencing. And we invite you to send your feedback via email to labtrainingneeds@cdc.gov.

And so if you have a question for today, we ask that you please use the Q&A function in Zoom so that we can address it during the call and not to use the chat function. Also, we like to ask that you please include your email, so that we can follow it up if we're not able to answer it during the call. And if you're from the media and you have questions about the presentation or would like to follow up with a speaker, please reach out to CDC media relations at media@cdc.gov. And of course, if you're a patient, please direct any questions to a healthcare provider.

And I'd like to remind everybody that the slide decks may contain presentation material from panelists who are not affiliated with CDC. Presentation content from external panelists may not necessarily reflect CDC's official position on the topics covered. And with that, I'd like to introduce our first speaker for today. We have Dr. Paul Rota from CDC's Division of Viral Diseases. And he'll be speaking about testing for viral vaccine-preventable diseases. Paul?

Paul Rota: Thanks, Sean. Good afternoon, everyone. I just wanted to talk to you a little bit today about testing for viral vaccine-preventable diseases (VPDs). Could I have the next slide, please?

And what I'm really talking about here are the viral vaccine-preventable diseases that are handled by my branch. And that's measles, mumps, rubella (MMR), and varicella-zoster virus, among other things. These are the viral vaccines that I want to focus on for this afternoon's presentation. Next slide, please.

And just recently, we've become aware that there is a greater availability of testing in commercial and clinical laboratories for measles, mumps, rubella, and varicella, either molecular testing by RT-PCR, or PCR in the case of varicella, or for serologic methods, including detection of specific IgM for case confirmation.

And I think the advantages of this expansion of VPD testing in commercial laboratories certainly has expanded the availability of testing and has also potentially faster turnaround times and quicker time from collection of sample and routing of specimen and reporting of results. The advantage is that the providers are familiar with commercial labs. And this really constitutes part of their normal specimen flow.
And of course, the results are linked to electronic medical record systems. Next slide, please, Sean.

However, there are some challenges with this expansion of testing by the private sector laboratories. And some of the challenges are here on this slide. One of them is that some of the sensitivity of the RT-PCR assays, the lower limit of detection, and the specificity might not be known in all cases.

And sometimes it's hard to interpret results if we don't know especially the sensitivity of these tests. There's often lack of detail about the serologic testing, particularly the assay format used to detect IgM. The assay format can be pretty critical in helping to interpret the results, especially if you suspect there might be a false positive reaction.

We want to make sure that the molecular tests have the ability to detect all circulating genotypes. And as you know, CDC monitors the genetic characteristics of these circulating viruses to make sure that we're not getting any genetic drift in the targets for the molecular tests. There's also a critical role when these tests are ordered to have integration with state and county departments of public health, since these are reportable diseases. And the state or the county will often need to do an investigation, contact tracing, and monitoring vaccination coverage if it's at schools, and things like that.

So it's really important that we don't lose the ability to advise the public health authorities or the public health infrastructure in the state to make sure that they're aware that they have to respond to this. And

also, the state public health laboratories are familiar with these tests and know how to interpret the results.

On a practical level, sometimes there's difficulty with acceptable specimen types, as you know. I think now after the large amount of testing for COVID-19, there are different types of universal transport medium and things like that used for PCR, which are a little bit different than the standard viral transport medium, which have been used. And it's important to make sure that the specimen types are acceptable for the test being used and OK for reflex testing.

One of the issues is that the positive specimens for these are not routinely genotyped because the commercial labs are offering detection methods, but not genotyping methods. And genotyping is also a critical part of our virologic surveillance activities at CDC. Specimens are sometimes unavailable for additional testing. So sometimes, we need to do some downstream testing in addition to genotyping.

There might be serologic testing, like IgG avidity or something that needs to be done. And sometimes it's difficult to recover these samples for downstream testing. In the case of measles, vaccine-specific assays are not available. And this is an important aspect because as you know, 5% of recipients of MMR vaccine can develop a fever and rash, which is clinically compatible with measles, if vaccination is occurring in an area that's had an outbreak.

So there's vaccination and an opportunity or possibility of exposure. It's very important to detect these vaccine reactions very quickly because if not, they would have to be counted as if they were an actual case. And that would mean a fairly lengthy public health response in terms of contact tracing, et cetera.

So rapid detection of especially measles vaccine reactions is very critical. And CDC has a Miva, a RT-PCR that's designed to specifically detect measles vaccine reactions and identify measles vaccine reactions in the three hours it takes to do a real-time RT-PCR. And then there's also issues with specimen storage and stability because if we're going to do downstream testing, we need to know that specimens have been stored and maintained in suitable conditions to allow further testing to be performed. Next slide please, Sean.

