Clinical Laboratory COVID-19 Response Call
May 3, 2021

Agenda

- **Welcome**
  - Jasmine Chaitram, CDC Division of Laboratory Systems (DLS)
- **Surveillance Testing of Wastewater for SARS-CoV-2**
  - Amy Kirby, CDC Division of Foodborne, Waterborne, and Environmental Diseases (DFWED)
- **Investigation of Linked Clusters of SARS-CoV-2 Variant B.1.351**
  - Kenneth Feder, Maryland Department of Health
- **FDA Update**
  - Tim Stenzel, U.S. Food and Drug Administration (FDA)

JASMINE CHAITRAM: Hello, everyone. I'm Jasmine Chaitram. I'm with the Division of Laboratory Systems in CSELS at CDC. Thank you for joining the Clinical Laboratory COVID-19 Response Call. The Division of Laboratory Systems has been hosting these calls since March of last year, and we're glad that you were able to join us again today.

Just to go real quickly through a couple of housekeeping things and just a couple of changes to our agenda for today-- unfortunately, we had some bad weather come through the Atlanta area today, and so Amy Kirby is without internet access and will not be able to join us for this call. We will reschedule her for another time. So, very sorry for those of you that were joining specifically to hear about the surveillance testing of wastewater. Hopefully you can hear that another time.

Hang on one second. I'm getting a message that folks can't hear me, so let's just make sure that they can hear me. Hold on. All right, we did we did a little sound check and folks here on my end are saying they can hear me. So if you cannot hear me for some reason, please try to dial back in. And you probably can't hear me saying that, so maybe somebody will put it in the chat for you.

Anyway, as I was saying-- so Amy Kirby is not going to be with us this afternoon, and we will schedule her for another time. We do have two other speakers. It may be a shorter than usual call, which is fine because I'm sure everybody has a lot to do and lots of things that they could do with their extra time. So with that, I will move into some of the housekeeping things.

Oh, before I do that, reminder about the Division of Laboratory Systems and who we are-- we are the division at CDC that has been focused on supporting clinical and public health laboratories. We were doing this before COVID, and we were doing this through the response. We do this in several topic areas, including quality and safety, bio repositories and data science, informatics, workforce development and training. And we also do this in the area of preparedness and response. And as I mentioned, we have

been doing this through the COVID response, serving as a liaison to the CDC's Emergency Operation Center, which is managing the response. And we are providing information and outreach to clinical and public health laboratories. And one of those avenues is through these clinical laboratory calls that we host every other Monday.

And so, another mechanism for us to share information is our Preparedness Portal, and I'm showing here the website for that portal. And these slides will be available after the call on this portal as well as the transcript and the audio from the calls. We also archive our LOCS messages. That's the Laboratory Outreach Communication System. That's how we send emails to all of our clinical laboratory partners out there. And we also have links to other CDC pages related to COVID-19. So, one-stop shop for information about the COVID response.

As I mentioned already, our next call will be in two weeks on Monday, May 17, from 3:00 to 4:00 PM. These calls are moving to schedule-- have moved to a schedule-- of every two weeks. And if you have any specific training and workforce development needs, please send those to labtrainingneeds@cdc.gov. We would love to hear from you on what those needs are.

And then finally, the reminder about how to ask a question-- please use the Q&A button in the Zoom webinar system. That's at the bottom. It says Q&A. And type your question there. We would prefer that you do not submit your question in the chat box. We do look back through the questions that we receive, and any questions that are unanswered during the call-- we do try to provide a response. And that's usually through email if we're not able to get through it during the call. And in order for us to do that, we need to have your email and your name-- at least your email. Sometimes we do not have the right experts on the call to answer your question. Sometimes we have more questions than we can answer during the call. And it's helpful to have that information in the Q&A box so that we can try to follow up.

And I do want to remind all of you that are on the call that this is a laboratory-focused call. So we really are trying to answer questions about testing or clinical laboratory requirements and regulations around CLIA and test reporting. So try to keep your questions around that subject matter. And with that, I guess we will go ahead--

DR. TIM STENZEL: Jasmine, can you hear me? This is Tim. There's lots of participants who still can't hear you. And they're showing that you're on mute. I have no idea what the challenge is. Hopefully they can hear me, because when I speak, they can be heard. But they're saying that you're shown as muted, so I just wonder-- it may not do anything, but if you could mute yourself and then unmute yourself, maybe the act of doing that would-- Oh, they say they can hear me, but they're still not hearing you. It may be silly, but if you can mute yourself, unmute yourself-- and see if that flips some sort of switch that then works for you.

JASMINE CHAITRAM: OK, I did it. So I'm not sure if anybody can hear me now. I see, "I can hear Jasmine." So it sounds like we've fixed the problem, I hope. A lot of people just missed my spiel, but

hopefully they are longtime participants and know the drill as far as asking questions and know who DLS is. And thanks, Tim, for your tip because it seems to have worked.

