Agenda
Clinical Laboratory COVID-19 Response Call
Monday, February 7, 2022 at 3:00 PM ET

- **Welcome**
  - Jasmine Chaitram, CDC Division of Laboratory Systems (DLS)
- **How It Works: COVID-19 Sequencing From Patient Swab to Variant Classification**
  - Justin Lee, CDC Laboratory and Testing Task Force
- **Increasing Community Access to Testing (ICATT) Pharmacy Expansion Update**
  - Daniel Parker, CDC Expansion of Screening and Diagnostics Task Force
- **FDA Update**
  - Tim Stenzel, US Food and Drug Administration (FDA)

JASMINE CHAITRAM: Hello, everyone, and thank you for joining the Clinical Laboratory COVID-19 Response Call. I'm Jasmine Chaitram. I am the Associate Director for Preparedness in the Division of Laboratory Systems, and we're glad that you're here with us today.

On screen, I'm showing the agenda, and before we get into our topics, I will go through a few updates and some housekeeping and some information about the division which I normally cover. So first, the Division of Laboratory Systems has been hosting these calls since 2020, I can't believe we're almost at two years, and we have a primary focus to support clinical and public health laboratories around the US, and we're supporting them in several areas.

And we were doing this before the COVID response, and we'll continue to do this throughout, during, and after. And this is in the area of quality, workforce development training, preparedness, and safe laboratories. And this is something that's part of these calls directly support, is the preparedness and response piece. We also work in informatics and data science area, making laboratory data accessible to others that need it.

The CDC Preparedness Portal is hosted on the division's website, and it is a one-stop-shop for a lot of the preparedness activities that we do and also helps to connect you to the COVID-19 response web pages that CDC manages. And also, on our page, we have information about these calls. We have archived the transcripts, the slides, the audio from all the previous calls that we've hosted, so if you ever miss a call, you can always go to this web page to get the information. It usually takes us about two weeks to post our slides and transcripts, so please be patient with us. But we will get them up there.

Also archived on this website are the Laboratory Outreach Communication System messages that we have sent out. All of our emails that we've been sending since the beginning of the COVID response can be found there, and I think that's everything I want to mention about this page. Let's see.

We also have a new web page, and this is the Increased Community Access to Testing-- ICATT-- program that CDC is helping to manage. This website is new. We just launched it last week.

You've heard about the ICATT program before on these calls, and we actually have an update today on the ICATT program. And basically, this program is the one that you'll be hearing about today. There are different aspects of ICATT, but the one that you'll be hearing about today is laboratory testings in pharmacies that have been made available at no cost to communities that have been disproportionately affected by the pandemic.

So, our next call will be on March 7, so that is actually a month from now. We do host these calls every two weeks. The next call that we would have had on February 21, I believe, is going to be a holiday, so we will skip that call and see you on March 7. And as always, we ask if you have any specific training and workforce development needs that you send those to labtrainingneeds@cdc.gov.

And reminder about asking a question-- please submit your questions in the Q&A button in the Zoom feature and do not use the chat. We do try to answer as many questions as we can on these calls, but sometimes, we run out of time. Or we don't have the proper subject matter experts.

If you want us to get back to you at some future time with an answer, please also submit your email address along with your question. The Q&A box also allows us to track questions so that if we don't get them answered, maybe it becomes a topic for a future call, and so we do appreciate you putting your questions in the Q&A box. If you're with the media, please direct your questions at media@cdc.gov, and if you're a patient, you need to be talking to your health care provider.

Reminder about the presentations for normally that we have on these calls-- I don't think it's an issue today, but sometimes, we do have speakers that are not affiliated with CDC and just a reminder that they don't necessarily reflect CDC's official position. And that goes for the presentations that have occurred in the past that we've posted to our preparedness portal.

And with that, I think we're ready for our first speaker. We've had many updates from the CDC Laboratory and Testing Task Force on variants and lots of numbers, and today, Justin Lee from the Laboratory and Testing Task Force is going to talk about how the COVID-19 sequencing works from when the patient specimen is collected up to the variant classification. Justin?

JUSTIN LEE: Hello, hello. Can you hear me?

JASMINE CHAITRAM: Yes, I can. Thank you.

JUSTIN LEE: Great. Thanks, everybody. I appreciate the opportunity to be here today. As Jasmine mentioned, it's my understanding that on these calls there's been several sequencing surveillance updates, but a lot of questions have come after those about how the sequencing process works as specifically relating to the classification taxonomically as variants of concern. So that's going to be the intent of the talk today, is just to walk some of you through how sequencing works and how we get to a variant call.

And I know that there's a range of experience, and probably, a lot of you have some experience with sequencing or maybe a lot. And a lot of you may not, so I'm going to try to hit the middle and make sure that everybody can follow along. Next slide, please.