So some of the considerations are, of course, turnaround time. And we want to make sure we have the quickest possible turnaround time, so that we can generate a public health response as needed for detections of these viral agents. Serum samples are useful. We sometimes do follow-up testing, including using different types, for example, for IgM detection. We might use an IgM capture assay, which is sometimes less likely to have false positive reactions in some of the direct IgMs.

And also, we use IgG avidity as a way to help rule out cases, especially for rubella. So we need to think of ways to do effective reflex testing, so that this follow-up testing can be performed. We also want to do routine genotyping for positive samples of measles, mumps, and rubella.

This is not terribly urgent because the genotype information are not really used in the outbreak control mechanism. But the data are needed to maintain an accurate sequence database, as the sequence data are used to track transmission pathway. And in the case of measles and rubella, the sequence data are submitted every year to show that we have maintained the elimination of measles, rubella, and congenital rubella syndrome.

So it's important that we be able to maintain a robust sequence database for these viruses that are circulating. And certainly, for varicella virus, we don't really do sequencing. But we have methods to distinguish between detections of the vaccine virus, Oka strain, and wild-type varicella. And this is important for our surveillance activities for varicella-zoster virus.

And it's important to mention here that the samples need to be collected, shipped, and stored in a manner that's consistent with the CDC test directory, which is available online. Next slide, please.

So the CDC goals are to continue to engage in the dialogue with commercial laboratories. We want to develop guidance for the commercial labs for submission of specimens to CDC for additional testing.

And we'll probably work with various partners, including the APHL to do this. The guidance will probably be posted on the CDC web page and contain CDC point of contacts for the tests, as well as references to the CDC test directory. So that would list the acceptable specimen types and shipping and storage conditions for the specimens.

CDC could possibly assist commercial laboratories with validation, and identification, and proficiency testing programs. For genotyping and vaccine reactions, we certainly want to help develop provider awareness on measles vaccine reactions. And when laboratory testing is required, it's still an area where we need to constantly update providers and develop provider awareness on the availability of vaccine-specific tests in public health laboratories, so that when it's necessary, they can get rapid confirmation of measles vaccine reactions.

We want to develop awareness for state and county public health laboratories, as well as develop workflows for routine reflexing of positive samples to CDC or the CDC vaccine-preventable disease reference centers for genotyping or for strain-specific PCR for varicella zoster virus.

And I think that's my last slide, Sean.

Sean Courtney: All right, thank you. I appreciate that update today. I guess a quick question I had was, the HAN that was recently released, it kind of mentioned there had been about 16 cases here in the U.S. Is there a concern, I guess, for more testing needed to be available or just to be ready for this summer for laboratories?

Paul Rota: I think the HAN was just to maintain awareness. Measles has been eliminated in the United States since almost 2000. But the virus, measles itself, is very infectious. It's one of the most infectious viruses known. And it travels very well.

And so, with now international travel rebounding to pre-COVID levels, actually exceeding pre-COVID levels, it's very common even for U.S. citizens to be traveling abroad and become infected with measles and bring it back. And we need to make sure that we can detect these importations of virus very quickly because we know that we have some communities and some pockets of under-vaccination in the US. And the virus can spread very quickly there.

So early detection of these cases is absolutely critical. And they can happen any time, but are frequently associated with international travel. And as you know, measles is endemic still in many parts of the world. And in fact, many places are now seeing a resurgence of measles. I hope that answers your question.

Sean Courtney: No, it did. That was really helpful, thank you. Yeah, so there's a couple questions that are popping up. I'll try to go through some of them with you here. The first one is, you mentioned about engaging in dialogue with commercial laboratories. How would you prefer that to go? Is there like a website, or email? Or do you want to set up specific conversations? What are your thoughts around that?

Paul Rota: Well, as I said, I want to try to develop some general guidance that we can make available through CDC, our outwardly-facing websites. However, we've had a series of one-on-one calls with various commercial providers. And I think we want to continue to engage.

I think it's good to have a quick call just to discuss these things. And we've had calls with some of the vendors. We have a few more to go. And certainly, we want to encourage open communication between CDC and the commercial labs.

Sean Courtney: All right, great. Thank you. Appreciate that. So next question was, how do you track genetic drift and shift, especially for viral pathogens that show extensive changes?

Paul Rota: Well, we do routine sequencing. We do whole genome sequencing on the representative samples. We are also a global specialized lab in the WHO measles and rubella laboratory network.

So we are looking at genetic changes, especially for measles and rubella, on a global scale. There's a global database that's maintained at the United Kingdom Health Security Agency, or UKHSA. We have access to that database. And so we can use that information to monitor genetic changes in the virus. And certainly we can monitor the target for the molecular assays.