OK, so we actually don't have any slide decks, but normally when we do present slides, sometimes those speakers are from outside of CDC and this is just a disclaimer the speaker or the panelist may not necessarily reflect CDC's official position.

All right, we are ready to get into our agenda, and as I mentioned, Amy Kirby was not able to join us today. So we are going to go to our next speaker, which is Dr. Kenneth Feder from the Maryland Department of Health. And he's going to be talking about an investigation of linked clusters of SARS-CoV-2 variant. And this was recently published, I believe, in a CDC MMWR. And Dr. Feder does not have slides today, so you'll just be listening, and I think he'll be joining us by camera. Kenny?

DR. KENNETH FEDER: Yes, good afternoon, everybody. Thank you. Before I get any further, please indicate in the chat box whether you can hear me speaking. Excellent. I hear lots of yeses. OK, so you can stop indicating whether you can hear me. I'm going to post a link to the MMWR in the chat box. And so that way, folks can follow along in the MMWR as I go. So thank you, Jasmine, for inviting me. Thank you, everybody, for joining.

As Jasmine said, my name is Kenny Feder. I am the CDC's Epidemic Intelligence Service Officer for the State of Maryland. And what I'm going to talk about today is an investigation that Maryland and several local health departments in the state conducted into linked clusters of SARS-CoV-2 infection that were ultimately identified to likely be the first linked clusters of SARS-CoV-2 infection of the B.1.351 lineage that were identified in the United States. And that variant, which I think many of you were aware, was first identified in South Africa and is designated a variant of concern because it carries a mutation that might reduce protective immunity acquired through vaccination or prior infection. And so we did write up a description of this investigation in MMWR, and so Jasmine invited me to come talk about it so you can hear how we were able to use the sequencing data in this investigation. And then I can answer any questions you may have.

Now, the focus here is not necessarily on the laboratory component, so I encourage you to afterwards please let me know what follow up questions you may have. OK, so just for some important context-- in Maryland, our contact tracing program attempts to conduct case investigations and contact tracing where all persons with COVID-19, immediately after they received their initial positive diagnostic test, case patients get interviewed, close contacts get elicited, those contacts get contacted, and they get instructed to quarantine. And all the investigations are documented in a central data management system via scripted electronic form, and then also as audio recordings.

So what happened in this instance is that in late January of 2021, a clinical laboratory that was on its own conducting genetic sequencing contacted the Maryland Department of Health to tell us that the SARS-CoV-2 variant of concern, B.1.351, had been identified in a specimen collected from a Maryland resident with COVID-19. And because of our heightened interest in this infection, those electronic forms and

recordings from the case investigation to that person were reviewed by the leadership of our contact tracing team, and that patient was also re-interviewed. And then the named close contacts from that investigation who developed COVID-19 were identified from the records in that same system. And we tried to get specimens from those people for genetic sequencing. So we tried to obtain the positive specimens from the clinical labs to see if we could obtain them for genetic sequencing, or if they weren't retained, to recollect them to sequence the recollected specimens. And then the contacts of these contacts who tested positive were also elicited, and so on, until we couldn't trace the chain of transmission anymore. And for everybody who we identified within that transmission chain, we made at least an attempt to obtain a specimen for sequencing.

So the index patient was initially identified as being infected with the B.1.351 variant. That patient reported attending an indoor social gathering prior to the onset of the symptoms with six other people two days before their symptom onset. And after reviewing records from our database of investigations, all six other attendees of that social gathering had also received positive SARS-CoV-2 antigen or NAAT test results, with specimen collection dates ranging from three to 13 days after the gathering. So we identified that as very likely the place where our index patient was infected.

Now, among the attendees of this gathering, the earliest self-reported illness onset date was actually the date of the gathering. The person got sick that very day. And that person was identified as the likely source of infection for the other people who attended the gathering. So a retrospective review of that source patient, or that likely source patient, revealed that the patient's workplace was a business that had been reported through an anonymous tip line established for reporting social COVID-19 safety concerns. So the local health department opened an outbreak investigation into that workplace. And that workplace had only seven employees. And six of them were symptomatic and had at some point, it turned out, received positive SARS-CoV-2 antigen or NAAT test results.

And those six employees named a total of eight nonwork close contacts links. And five of those nonwork close contacts also received SARS-CoV-2 positive test results. And then three of them never experienced any symptoms and did not ever have a positive test. So in total, we found these two linked clusters-- one in the workplace, and then one in the social gathering. And in total there were 17 infections. So that was seven people who attended the social gathering, six employees from the workplace including one person who was at both, plus five of the 11 close contacts named from all of the people in either of those two settings-- ultimately tested positive. And none of these patients reported any history of international travel or close contact with somebody with a history of international travel.