So, there's been a lot of advances in the technology that underlies sequencing over the last 10 or so years, and what that means is that it's easier than ever to generate and analyze sequence data. And that's a really good thing. Fortunately, what this means is that there's a lot of applications for this in biomedical research and specific to this call in public health and that it's become more accessible to more and more groups. Next slide.

The difficulty comes in that just because it's easy to generate sequence data, and it's easy, well, easier and easier to analyze the data, that doesn't necessarily mean that you're always going to get high-quality results out the other end. And so, I like to emphasize the point that generating good, high-quality sequence data that is informative at the level of public health can still be relatively challenging. Next slide, please.

So, I put this slide in here to try to hit home the point that really sequencing workflows, as far as how samples move and how data moves, they're no different than other laboratory workflows that many people on this call will be familiar with. At a really high level, what we're doing is receiving and accessioning samples, doing some preprocessing such as RNA extraction, and then doing some series of biochemical tests or molecular biology tests on them or assays to convert them into a form that will go on to a next-gen sequencing instrument and generate the data that we're going to use in our downstream analyses. And so really, there's a lot of underlying principles from things like real-time PCR or conventional PCR that are mirrored here and really have been used to form the backbone of how sequencing workflows work.

And once the data is generated, we do the same process as would be done in any other diagnostic lab. We track everything from start to finish in a LIMS system. We can then combine the sequencing data with the metadata about the specimens and perform some analysis, which we typically refer to as a bioinformatics pipeline to ultimately end on something that we can deposit into public repositories for nationwide surveillance efforts. So, throughout this talk, we'll be going over each of these points in a little bit more detail. Next slide, please.

OK, so as I mentioned, a lot of the science and the laboratory work that goes into a sequencing workflow is very similar to diagnostics, especially here with PCR. So, for most of the SARS-CoV-2 library preparation or sample preparation methods that are used to generate sequencing-ready samples, many of them rely on a multiplex PCR, so it's not that different than the diagnostic tests that a lot of people are running for COVID. The complexity is different, and some of the details are different. But ultimately, it's the same process.

And so, the purpose of the sample preparation, which we call library preparation when we're dealing with sequencing, is to use a multiplex PCR to amplify the full genome of the SARS-CoV-2 virus, and then

beyond that, the most important step is to convert those molecules after the PCR into a form that will be accepted and read by a sequencing instrument. And so, what we do is we just attach adapters to the ends of each molecule that come out of the multiplex PCR reaction, and those do two things. They make those molecules compatible with sequencing instruments, and they also attach a unique identifier onto each sample in the workflow so that we can pull samples together, generate data, and then separate the data back out at the end based on which sample it originated from.

So, in other words, we can prepare 96 samples on a plate, and each sample gets a unique identifier. It's a molecular sequence that gets added to the end of the molecules, and then when we pull those 96 samples together and sequence them, then we can tell which data point came from which sample because each one has a unique identifier in it. Next slide, please.

So, the reality is obviously a little bit more complex than that schematic on the left. The schematic that we use at the CDC right now for sequencing relies on about 300 primer pairs in a single tube, so it's a massively multiplexed PCR reaction. But again, it needs to amplify 30,000 base pairs of the virus genome and do so in a format that we can then put onto a sequencer.

And so I often use this analogy-- you'll see in the upper right there-- of glitter because it's kind of molecular glitter that we're generating at this point, where we're doing all of these short amplicon reactions generated by PCR in wells of a 96- or 384-well plate that are right next to each other, and it's at this point that the risk for contamination from sample to sample is really high. That's where a lot of labs get off track. OK, next slide, please.

All right, so there's a lot of information out there about how sequencing actually works on the instrument. I'm going to paste some links here in the chat (https://www.youtube.com/watch?v=fCd6B5HRaZ8; https://nanoporetech.com/how-it-works; https://www.pacb.com/smrt-science/smrt-sequencing) I would recommend everybody just take a few minutes to watch a couple of these videos that I just put there. They're fascinating, and oftentimes, it seems like science fiction. And it doesn't even seem like it should really work, but it's very precise and very sensitive and specific. And it's just an amazing resource that we have.

But the idea behind all of them is pretty much the same. We can pool a lot of samples into a single tube. Again, we've indexed them, so we know which data are going to go back to which sample. And then we're going to put the material from that, the libraries onto a flow cell or something analogous to a flow cell, and it's called a flow cell simply because the liquid flows in one side through it and then out the other side.

And in the process, it's going to go on an instrument that's going to generate the sequence data, and in most cases, sequence data boils down to, as I've depicted here on the right, an image from Illumina sequencing. It's kind of similar to a Sanger sequencing reaction in that you're going to add one base at a time over the course of multiple cycles, and each base has a characteristic color that's going to appear and be detected by a camera. And so really, what the most simple form of data from a sequencing run is just camera images of the presence or absence of each of the four different bases at each position.