A lot of the molecular assays that are used globally, we are quite familiar with the primers and probes that are used. We actually run a global proficiency testing program here at CDC for over 100 global labs. So we would be in a good position to know if there was any decrease in assay performance because of genetic drift in the viruses.

Now, it turns out that measles and rubella are actually genetically quite stable. These lineages stay stable for a long time. So it's not the same level of genetic change as you would see with like SARS-CoV-2, or influenza virus, or anything like that. But we are monitoring the change.

Sean Courtney: Great. Thank you. All right, so the last question I see is, can you elaborate on sequence databases for measles?

Paul Rota: Well, yeah, I just said the sequence database, the global WHO sequence database is maintained by the UKHSA. And it's maintained for members of the global measles and rubella laboratory network. A lot of the data from that database are also exported into GenBank. So one can do a GenBank search and have access to a number of these sequences if you wanted to.

If there was a need to look at the database, if you contact me, I can put you in touch with the the global database, the individuals who are running the global database.

Sean Courtney: All right, great. Thank you. All right, one last question. And it is, for resource-limited countries, how could we deal with genotype and vaccine reaction? Not sure if you can answer that.

Paul Rota: Well, I mean, we are trying to establish genotyping, just routine genotyping, especially for measles and rubella in resource-limited countries. The idea is that we need to establish genetic databases in these countries because every region has a goal to eliminate measles and rubella.

And so establishing a genetic database, even though they may not need to sequence-- just sequencing a small proportion of the cases, so we know what's circulating because we look at the shift in those genotypes to look for evidence of interruption of transmission chains. The detection of vaccine strains, some international countries, some countries are using various versions of a vaccine-specific real-time RT-PCR. While others would be using actually sequencing to identify vaccine strains.

Unfortunately, vaccine reactions, we can't use serologic methods to confirm vaccine reactions. Resource-limited countries have the ability to forward samples to, say, a regional reference lab or one of the reference labs for additional testing, if they don't have capability for, say, sequencing or sequence analysis on site. So I hope that answers the question.

Sean Courtney: Yeah, I think so. Thank you so much. Really appreciate that. And really appreciate you joining our call today. And if any other questions pop up, if you're available and still online, if you could just maybe answer them within the Q&A function as they pop up. That would be great. But again, thank you for joining us today and really appreciate the presentation.

Paul Rota: Thanks, everybody.

Sean Courtney: Thank you. All right, so up next, we have Dr. Brian Raphael and Dr. Molly Freeman from CDC's Division of Parasitic Diseases and Malaria. They're going to be providing us with an update on locally acquired malaria cases and recommendations for laboratories. So Brian and Molly, I will turn it over to you guys.

Brian Raphael: OK, well, thank you for joining us. And my name is Brian Raphael. I'm the Acting Chief in the Laboratory Science and Diagnostics Branch. And I will be providing some information along with Molly Freeman, who leads our Malaria Laboratory Research and Development Team. Next slide, please.

So as you may have heard recently, there have been some cases of locally acquired malaria since May of 2023, six cases in Florida and one case reported in Texas in June of 2023. These cases were determined to be caused by *Plasmodium vivax* through multiple testing. And ongoing state public health responses include a variety of activities, including ongoing active surveillance for additional cases of malaria.

There's activities involving mosquito surveillance and control measures, and also outreach and prevention education to the public. CDC is also doing some activities related to these locally acquired cases. CDC is providing recommendations to U.S. residents, to clinicians for malaria prevention and treatments. Some of this material is available on the CDC website.

We're also responding to requests for information and supporting local investigations through laboratory testing. One of these activities includes remote telediagnosis, which is really a rapid service to look at images and provide back a report on those images to folks who are doing the testing locally. We are also receiving some specimens to conduct confirmatory testing primarily through PCR.

Our epidemiologists are involved with performing technical assistance to the state and local public health agencies that are involved in these responses. And also, our entomology laboratory here at CDC has been actively involved in mosquito testing and also providing additional technical assistance. Next slide, please.

So we really wanted to use this opportunity to share with you some important updates or information on malaria diagnosis. I'll just give this slide and then turn it over to Molly for some additional details. But just overall, microscopic examination of both thick and thin blood smears is considered the gold standard. And where patient care is offered, this should be available 24 hours a day or referral to a laboratory very quickly.

It's not always possible to differentiate species, even with well-experienced morphological analysis. PCR comes into play, especially in these cases, where it's difficult to differentiate species. These are generally laboratory-developed tests.