So we were able to successfully obtain four specimens for sequencing, including the index patient, so we got three more specimens. So two of those patient specimens were from patients exclusively associated with the social gathering cluster. And one specimen was from a patient exclusively associated with the source patient's workplace cluster. In other words, they were actually a degree removed. So we had three specimens from people who attended the social gathering. And then we had one specimen from a person who was in close contact of someone who worked in the workplace. And someone else in that workplace went to the social gathering. So they were sort of like three links removed, but when they were

sequenced all were identified as B.1.351 lineage. Two of the specimens were identical to the index patient's. And then one of the specimens differed by a single SNP.

And then finally, just quickly-- two patients were hospitalized, including one employee of the workplace, one close contact, and one of those patients died. Neither of the people who were vaccinated-- excuse me, neither of the people who were hospitalized or died had any vaccination history. But two of the infections did occur in people who had received the first of their two-dose COVID-19 vaccine series, 11 and 12 days before their likely exposure. So they were vaccinated 11 to 12 days before the exposure.

And one infection occurred in a person who had already tested positive for COVID-19 five months earlier. So that's two cases in people with a single dose of a two-dose COVID-19 vac series, and then one case of suspected reinfection or positive test in a person who had been previously positive five months earlier.

So we think there are three key takeaways from this that we highlight in our MMW. So first, most of the case investigations took place before the people in these clusters were identified as having been infected by the B.1.351 variant. And that's because sequencing usually takes several days or weeks beyond the time needed to do the PCR testing. And so consequently, most successful variant case investigations and contact tracing will probably happen before it's actually identified that it's a variant case investigation by sequencing. And so that means the key to controlling these variants of concern is consistent implementation of best practices for case investigation and contact tracing for all SARS-CoV-2 infections to control the spread of SARS-CoV-2 variants.

And then second, the index infection was identified in a person whose specimen was sequenced even though they had no history of international travel. In fact, they were just sequenced at random by a clinical laboratory that tries to sequence every specimen below a certain Ct value-- excuse me, with a Ct value below a certain cutoff. And so, our takeaway was to maximize identification of variants of concern. It's important to both prioritize cases with some risk factors that we think might be indication of a variant of concern infection, like vaccine failure, travel, unusual clinical presentations, but also perform random sequencing of specimens with a low enough Ct value to be sequenced.

And then third, a couple of practices from this investigation we think are potentially useful to other local health departments-- so, audio recording the interviews turned out to be very useful because we were able to review them and find there was information in there that wasn't necessarily entered in the e-form-- searching for the potential source of infection, as opposed to just searching for people who might have been infected by a case. So in other words, we found that you have COVID-19. Instead of just trying to figure out who you infected, let's also try to figure out who infected you-- back-tracing-- and then the anonymous tip line, which helps figure out that the workplace was a very likely source of exposure for that source patient at the social gathering.

So that's really it. That's the crux of the investigation. And I apologize to everybody for not having slides. I was not able to get them prepared in advance. I encourage you to look, though, at the MMWR itself, because in there are three figures that sort of summarize a lot of what I'm saying in terms of the timeline

and then the relationships between the different people. And in fact, if it's OK, I'm happy to share my screen if questions come up, and that may be helpful. So I'll stop there because I really just want to open things up to you. Did you have any questions about the investigation, about things you'd like me to say over again because of the absence of slides, about ways the laboratory data was used?

Have there been-- OK, great, here we go. So have there been additional clusters in Maryland of this variant? So there have been additional cases of this variant in Maryland that were not linked to this cluster. And some of them have themselves been part of linked genetic clusters, but others are isolated. So at the time we did this investigation, this was the first linked cluster of this variant in the United States. And so we did this real in-depth investigation, and we actively tried to obtain these specimens for sequencing to confirm that these were genetically linked cases.

During the time that we were doing this, a number of cases emerged that weren't linked to other known B.1.351 cases, and our conclusion is that this variant is spreading in the community at some level in Maryland. And I think now it's maybe about 2%, 3% of our cases in Maryland, although I'm not sure off the top of our head. So yes, there have been other cases and there have been other clusters, but in some ways, the isolated cases are more concerning to us than clusters because it means this variant is out there and we don't have it linked to any confirmed clusters.

JASMINE CHAITRAM: Kenny, we do have some questions for you in the Q&A box, and I can ask them of you just to help move them along. OK, so first of all, what is the rationale for applying the Ct cutoff for testing?