And I'd be happy to discuss that more, but it takes a little time to go into more detail. So, I thought I'd just skip over this part for today. OK, next slide, please.

OK, so we've done the laboratory work and made libraries. We've put them onto a sequencing instrument, and now the question is, what do we do with all that molecular glitter that I was talking about? So, most of these workflows for the analysis start with a reference sequence, so that's this sequence here. I realize the bases are a little bit small, but each of the string of nucleotides As, Cs, Gs, and Ts at the top, are in four different colors. And that represents the Wuhan reference sequence from the original SARS-CoV-2 virus that emerged at the beginning of the pandemic.

And we use that sort of like the top of the box cover of a puzzle. So, this is the map that we're going to be using to align our data to figure out where it goes and how the sequence from our samples differ from that of this reference sequence. And then I've also highlighted here that we maintain the information about where our primers bind from that original multiplex PCR reaction so that we can see how the data line up relative to the primer-binding sites. Next slide, please.

OK, so now we add in our data, which is that glitter. At this point, the data is called reads, NGS reads is what you'll often hear. And the way to view this is that each row on this figure is one read that originated from one molecule and the original sample or the PCR reaction. So, because we know the sequence of the reference strain, we can take those NGS reads and map them to the correct location on that reference sequence, so in this way, we're starting to build our body of evidence about what kind of virus we're sequencing. Next slide, please.

Now if you let your eye do a little bit of work here, you'll see that there are two different types of colors throughout the NGS reads. In this case, anything that's white matches to the reference sequence, so it's identical. Anything that is red, green, yellow, or blue differs from the reference sequence, and there are two different forms of that.

The first I've highlighted here is due to sequencing errors. So, you can see that they're pretty rare. They're randomly occurring. They occur about 1 in every 1,000 bases or so for most of the data that we look at, but they're kind of just sprinkled throughout. And because each base in the genome has been sequenced so many times, it's pretty easy to pick them apart from the real data, the correct data. Next slide, please.

And then there's this other string here, which is one position in the reference genome that was originally a G, and now it is being called as a T in almost all of the next-gen sequencing reads that came from this sample. And so, this is a mutation. This is where the sample that we're sequencing differs from the sample that we're comparing it to or the original reference sequence, and I hope that you can appreciate that in most cases, when the data are this clean, they're pretty easy to distinguish between sequencing errors and actual mutation in the sample. OK, next slide, please.

So, from all of that, what we do is we collapse down the sequencing data into what we call a consensus sequence. So, this is going to be one string of nucleotides that represent the most common base calls at each position in the sample that we're sequencing, and so I've just put boxes around about six bases each and shown you what those sequences will ultimately be called. And in some cases, there might be a couple of different bases present in the NGS data, but we're calling the most common base at each position. And I've underlined that T in the upper left where it was originally a G, but the mutation has converted it over to a T.

And then next slide, the last thing to do before we go on and start to taxonomically classify this sample is to convert it into an amino acid sequence, so we translate the nucleotide to amino acid. And amino acids are what is most commonly used for SARS-CoV-2 taxonomy mostly because it's the most evolutionarily informative. And in this case, the mutation of a G to a T converted the valine amino acid to a phenylalanine. OK, next slide.

OK, so we've now got a single consensus sequence that is present in both nucleotide and amino acid form, and we've noted a mutation that has changed the amino acid sequence. So, what do we do next? So, there are a couple of different organizations that have come up with taxonomic classification schemes for this virus. One of them commonly used is Pangolin. You'll hear that a lot, and another one is called Nextstrain or Nextclade. There are several others, but those are the two most commonly used.

And so really, once we have a string of amino acid sequences, the next step is just to compare it to all the other sequences that are out there and identify what it's most closely related to and where it might differ. And so, if you'll hit the next slide, the way that this typically works is we want to ultimately get down to what we would call a viral lineage, which is just a branch on the tree that has neighboring related viruses on it. Again, we're comparing this at the amino acid level.

And so, if you look down at the bottom here, this is a depiction from a website called outbreak.info where it's got-- this is the spike protein, and all of the mutations are amino acid changes that are present in these variants of concern here relative to the reference sequence in Wuhan. And so, each one of those reads is a little designator. For instance, at the end on the right in the red box, we have V1176F, and that is the mutation that we depicted in the previous slides when we had a G to a T mutation that resulted in a valine to a phenylalanine amino acid change.

And so, what that is it's the original amino acid or valine, the location within the spike protein, which was 1176, and then the phenylalanine, which is the resulting amino acid call after the mutation. And so, we can look across the different variants and the taxonomic classification and figure out what this is the most closely related to, and that's how the classification of variants and variants of concern works. OK, I think I'll go on to the next slide.