There are some tests that are available that differentiate *Plasmodium falciparum* from other *Plasmodium* species. There are other assays that may not be able to differentiate two or more *Plasmodium* species.

Assays really should be selected for this response that can definitively differentiate human-infecting species, including *P. vivax*.

And then rapid diagnostic assays are available. These detect malaria antigens in the blood specimen. They may be able to differentiate [*P.*] *falciparum* from other species. In the U.S., there's a single FDA-cleared assay. This is not CLIA-waived. And Molly, I think, will present a little more details on that. Next slide. Molly, over to you.

Molly Freeman: Sure. Thanks, Brian. So the BinaxNOW Malaria RDT is from Abbott. And as Brian said, it is the only malaria RDT that is approved for use in the U.S. by the FDA. It is an in-vitro immunochromatographic assay for the qualitative detection of *Plasmodium* antigen in human blood.

It uses histidine-rich protein two, or HRP-2, antigen that is specific to *Plasmodium falciparum*. And it also uses a pan malaria antigen that is common to all four malaria species capable of infecting humans. So *P. f*[*alciparum*], *vivax*, *ovale*, and *malariae*. So RDTs can be used to aid in the rapid diagnosis of human malaria infections, so that proper and immediate treatment can be started, and also to aid in the differential diagnosis of *P. f*[*alciparum*] from *vivax*, *ovale*, and *malariae*.

For malaria cases that are caused by *P. vivax* infection, the samples may test positive by the pan malarial antigen. But the RDT will not distinguish it from *P*. *o*[*vale*] or *P*. *m*[*alariae*] -- so *ovale* or *malariae*. And that's true whether that's a single or a mixed infection. The BinaxNOW Malaria RDT is not a screening tool for asymptomatic populations.

It really is only approved for use for testing individuals with signs and symptoms of malaria. When implementing RDTs, plans should be made for adequate specimen collection and follow-up testing or confirmation at an appropriate laboratory. The test uses venous blood. And that's tested within three days of collection or capillary EDTA whole blood tested immediately after collection.

Because the RDT does not differentiate *P*. *v*[*ivax*] from *P. malariae* or *P. ovale*, additional testing is needed to confirm *P. vivax* or any mixed infections. Importantly, negative results as well must be confirmed by thick or thin smear microscopy. Next slide.

So again, just to emphasize that it is critical to be able to differentiate the *Plasmodium* species. This helps to ensure that appropriate treatments for *P. vivax*, or *P. ovale* especially, are started in order to prevent relapsing illness. Genotyping for *Plasmodium* is possible.

Analyzing the sequence data from certain markers may help to elucidate the genetic relationship between strains and can also help describe if individuals are infected with multiple strains. Most of the published reports for molecular genotyping for *Plasmodium* have been from endemic areas for population-based studies of malaria.

Currently, the CDC is assessing multiple genotyping methods, including high-resolution genomic approaches to analyze specimens from individuals in a non-endemic area. Next. Brian?

Brian Raphael: I think there was one-- was there one slide before this? No, this is right. So what can laboratories do? We want to point out that ensuring the availability of those thick and thin blood smear analysis and RDTs in hospital clinic laboratories. As I mentioned at the beginning, there's availability of rapid telediagnosis of blood smear images.

This can happen very rapidly and provides back a test report through uploading onto our secure server, the image and sample requisition. If you have questions about that, please send an email to DPDx@cdc.gov. Also, submitting specimens to the public health laboratory for confirmatory analysis, including PCR, can be very valuable.

And I also like to remind folks that retaining positive samples for genotyping analysis can be helpful for those types of analyses that Molly spoke about in terms of looking at the genetic relationship of strains from these current cases and also the relationship to other strains for which there is data available in public databases. Next slide.

There are several resources and contact information. We have both mine and Molly's email points of contact here. Also, there's more information on the CDC website regarding malaria, diagnostic procedures. That DPDx website does have several very useful sort of PDF worksheets and additional information on malaria diagnostics in general.

Questions about cases, case investigations can be sent to the malaria@cdc.gov Malaria Hotline. And specimens for diagnostics, you can send questions to parasiteslab@cdc.gov. And also for surveillance specimens get sent to malarialab@cdc.gov. I think with that, we are at our last slide. Thank you.

Sean Courtney: All right, thank you both for that update today. So there are a few questions. And I'll kind of go over some of them if you're able to answer them here on the call. So the first one is, how often do we detect imported *P. knowlesi* infection from Southeast Asian exposure?

Brian Raphael: So that may be a question that would be better answered by our epidemiology and surveillance colleagues. So I would be happy to follow up on that question with our colleagues.