DR. KENNETH FEDER: For sequencing? So I think the rationale for applying the Ct cutoff for sequencing is that it's more likely to produce a viable sequence. That's my understanding from my colleagues in the labs, is that essentially they said, we won't get good enough data if it's a Ct value above-- I think our lab uses a cutoff of 30. So we're very-- just to provide some context-- we're very lucky in Maryland. Our state lab has significant sequencing capacity. And then there's two large local clinical laboratories that also have a lot of sequencing capacity, partly under contract with the State of Maryland. That's Johns Hopkins University and the University of Maryland. And then we also get sequencing from labs under contract with CDC, like LabCorp and Aegis Labs.

They all use slightly different cutoffs, but I think our lab will sequence things only if they have a Ct value below 30, because they think below that they just won't be able to get a viable quality sequence that can be used in phylogenetic analysis. I think Johns Hopkins might use anything below 28 or something like that. I think University of Maryland uses-- 32 is their cutoff, but it may-- I'm not sure if that's been flexed since we last spoke about it.

JASMINE CHAITRAM: OK. For those who were vaccinated, was serology performed to determine if they had antibodies?

DR. KENNETH FEDER: That's a great question. I think the answer is no. I think the answer is no, serology was not performed to determine if they had antibodies.

JASMINE CHAITRAM: OK, did you share the results of the positive SARS-CoV-2 across different individuals with whom you contacted in your follow up at the social gathering cluster and the workplace cluster?

DR. KENNETH FEDER: No, no, so people's anonymity is protected. So if you test positive, we ask who your close contacts were, and then we can review the test results that we received. So we know those people tested positive, but we don't disclose to the index that those other people tested positive. And we also don't disclose to people the source of their infection. So if we warn you that you were in close contact with someone with COVID-19, we don't tell you who was the close contact or anything about where you were exposed. We just say, we believe you may have been exposed to COVID-19 and you have to quarantine.

JASMINE CHAITRAM: Right. Sorry, Kenny, I'm still seeing people telling me in the chat box that they can't hear me, but apparently, they can hear you. So if you're able to look in the Q&A and read the question, I think that would be more helpful because then people will know what the question is when you're answering it, because for some reason--

DR. KENNETH FEDER: I can definitely do that. OK, open-- OK. I'm not a laboratorian, so I apologize right off the bat. I realize that different PCR testing platforms may run different number of cycles. We've evidence on how Ct values correlate to infectiousness at the time of specimen collection or even with evidence of how viral load correlates with infectiousness. Other people on this call can probably answer that better than me, I think the answer is they are correlated. They are not a perfect proxy for each other, but I think they are correlated-- that lower Ct values are associated possibly with greater levels of infectiousness. But the other point is that that's not necessarily-- the key point here is not-- what we're concerned about is not necessarily infectiousness. It's just literally, can we sequence the specimen? Right, so you have your positive PCR test. Now we want to run the full sequence for phylogenetic analysis to determine the lineage to determine if it's a variant of concern. In all likelihood, you're not going to be able to do that sequencing if there's not a strong enough signal there. Okay, I'm not sure how to mark it as--

JASMINE CHAITRAM: We'll take care of that. We'll take care of marking them as done.

DR. KENNETH FEDER: All right, thank you. Which NGS platforms have been used successfully? I don't know. I apologize, I don't do the sequencing. I am the epidemiologist, not the laboratorian.

Stated in MMWR, SARS-CoV-2 B.1.351 one variant might elicit a reduced neutralizing antibody response. Do you have objective evidence of this? I don't see it presented in the body of the report. Thanks. Yeah, that's a great question. So we did not generate that evidence. So we didn't show that B.1.351 is associated with reduced neutralizing antibody response. There are other published studies out

there that show that SARS-CoV-2 of the B.1.351 lineage is associated with reduced neutralizing antibody response. So we are accepting that research at face value, essentially, and accepting that we are concerned about this variant when we followed up on it.

What percent of COVID currently is the new variants? I've heard a month or so ago, it was over 50%. Your thoughts? So, I don't know what it is nationally, but I think it is over 50%. In Maryland, I think-- so my role in this investigation is not doing the actual case investigations, except for a few of them I actually helped coordinate a lot of logistics of Maryland's genomic sequencing. So I look at this data a lot. I think we are well over 50% of our sequences being one of the CDC's designated variants of concern. I think we could be closer to 80%, but there's some diversity in there.

So well over half of that is the B.1.1.7 variant, which is not what I'm talking about here, which is now the dominant strain in the United States, certainly is the dominant strain in Maryland. Whereas B.1.351 exists, it's spreading in Maryland, but it's not that common. Some of the other common lineages in Maryland that are designated variants of concern by CDC are B.1.526 and B.1.526.1, which originated in New York. You may have heard of them as known as the New York variant.

And in Maryland, a lot of those 526 cases-- actually, this may be too much context. I apologize, but if folks want me to follow up on this more, I can-- but a lot of those 526 cases actually carry the same mutation as the B.1.351 strain that's concerning, this E484K mutation on the spike protein. So we're similarly concerned about those 526 cases. This investigation just happens to talk about 351 because that was what we identified first, and we already knew it was a concern because of the experience in South Africa.