After that, we have thousands of samples being sequenced by lots of labs across the country, and ultimately, the work goes into the national surveillance program that you've already heard about. And it moves from an individual level to a population level, and it becomes summed and summarized for the

metrics that we put out on the CDC's variant tracker. So, I think I'll go to the next slide and just remind everybody that now what looked like this at the beginning-- if you can go to the next slide, hopefully, these images-- next slide, please.

Hopefully, these images would make sense to everybody here now. So, we go from our swabs through our multiplex PCR and library preparation reaction. We put them on a sequencer and generate data that ultimately feeds into a mapping algorithm where we map the reads back to the reference sequence and identify mutations. We figure out what those mutations are related to, and then we can come up with our strain surveillance metrics.

And I think that's my last slide, so if you go to the next slide, I'd just like to acknowledge all the people that I work with on these projects. And if there's time-- I don't know if we're doing questions now or at the end, but I'm happy to carry on this conversation. Thank you.

JASMINE CHAITRAM: Hey, Justin. Thank you so much. That was an excellent presentation. We do have a couple of questions that I wanted to ask you. The first one is, are you-- and that means you, CDC-- working on a CLIA whole-genome sequencing protocol to report the variant for diagnostic purposes?

JUSTIN LEE: We have done that. It's not currently an active protocol. We did some work to validate something like that earlier, I guess, last year, and there are other groups that are working on it as well. And we decided to put it on pause for right now to make sure that we were complying with the FDA rules around LDTs and EUAs and make sure that we have all the right documentation in place, but that is something that we have worked on in the past and we would potentially consider bringing back to a functioning state.

JASMINE CHAITRAM: Thank you. Another similar question is our providers have asked me repeatedly if there's a way to find out if their COVID-positive patients have Omicron or any other variant. What I've discovered is that even if a specimen is sent off to a lab that sequences for the specific variant, there is no way for our providers to get that information. So, I guess, what is the reason that we're not giving out specific information for patients when sequencing is being done? And I can also add to that, but I'll let you take it first.

JUSTIN LEE: OK, yeah, I'll just do a real quick start. Again, this kind of goes back to the first question, also. Any test that was going to provide results back to a patient of informed care would need to be done under CLIA regulations and also under FDA EUA approval. And we checked the first box of that last year, but we never got around-- we didn't make it through the second process yet.

And so, I think that there are a lot of potential use cases for having those data inform clinical decision making. I think one hard thing that has limited its usability in the past is the turnaround time that's often required to get sequencing results and the time to action that's required in a clinical setting. And so, this is something that we've talked a lot about, and we continue to try to figure out the best way forward.

JASMINE CHAITRAM: Thank you, and I agree that other laboratories also are in the same boat that are doing sequencing. So, another question, and there's two that are related. So, I'll read them both. Do you sequence all positive COVID tests results-- I guess, samples from positive COVID tests-- and is the data representative of actual positivity in the US? That is, is it a sample, or are you sequencing all of the samples received at all labs?

JUSTIN LEE: That's a great question. So, my lab does the sequencing efforts for the CDC currently, for the surveillance efforts, and we are part of a large program called the National SARS-CoV-2 Strain Surveillance Effort, which has multiple prongs, including commercial laboratories, universities, state public health labs, importantly. And everybody is doing sequencing efforts, and all of those data go into the same bin for this program. And so, the answer is, no, we're not sequencing all positive specimens, and I don't think that there would be great returns on the amount of effort that that would take.

There's some discussion about what an ideal number is for surveillance. Some people throw around a number like 5% of samples. Some people throw around numbers like 300 samples per week per jurisdiction. It really depends on the goals of the surveillance effort and whether it's to catch brand new emerging variants that could potentially become variants of concern or whether it's to track the circulation and changes in genetic diversity over time of existing variants. But I think that for the time being, the sequencing surveillance efforts that we're trying to shepherd here at the CDC are going to continue as is, which is a multipronged effort with a lot of collaborators involved, which is really underpinned by the state public health labs.

JASMINE CHAITRAM: Thank you, and you kind of already mentioned this but, I guess, clarification about who is doing this sequencing procedures. I know you mentioned public health labs, but are hospitals also performing these procedures?

JUSTIN LEE: Yeah, there are some hospital labs that are doing sequencing, for sure, especially a lot of those affiliated with academic teaching and education programs. But I think that that makes up a relatively small percentage. The majority is through some federally funded contract sequencing efforts and state public health labs.

JASMINE CHAITRAM: Great, and then how does the evolution of the COVID-19 variants compare with other pandemics? Are they typical or without precedent?

JUSTIN LEE: I think that most people would say the types of evolution we've been seeing so far are fairly typical for an RNA virus that's transmitted by respiratory and has infected this many people. If you think about it, each infection is an opportunity for the virus to mutate and try something a little bit different. Then those things that infer any kind of fitness advantage can be propagated throughout the population, so I don't think that there's been any major surprises to most people. If anything, probably the only surprise is that it took so long for these variants of concern to start popping up the way they did.