Sean Courtney: OK, great. Next question is, are there any PCR tests for malaria being developed on machines such as the Cepheid or BioFire instruments?

Brian Raphael: Molly, do you have information on those two platforms?

Molly Freeman: I do not. But I am happy to look into that one as well and get back to you.

Sean Courtney: All right, next question is, which 8-aminoquinoline do you recommend for the *P. vivax* patients in the US?

Molly Freeman: This is Molly. I would say that's another one for our med epi colleagues.

Sean Courtney: No problem. I appreciate it.

Brian Raphael: And Sean, also that is also a really good question to send to our Malaria Hotline.

Sean Courtney: Right. Great. OK, yeah. Thank you. All right, so the next question is, if thick [and] thin blood smears are the gold standard diagnostic test, is CDC having any communications with ASCP to recommend this to be included on their MLT, MLS board exam content?

Brian Raphael: So we are starting to engage with external partners on these types of questions. But I will also add this to our list of outreach.

Sean Courtney: OK, great. Thank you. I understand some of these questions can be difficult. So I appreciate any guidance, like you've mentioned, of additional outreach. Let me see here. Another question is, does the CDC currently provide any resources for surveillance of *Plasmodium* drug resistance?

Molly Freeman: Yes. You can email malarialab@cdc.gov. We do accept specimens for surveillance purposes only. And we will sequence the molecular markers of resistance.

Sean Courtney: Great. Thank you. So another question is, what are your thoughts on next generation sequencing in malaria?

Brian Raphael: We may both have comments on this. So these are complex genomes. They're very large genomes. And so we are working at CDC to develop targeted approaches for genomic sequencing of *P. vivax*. And Molly made a really good point in the slides that there are several studies in the literature, which have looked at a multitude of different markers for sequencing, different types of approaches to sequencing.

And one of the things that we're really trying to evaluate very quickly is, what is a set of markers that will give us the type of high-resolution ability to resolve strains with high resolution to actually have some kind of useful information that can be used to guide public health responses? And that's an ongoing effort.

Sean Courtney: All right, thank you. Next question was, are there guidelines for clinicians on patients presenting with no travel history? They've seen a large increase on malaria requests for testing on non-travel patients with non-specific symptoms.

Brian Raphael: Right. So there are very specific guidelines that are available on the CDC website for evaluating patients in this context. Also, I would strongly suggest the Malaria Hotline for additional information.

Sean Courtney: All right, thank you. Next question is, is the rapid test acceptable to be done initially? So if there's a negative, is there a manual smear required? Or can you report by next test and stop there? And I think Molly would like to answer that one.

Molly Freeman: Sorry, I started to type, but pressed the wrong button. Because the RDT is being used to screen patients who are symptomatic and with suspicion of malaria, then yes, that negative should be confirmed with a thick or thin blood smear.

Sean Courtney: All right, great. Thank you. All right, so I'll do one more question. It's been a really hot topic. So really appreciate you two hanging out here and answering a lot of questions. So really appreciate that. The last one though is, a PCR has shown that asymptomatic malaria is far more common than previously thought. Are you doing serologic surveillance for subclinical *P. vivax* in the US?

Brian Raphael: Molly, do you want to take that one?

Molly Freeman: Yeah. I can start. So we have used serological assays in surveillance studies before. And so there have been some discussions on how this could be used for the current situation. It has not started yet. We'd want to make sure that we've got really an excellent test to be able to roll this out.

And so for now, we are focusing on the current situation and responding to the outbreak and anticipate additional discussions about doing serosurveys.

Sean Courtney: All right, thank you. Thank you for that. All right, well, we'll go ahead and wrap that up. If there's additional questions, if you guys could kind of maybe reach out to them through the Q&A function, that would be great. There were a few in there. And I tried to try to get to some of them that we could and some of them that we couldn't. So we appreciate that.

But with that, we'll go ahead and wrap up for today. So I just want to, again, thank all of our speakers today. And as a reminder, we typically hold these calls on the third Monday of each month. And they're scheduled for one hour.

Our next call is scheduled to take place on Monday, August 21st, from 3:00 to 4:00 PM [Eastern Time]. And also, please let us know if you have any suggestions for topics for future calls, as we look forward to continuing to discuss these along with any of your laboratory and testing community needs. And as we mentioned before, we'll try to post this audio, transcript, and slides from today's call on the website within the next two weeks.

As always, you can find us on social media. So you can find CDC on Facebook, Twitter, Instagram, and LinkedIn. And please follow them to stay up to date with the latest news and recommendations. And with that, we just want to, again, thank everybody for your time. And again, thank you for our speakers. And we will see you guys in August. Thank you. Have a good one.