What gene in COVID do we see the mutation occurring in? Sorry, so the question is, what gene in COVID do we see the mutation occurring? My colleagues in the lab have graciously educated me that there's not a one-to-one correspondence between lineages and mutations. So not every member of the lineage carries a particular characteristic mutation. But in general, common lineages have a common ancestor. They often carry similar mutations. In this case, one that's really stood out as being of concern is a mutation on the spike protein at the 484 position. It's an E484K substitution, and that's the mutation that I think is hypothesized to be associated with the reduced response to neutralizing antibody.

And some of you may have been following the large increase in cases in India. There's a variant in India, which has been labeled by the media as being a double mutant. I'm not sure exactly what that means, but one of those mutations that's concerning is E484Q. So it's a different mutation, but at the same position. And so somebody on this call may be able to explain better than me, but there's a lot of concerns about that 484-position playing a key role in the spike protein finding-- sorry, a key player role in antibodies binding to the antigen. And so the mutation there-- that being the reason why it's potentially associated with reduced responsiveness to neutralizing antibody. So that 484 mutation is the one here that we were concerned about.

Is all the hype about variants in cycling during testing really a major issue? I don't totally understand that question. I think maybe it's a question about whether there's concern about variants that maybe would be

missed by certain PCR tests, and I'm afraid I don't know the answer. I do think that, generally, concern about these variants, particularly those that maybe have reduced responsiveness to neutralizing antibody is warranted from a public health perspective.

Have there been any cases of B.1.351 variant infection in Maryland in two weeks after full two-dose vaccination? The answer to that is yes, but not a lot. We don't have published numbers yet, but I can tell you the answer is yes, in other cases. But cases of infection, of any infection, after full vaccination are very rare. So I'd be sort of reluctant to draw any trends from that. I mean, we do see-- we're the health departments, so we see every infection. So we see infections after full vaccination, but they are rare.

Question-- I tested COVID positive six weeks post full vaccination. I assume I got the variant. Is that correct? Not necessarily, no. There are different-- so first of all, I'm sorry. I'm very sorry that happened. It's unfortunate. And I hope you made a full recovery. So no, you shouldn't assume you had the variant, or this particular variant, or really any variant that carries the E484 mutation. The vaccines are not perfectly effective. They're extremely effective, but they're not perfectly effective. We expect there will be some people who are infected after being vaccinated. And also, in clinical trials, the outcome against which the vaccines were tested was symptomatic infections.

So we don't fully know, I think, the extent to which the vaccines protect against any infection, including those that are asymptomatic, or that they necessarily protect against PCR positivity, per se. So certainly, if you test positive after being fully vaccinated, we think the vaccine probably is protective and you got very unlucky, but also it's not necessarily true that it was one of these variant cases. It is possible that it was a breakthrough case for some of the reason we don't totally understand.

Did the patient who passed away have underlying comorbidities? That's a good question. I don't know if they had underlying comorbidities, but they were in their 70s. And they had no history of vaccination. Recently saw a positive COVID test in the clinic, and the report indicated that patient maybe positive if they were recently vaccinated. Is that always the case in terms of getting a vaccine and then possibly being positive later? I don't-- oh, might be, I apologize. The report indicated the patient might be positive if they were recently vaccinated. No, so recently receiving a SARS-CoV-2 vaccine should not cause you to test positive for SARS-CoV-2 by PCR or antigen tests because the vaccine does not contain viral RNA, and it does not contain the SARS-CoV-2 antigens that are the target of the antigen test.

So if somebody tests positive for SARS-CoV-2 by PCR or antigen, they were most likely infected, unless you were doing an antibody test-- which, I apologize, I don't know from the question-- in which case you would be looking for a history of previous infection. Or that vaccination might cause you to test positive for antibodies because vaccines are supposed to produce antibodies-- or cause your body to produce the antibodies, excuse me. But you should not test positive by PCR because you were vaccinated-- the opposite. It should prevent you from being infected.

Despite these variants, cases in Maryland are dropping every day and positivity rates related to vaccination plus zero prevalence in our population? Probably-- I think-- so, you know, they went up for a

little while, and then now they are going back down again for the last couple of weeks, which is encouraging. So this is actually a really important point. So different mutations to the SARS-CoV-2 virus are going to cause-- may give the virus some relative competitive advantage. So for example, B.1.1.7 clearly has some mutation that gives it a competitive advantage over other strains of the virus in the environment that existed several months ago. And in Maryland, that variant went from being non-existent or undetected at the start of the year-- obviously not truly non-existent, but undetected-- to being 60% or more of our sequenced cases within a span of four months. And it's become the dominant strain in the United States from being barely detected a few months ago.