JASMINE CHAITRAM: Thank you. Somebody's making a comment about the amount of paperwork to submit samples to their state health department for sequencing, and I don't think this is something CDC can necessarily solve. I think it's, unfortunately, individuals, laboratories, facilities are going to have to work through their state health departments to figure out a good process, so apologies for that.

I don't see any other questions for you at this time, Justin. I want to thank you again for taking the time to put this presentation together. It's really interesting, and I think a lot of folks really enjoyed it. So thank you.

JUSTIN LEE: Well, yeah, and I know it's a lot to cover in a short amount of time. But if people have additional questions, please reach out to the meeting organizers, and I'd be happy to try to clarify anything.

JASMINE CHAITRAM: OK, great. Thank you. We are going to go to our next speaker. I'm moving my slides forward to catch up.

All right, and as I mentioned, we're going to have somebody talk about the Increased Community Access to Testing-- ICATT-- program, specifically the pharmacy expansion. And so I invite Daniel Parker to go ahead.

DANIEL PARKER: Hey. Thanks, everybody. I appreciate the opportunity to speak with you guys today. So, I'm one of the pharmacy leads, particularly the expansion lead for the Increasing Community to Access Testing team here at CDC, which is part of the COVID-19 response for the agency. Next slide, please.

So, to just give you a little bit of background, our team is-- the name really says it all. Our goal is to increase community access to testing, and to be honest, a way of saying it is our goal is to make testing ubiquitous across the country, as many sites, as much applicability as we can. And so currently, for the past number of months, we've partnered with pharmacies, particularly large pharmacies-- so think Rite Aids, CVS, and Walgreens-- to provide no-cost testing to communities across the country. One of the mandates that we have is to do that particularly not limited to but in particular to give particular focus to those areas that are disproportionately affected, so this includes racial and ethnic minority areas as well as, based on census tracts, areas that have a high rating for the Social Vulnerability Index.

And so, to date, we've got about 10,500 partners or so pharmacy testing sites across the country, over 30 million tests that we've resulted during the time of our program. And from a presidential mandate, our goal now is to take from 10,000 sites and double it up to 20,000 testing sites across the country. And so, we say pharmacy testing, but it's really not limited to that. It can be pharmacy partners, retail partners, and I'll speak a little bit more about this in a minute. But it can really be focused in just about any retail partner that you can think of.

And so, in addition to the three pharmacy partners that I mentioned, there's another company called eTrueNorth, and they held a considerable space in the pharmacy area prior to COVID-19 and then obviously a bigger area since then. But we're able to partner with them to reach independent pharmacies and to subcontract to other retail partners for ICATT testing. Next slide, please.

So just to give you a little bit more background on eTrueNorth, so again, their ability to subcontract with almost anybody, any retail partner, independent pharmacy, and more than that-- they are a huge focus of our expansion efforts to get to that 20,000 goal, which we hope to reach by the end of March, I should say, which is why we're talking to you guys today, to kind of spread the word and get awareness out there. And so, what they have developed is a host of programs, testing models, and I'll speak about that here in just a minute.

But they really kind of have a bit of a turnkey process for what they've developed, and so they're able to do everything from staff training for the sites that they partner with. They manage all of the back-end IT resources for patient registration, scheduling, and that also includes the testing results and how they get back. They manage all of the shipping components for the kits themselves that get sent out that aren't POC tested there on site.

And so again, it's a turnkey process. They manage from start to finish the entire process, and so between testing options and models, they really have kind of a suite of options that they can tailor to whatever the site need is. And so that can include laboratory-based NAAT tests with either observed or unobserved self-collection options, point-of-care testing so the patients get the result there on site. Those are options, and they've even got one more. It's a third option that I'll discuss in the next slide that involves a home collection model, which is where we're really interested in terms of expansion.

But all of these options are kind of tailored to whatever the site needs are for the individual either pharmacy or retail partner. So, if they have a drive-through, they can have a few different options. If they don't have a drive-through, there are still options. Are there technical staff that can assist with the swabs and give instructions, or does a particular site not have technical staff? Either way, there are testing options available to kind of accommodate whatever those site needs are. Next slide, please.

So, this graphic here at the top kind of explains that just a little bit more detail. So again, if there's a drive-through model, we can potentially partner with eTrueNorth to have POC testing that gets processed on site or have some type of observed self-collection through the drive-through that can then be transported through FedEx or UPS, some type of courier, to attest for resulting. The third option is, I think, really a very clever way of doing this, and this is a brand-new option that they've just started rolling out within about the past three weeks once the EUA was approved to do this.