But just because it's growing as a share of infections, that doesn't mean that overall infections are increasing. So it could be that, for example, on one day there are 20 infections and 10 of them are B.1.1.7. And on the next day there are 10 infections and nine of them are B.1.1.7. So there, B.1.1.7 obviously grew as a share of infections. It went from 50% to 90%. It had some competitive advantage maybe, but overall infections still went down. And that's because of the other measures that we take to control the spread of SARS-CoV-2, which are largely effective against these more dangerous mutants, we think, as well. So that's vaccination, prior immunity, and then the other control measures we have in place-- so continued use of masking, continued use of social distancing, contact tracing, and other public health measures that are used to control the virus.

All of those measures together, at the moment, appear to be driving cases down in spite of these dangerous mutations. But the mutations are concerning because they represent new opportunities for the virus to break through the control measures that we are putting in place that appear to be successfully driving cases down now.

Can the variants produce a false negative? Do reagents need to be specific to search for different variants? That's a great question. I don't know, and I'm sure it depends on the platform. I know early on there were some concerns about the B.1.1.7 variant producing false negatives, but I think those concerns turned out to not really come to fruition, in a good way. I apologize, I'm having trouble phrasing this, but in theory, I assume that the virus could mutate in such a way that they could produce a false negative. But so far as I know, none of the CDC's designated SARS-CoV-2 variant of concern lineages are designated as such because they could produce false negatives. Instead, they're designated as such either because they are thought to be more infectious, like B.1.1.7, or they're thought to be more severe, like B.1.1.7, or because they're thought-- maybe neutralizing antibody is less effective against them, like B.1.351, but thankfully, not B.1.1.7, against which the vaccines are thought to be fully effective.

JASMINE CHAITRAM: Kenny? Sorry, I'm just going to jump in here and suggest that you answer maybe one or two more questions, and then we're going to go to our next speaker so we have enough time for FDA.

DR. KENNETH FEDER: Totally fine. So GISAID shows that B.1.351 variant all along the southeast coast states of the United States. Do I have a comment? I don't know that I have a comment, but I do know that pattern, and we are in that region.

Question about what is the best way to monitor emerging variants-- I don't know that I'm the best person to offer the best way to monitor those, but increased sequencing capacity is obviously an important part of that. And sequencing capacity in the United States has expanded dramatically over the last several months because of new investments from CDC and because of onboarding from clinical laboratories. And it is making a world of difference in terms of our ability to quickly identify new potentially dangerous variants and provide more lead time for vaccine developers and other things to respond to potentially concerning mutations.

Let me-- I have two more questions. Do you want me to take them or should I stop?

JASMINE CHAITRAM: Yeah, go ahead.

DR. KENNETH FEDER: OK. Are all states doing some type of variant studies? I'm in North Carolina, and a coworker had COVID about six months ago and his first COVID shot about 10 days after the first shot, ended up with COVID and was very sick. His wife then had both shots, but not as-- got COVID, but on his bed he called the health department and they really didn't seem to care. I would bet he had B.1.351. We don't think that's the case. It's impossible for me to say. You know, I apologize-- you know, when we say some-- yeah, it's impossible for me to say.

The vaccines are not fully effective, and there's still a lot that we don't know about the SARS-CoV-2 virus. On the flip side of that is thankfully the vaccines are extremely effective, although not perfectly effective, at preventing symptomatic infection and then, most importantly, at preventing hospitalization and death. And critically-- and this will probably the point that I want to end on-- the vaccines remain extremely effective, particularly in preventing hospitalization and death against all of the mutations that we've identified so far. So there's no mutation here that we think provides full or a substantial amount of an escape from vaccination. And so high levels of vaccine coverage remain among, if not the most important, tool to getting out of the pandemic and reaching a manageable and safe state.

And so we're focused on this B.1.351 investigation here because we think, OK, well maybe there's some reduced protection from acquired immunity. And so if we can, we're going to try to control this as much as we can through other public health measures because it's starting down the line of being concerning and the vaccines possibly being less effective-- we're not sure. But they're still very effective, and they're still the linchpin of our campaign. And as somebody previously pointed out, cases have been falling in Maryland for the last couple of weeks despite the fact that other variants have proliferated. So I find that encouraging, to end on an encouraging note.

JASMINE CHAITRAM: All right, well, thank you so much, Kenny, for being with us today. And I apologize for all the technical difficulties, and having you do all the extra work at reading your own questions, but I do appreciate you answering so many of those. I think folks appreciate that, too. We're going to go to our next speaker and last speaker for today. And Tim, I'm going to suggest that you introduce yourself, because I'm still not sure folks can hear me.

DR. TIM STENZEL: Will do. Thank you, Jasmine. Jasmine wanted to introduce me. I'm Tim Stenzel. I work at the FDA, and I run the office at the FDA that reviews the SARS-CoV-2 tests, molecular antigen and serology tests. And Jasmine and the CDC wanted me to cover a new amendment, a new regulatory pathway that we announced on April 20, and that pathway is going to allow, we believe, significantly more testing to occur in a shorter amount of time. And that is because it will allow easier validation and authorization for adding pooling and serial testing for asymptomatic carriers. And I'm going to go through those details now. And there may be some questions on this pathway.

First of all, it is what we call an umbrella pathway. So if you think about an umbrella that protects you from rain, this umbrella-- it covers specific areas in defined areas of authorization. And it's because the FDA has generated enough experience looking at data having to do with pooling and asymptomatic testing in order to offer this pathway. It does have limitations, though. Only specific tests will fall under this pathway. So the conditions are laid out at the FDA website for this new pathway. I'll go through the key ones on this call, but it is several pages long, so I can't go through all of the details. But I'll touch on the most important ones.

The other thing is that changes cannot be made by test developers to this pathway. It's sort of a cookie-cutter approach. And we follow this for a number of reasons, but it makes the process much easier to do this. And it ensures a high-quality result by everybody that follows this procedure.
Other pathways are still open to add asymptomatic testing to an authorization and/or pooling. This is just a streamlined pathway for the tests that fall under this. So I'm going through some of these conditions now. First of all, the test must have been previously authorized by the FDA for SARS-CoV-2 testing, and must use, when they pool under this amendment, only an anterior nasal swab specimen from asymptomatic individuals when they test in a serial testing program.

Developers who want to follow this pathway must submit notification to the FDA, which includes any validation data that is required. There is one condition that they can follow where no validation is required. I'll go into that a little bit later. These are only relevant to tests that are authorized for performing in high complexity labs only. The serial testing must be at regular intervals, at least once per week. And we have provided three different options on pooling. You can pool either up to three, or up to five, or up to 10. If you only go up to three, no additional validation is required if you meet the other conditions. And that is because our experience with looking at data from those who pool up to three-- we do not see any significant falloff in performance.

However, we have seen significant falloff in performance for those who try to pull five, and for those who try to pool 10. And so for five and 10, validation is required. We have provided multiple different ways to validate five or 10, depending on what the test will allow. And again, these validation recommendations for this pathway must be followed. Otherwise, another pathway is open to the developers.

And also, the pooling can be done by both media pooling or swab pooling. And recommendations for validation for those different sample types and pooling methods are present in the communication that the

FDA made on April 20 and is posted on our website. Going into a few more details about the requirements for this pathway-- first of all, it must be a reverse transcriptase PCR test. And it must have a sensitivity or a positive agreement of greater than or equal to 95%, based on validation with actual patient specimens. So for example, those tests that were authorized early in the pandemic when samples were not available may not have used actual patient samples because they weren't available. So this pathway is not open to those tests-- only those tests that were validated with actual patient specimens and reach that sensitivity.

Second is that these tests must detect two or more viral targets. It turns out that the vast majority of molecular tests that the FDA has authorized, well over 250-- the vast majority do target two or more viral targets. The obvious reason for this is because of the viral variant mutations that are occurring. We want to make sure that these tests are robust in the face of so many mutations.

Third, there must be a specific extraction method within the test. It can be automated or it can be offline, including chemical lysis, and some sort of solid phase extraction, like with the silica beads. And that's just to ensure that the inhibitors are removed and that there's a concentrating step for the nucleic acid. Fourth, this is available only for tests that have been validated and EUA-authorized to detect only SARS-CoV-2. So any of the panels for other viruses are not allowed this pathway. They want to do that-- there are other pathways to seek authorization for pooling or asymptomatic testing.

Fifth-- there's two additional conditions for this fifth thing, and that is either a validation has not been submitted for pooling to the FDA, or if validation data has been submitted for the test needed to be authorized for pooling. This of course eliminates any tests that submitted data to the FDA for pooling, but the FDA was unable to authorize that test for pooling.

And sixth, the last major condition, is that either validation has not been submitted for asymptomatic individuals, or if such data has been submitted to the FDA, the FDA authorized for asymptomatic screening. And that, again, eliminates any tests that have submitted data prior to this pathway, and since, where the FDA was not able to authorize the asymptomatic claim for that test. So I've taken up some bit of time going through this pathway and hitting on the major issues.

There may be a lot of questions that developers have about this pathway and this amendment. And our templates email address at the FDA is always open for business and is also open for business in addressing any questions regarding this new amendment. And we look forward to any questions that come in and for which we can clarify. But obviously, some things have been left out of this authorization. This is not for antigen tests. This is not for any test that doesn't have RT-PCR, and also for other sample types. So this isn't for saliva, or sputum, or aspirates of any sort, or oropharyngeal swabs, or even nasopharyngeal swabs.