But the way the model works is a patient can essentially conduct unobserved self-collection and then drop off the sample at the store, and the way it works is there's a website that the patient can go to within about three to five minutes, register, and select what store they would like to receive a test kit from. And the other option is to go to a store, the physical location, and with the signage that's in place scan a QR

code and again, within three to five minutes, complete the registration process and get a voucher on their phone, for example, show it to the clerk there at the store counter, and receive a test no charge. And they can go out to their car in the parking lot, for example, and do the swab, the self-swab at that point, or potentially take it home whenever they're ready to do the swab, and there's very clear instructions for both how to do the test as well as how to package up the sample to return it to the store.

And then once a day, the store collects all the samples with prepackaged boxes and shipping labels to return through FedEx, and so it's really a very simple, very clear and accessible way that either patients can go get a test. For example, do they just need a test to return to work or some other means like that? Or if they're symptomatic, they can ask a friend or family member to take their voucher to the store and collect a test on their behalf.

And so, a very clear, simple model that is, I think, of particular interest to a lot of stores because one of the consistent things that we hear is, we'd love to increase testing, but we just don't have the staff to manage it. And so, this particular model requires very little staff involvement and is really easy. All the materials for promoting it, for signage, things like that are all provided by eTrueNorth, and the partnering either retail establishment or pharmacy gets a reimbursement for every test that's resulted. So, there's a little bit of financial incentive in there for sellers to participate. Next slide.

And so again, our goal is to get as many of these sites online as possible just to make sure that we have tests available in the hands of people across the country if they want one. And so again, there's an email address listed here that if you guys have questions, you can submit any time, and we'd be happy to help answer. So, I would encourage you guys to think through partners. If you think this is something that you may know of particular stores or particular geographic locations that could benefit from this type of testing model, then we'd certainly love to hear about it and love to work with these folks to do.

Again, so the way we think about these things, we look for groups or areas that are relatively high to high or just have 0.5 values on the Social Vulnerability Index and higher. There's a number of other factors that we look at in terms of testing demand, community transmission, vaccine coverage, just a host of factors that we look at from a data perspective in trying to select sites. Again, it's not limited to these things, but those are certainly high-focus areas for us from a testing perspective. And again, there's an email address here (eocevent586@cdc.gov), and I'd certainly be happy to take any questions that you guys may have. Thank you.

JASMINE CHAITRAM: Thanks very much, Daniel. One question is about home health agencies, and could they be included in the model?

DANIEL PARKER: I think that's a great question. I think if they have the ability to partner in a subcontracting way with eTrueNorth, then potentially, I think the vouchers require that they be tied to a specific person. And so that's a potential model that could work. I think there's ways that we could make that work. We probably need some further discussions on it, but I do think there's potential, yes.

JASMINE CHAITRAM: Yeah, they may be able to help with specimen collection or getting shipping of specimens for individuals that might be homebound or something like that.

DANIEL PARKER: Absolutely, yeah. We'd love to engage in that conversation and see if we could do it, yeah.

JASMINE CHAITRAM: OK, and then can you make a comment about turnaround time? There's a comment in the Q&A box about going to a pharmacy and asking questions about this testing, and the turnaround time was like three to five days or up to seven days if it was a weekend. And I'm not sure-- pharmacies offer a lot of different testing, and some may not be participating in the ICATT. But can you just talk in general about turnaround times that you guys are observing in this particular program?

DANIEL PARKER: Sure, absolutely. Yeah, turnaround time is a big focus. I feel like I-- sleep turnaround time sometimes.

This particular program, I think, the data that I saw late last week was an average turnaround of 1.25 days for this particular program. Part of that is location or geographically dependent because of the availability of FedEx shipments, how quickly samples can get to the lab, and so that 1.25 time is an average time across that location-specific data. But the weekend is a factor that we have to be concerned with, and so for example, FedEx doesn't pick up on Sundays anywhere. And they're limited to times that are, I think, maybe up until 1:00 PM on Saturdays.

And so, weekend testing is a factor in this, and so there's a handful of stores that are already on board with this particular program that choose not to test on a Sunday, for example, because they don't want to risk losing the viability of a specimen. So, there are some factors like that that are concerns, and eTrueNorth can work through those factors to help assess what is best for a particular store or retail location. But generally speaking, we're seeing 24- to 48-hour turnaround times.

JASMINE CHAITRAM: Thank you. I'm going to put a whole bunch of questions together. So, the first part of it is, which labs do the actual testing? Is there a cost for pharmacies that are participating in the program? And is this only for pharmacies, or can schools participate?

DANIEL PARKER: Yeah, so there is not a cost for the pharmacies that participate or retail establishments to participate. In fact, they receive a reimbursement per test that is resulted, so there's financial incentive for them as well. Schools is a bit of a different conversation. So, there's another team at CDC called Operation Expanded Testing-- OpET-- that manages school testing, and so right now, this particular model isn't geared towards schools. There are some overlaps and some potential there for the future, but that is a different group at CDC that manages school testing right now.

JASMINE CHAITRAM: Thank you. Are you looking for additional laboratories to participate in this program and maybe help lower turnaround times?

DANIEL PARKER: So not at the moment, I think. I say that from my perspective. eTrueNorth is always looking at opportunities there, and that's a question that's probably better geared for them as the need arises for specific areas. They are somewhat limited in terms of the number of labs that they can work with based on what they were approved for in terms of the EUA from FDA, and so there are some limitations there. But generally speaking, the data that we've seen from them for turnaround times is on par with all of the major retailers and labs that we've seen.

JASMINE CHAITRAM: Thanks. And then again, two questions that are kind of related-- the first is a comment question. So effectively accessing rural underserved areas may need a single independent pharmacy, not a large chain, because those are the ones that are providing care within a 10-mile radius in those communities. And then is there any specific efforts to cross-reference the SIC with limited English proficiency data to serve mid- to small-sized pharmacies that reach the underserved minority population? So really getting to the minority populations and rural communities-- how are we doing that?

DANIEL PARKER: Sure, that's a great question. So, the materials that are given out by eTrueNorth are given out in both English and Spanish to try and help with any language barriers that exist on that front, and we do have the ability through eTrueNorth to partner individual, independent pharmacies that could be in rural areas. We do some of that, just for example, in northeast Oklahoma and some other areas that are more rural. And from a big-picture standpoint, our goal to get to 20,000-- so we'd love to sign on as many big-banner associations as we can just to try and have as much reach as possible. But we certainly both have and do continue to have the ability to sign on individual stores as well.

JASMINE CHAITRAM: OK, and the next question might be better answered by the FDA SME that's on the phone, Tim Stenzel, but I'll read it out in case you want to take a stab at it. It says, aren't the tests that are based on unobserved self-collect and shipped via FedEx required to have an EUA for those processes?

DANIEL PARKER: Yeah, they are required to have an EUA for that process, but I'll leave any conversations on that for FDA.

JASMINE CHAITRAM: Yeah, Tim, do you want to weigh in on that one?

TIM STENZEL: Yeah, I think there was a home collection, self-collection. So, there are many such collection kits that have been authorized. So yes, the Home Collection does require EUA.

JASMINE CHAITRAM: OK, thank you. OK, Daniel, thank you so much for joining us. I hope you get some good feedback to that email address that you provided.

DANIEL PARKER: Thank you.

JASMINE CHAITRAM: All right. Next, we're going to go to Tim for our FDA update. Tim, you're on mute if you're talking.

TIM STENZEL: Thanks for the reminder. Double muted, in fact. Yeah, I've got a number of questions here. I think six that I want to go through. The first question is, can FDA address the current recommendations regarding the use of SARS-CoV-2 antibody tests-- so these are serology tests-- and whether or not they can be used to assess immunity?

So, the tests are currently authorized to be able to detect immunological response, whether you've developed antibodies to SARS-CoV-2 or not. However, we don't currently recommend that they be used to determine if you have immunity. A positive antibody test does not necessarily mean you are immune or have immunity that will prevent or mitigate a COVID-19 infection.

More research is needed to understand what an antibody test result means and the role of antibody testing in evaluating a person's immunity or protection against COVID-19 and understanding if this test result could be helpful in deciding how to treat that patient. Additionally, SARS-CoV-2 antibodies detected in blood are effectively a part of the immune system, which also includes T-cells and other components of the immune system, so it's kind of an incomplete picture. I'll get into where we're hoping to go, although there are numerous challenges with COVID that make this difficult.

Further, all but one of the currently available antibody tests are qualitative or semi-quantitative. That means there's only one that's truly quantitative, and the difference in test design mean that the different antibody tests may detect different antibodies and different levels of antibodies. So, there's no way for these qualitative or semi-quantitative antibody tests to yield any universal information that will exactly match any other similar antibody tests. So, there's only one test that's really quantitative linked to the international standard and reporting out in international units.

It is really a limited amount of tests that can start to really ask and answer these questions, and this is really important because some of the studies that are being done out there right now are not using the quantitative tests. So, we'll have to try to link the results and outcomes of those studies on banked samples back to the international standard and/or at that time any fully quantitative tests so that we know international units based on the international standard. What is the level that offers various levels of protection, either complete or potentially mitigating outcome?

So, I think I went through that pretty well. So, it's very difficult for even semi-quantitative test because they're not really traceable to that standard. So quantitative tests which are traceable provide the greatest ability to compare results between tests. I think that's probably obvious from others.

And finally, there are neutralizing antibody tests. I think there's two currently authorized, but our knowledge about what neutralizing antibodies mean is limited and in various studies have shown that not everybody necessarily develops neutralizing antibody levels. So, this doesn't mean they're not going to have some protection from COVID.