This is designed as a streamlined way to increase testing capacity in the United States, testing capacity and authorization to help with reopening schools and workplaces and other places and make it easier for more tests to get pooling, asymptomatic claim for the use of serial testing. And I think that ends my

prepared remarks. I will take a look at the Q&A to see if there's any questions regarding this new pathway, and I'll read them out and attempt to answer them.

The first question is, are there any swab pooling assays that currently have an EUA? Not to my knowledge. We certainly have had a number of discussions with developers who are interested in swab pooling, and we have provided recommendations prior to this new pathway on how to validate swab pooling. Swab pooling has different considerations than media pooling. Actually it has some benefits, but also some new risks. The benefit of swab pooling is there's less of a drop-off, if any, in the signal due to dilution of the sample. If you're pooling media, you're pulling liquid, and you dilute each sample. With swab pooling, you're not doing that, but you could accumulate more inhibitors in swab pooling than you do in media pooling. And so we have specific recommendations how to eliminate the fact that there are inhibitors that happen with this.

The other thing is if you happen by chance to pool a number of swabs that have very high positives, you could actually overwhelm the reaction, or the reaction could go too fast, and some systems may not actually detect an extremely high positive. So we have a method in the validation recommendations to test for that to make sure that's not happening. The downside of swab pooling, and it's the reason why most developers have not gone this route, is that it is difficult to de-convolute the results of swab pooling, unless you take two swabs from each patient. And then there are all sorts of logistical issues with that, including supply issues for the swabs.

So typically when swab pooling is entertained, it would be in this manner. You would collect swabs from each pool, you would pool them, you would test them. If the swab pool is negative, then there's no problem. You can let those know who are tested that they tested negative. If there is a positive result, for example, though, those individuals will either be re-swabbed by whoever's doing the original testing, or they'll be asked to go to get another test. Hopefully, the number of positive pools is very low, and this is not a big issue. But those are the upsides and downsides of swab pooling.

Let's see. So there is a question about whether this applies to independent labs. So if those labs previously had an EUA authorization, this would be open to them. There's a question about seeing rapid tests for home, and a question about pricing. This is a little bit off topic, but it is a very frequent topic, and the FDA does not have the authority to set pricing. We live in a free market enterprise system, and that is set by the test developer and the FDA has no authority over that. What we can do is try to authorize tests that are lower cost to manufacture, which there's no prohibition against that. And so we like to authorize tests that can perhaps be used in the home and can be very inexpensive because the cost of developing them is very expensive. But we also cannot compel developers to develop anything.

The only thing we control is if they develop something and send it to us, we look at the quality of the testing. And if the quality of the testing is sufficient, then we can authorize it. And we hope that the FDA seal of approval in these cases gives reassurance to not just the labs, and clinics and health care centers and employment centers and education centers have confidence in the test for their purposes. We also monitor all these tests after they're launched to make sure they continue to be safe.

There are studies and home tests in North Carolina and Tennessee. We're well aware of those studies that are ongoing. Once those studies are complete, the study organizers have assured the FDA that they will make this data public as soon as possible.

This only applies to labs that are already doing swab pooling? No, this applies to any previously authorized RT-PCR test that meets the condition and wants to add pooling or wants to add asymptomatic testing. If they're already doing pooling and don't have an asymptomatic claim, they can follow this. If they have an asymptomatic claim and they want to do pooling, they can follow this amendment as well.
I'm trying to-- there's a few changes to the chat Q&A, so I'm not sure which questions have not been answered already. But again, the name of this new pathway-- and perhaps I can finish there-- is Pooling and Serial Testing Amendment for Certain Molecular Diagnostic Tests. Again, that's Pooling and Serial Testing Amendment for Certain Molecular Diagnostic Tests. And that is at the IVD EUA authorization page at the FDA website. And I'll turn it back over to you, Jasmine.

JASMINE CHAITRAM: Thank you, Tim. Really appreciate the help there, the assist with trying to get through the call. And thank you again for being on the call with us today. We appreciate your time. I want to apologize to everyone out there that had technical difficulties, but we think it might be if you used the app. There was some kind of sound issue. Unfortunately, that's out of our control. But we do record all of these calls, and the audio and the transcript will be posted usually about a week after the call. So if you did miss the portion of the call, you can always go there to hear the information.

Just a couple of reminders that I had given at the beginning that you may not have heard because of the audio issues-- our next call will be on Monday, May 17, and so please join us for that. Our speaker Amy Kirby was unable to join us today-- internet connection problems because of storms that came through Atlanta. We will schedule her for another time on a future call, so look for that agenda item again in the future. And I think that was all I wanted to mention. Again, so sorry about the issues today. Thank you for joining us and stay safe.