So, we're in the situation where we're awaiting the data that allow us to make an assessment about how to define immunity using serology tests, and we certainly welcome more neutralizing antibody tests and more fully quantitative antibody tests as well. I'll try to help the situation by having those kinds of assays out there and available for research and then ultimately when correlations are made that we can link them back to either neutralizing antibody detection or international units and fully quantitative tests.

Next question, why is there an age restriction for some tests such as no one younger than age two? So, first thing to say is that any test that's intended for the health-care-provider-collected specimens do not typically include an age restriction. So, health care providers, doctors, other clinicians, nurses have been appropriately trained in sample collection from any age of patients they've seen, and so there is typically no limitation on this. So, this is generally all point-of-care tests, laboratory-based tests that involve collection in a health care setting by a health care worker.

So, unless any specific limitation is provided in those kinds of tests, there is no age restriction. So where age language is silent, the FDA is saying that there is no limitation.

However, the home collection or self-collection situation is different, so tests that are intended for self-collection or collected by a parent or caregiver have been studied in this way. Home collection kits and home tests are all validated with self or caregiver-collected specimens to ensure adequate performance. User studies are also completed to ensure that the lay user can appropriately follow the instructions and safely collect an appropriate sample. These studies have typically been performed in individuals age two and over, including those that are collected by a caregiver-parent, since it is more challenging to collect a sample from a child under two. And so that is sort of the update on that question.

All right, next question is, what platforms PCR or rapid antigen tests are authorized for testing saliva? There's only one antigen test authorized for saliva. It's a central lab test. No point of care and no home tests have been authorized for saliva.

There are many, many PCR tests that have been authorized for saliva, and I did go ahead and put the link in to the FDA EUA authorization page so that you can look to see in the chat. So you can look to see which tests have these authorizations specifically in case you want to know and acquire those tests for your lab in your situation.

But even with PCR tests where there are many authorizations, we have seen performance issues on saliva for sometimes unknown reasons. Antigen tests, those that are point of care and at home so far that we've looked at, have generally had very poor performance with saliva. Could be for the lack of a concentration of the target for those tests, and that could be impacting performance. However, the FDA is open to all these samples, including saliva, and if a developer wants to develop a saliva test, we encourage it. We just want to see that it is performing testing accurately.

Next question is-- this question is related to the continuous lack of supplies reagents in the lab due to shortage or back orders. Has there been any change whereas with FDA stand on using expired reagents other than on the COVID reagents? So the FDA does not recommend using test kits or test components past their authorized expiration date, as the performance of the test will be unknown. The FDA advises that COVID tests should be used according to their authorized labeling, including any potential expiration dating.

The FDA works with manufacturers to extend dating, and we work closely with them for any of those manufacturers that wants to extend it when they have a support for that extension. So they submit data to the FDA. We review it and make sure it is stable up to that date and then allow the test manufacturer to update their labeling. We've also been very flexible that when there's a lot of kits in the field already that are closing in on their expiration date to allow the manufacturer to make users aware that the expiration date has been extended.

We recommend that any lab with expired product reach out to the developer to determine if they have been working with the FDA on extending the expiration date with additional stability data. It may be a situation where you're approaching kits with an expiration date, or you're beyond it. And so it's good to check because the manufacturer may be very close to submitting that data, or it has been submitted to the FDA. And it could very well be that once that expiration dating is updated that your kits can now be considered in date again, so again, reach out to the manufacturers in that situation.

Next question, what is the current data on throat swab sensitivity? So currently, the FDA doesn't have any data yet suggesting that throat swabs are a more accurate or appropriate method for at-home tests or for antigen tests specifically. We haven't authorized-- I think as of a week ago, we hadn't had any submissions in this area. There is some data that's accumulating that suggests that it may not be warranted to use an OP swab, a throat swab even for Omicron, as it may not be helpful enough, and that it does that additional risk, especially if it's self-collected. So, we're not recommending that home tests be used in a manner that utilizes self-collection of oropharyngeal swab.

The FDA will continue to monitor the situation. We look at literature. We also receive data that's not public, and looking at this, there is a recent UCSF paper using an Abbott Binax test that showed that adding an oropharyngeal swab, even when it was health care collected, did not substantially increase the sensitivity. The throat swab alone showed greatly decreased sensitivity, so the authors of that paper did not recommend adding a throat swab. And the FDA has additional nonpublic information that suggests that's probably the case as well.

So, while we remain open to different sample types both for antigen tests and molecular tests, nasal swabs still remain likely the best option. They're easier to obtain. There's a little more ease of use, a little easier on patients. So, I know it's not the easiest for any type of swab.

And so, if developers want to look at these other sample types, go ahead. But I would recommend that they don't just put all their eggs in a non-nasal swab basket but also include nasal swabs along with other

sample types they wish in their test development program. I forgot to mention, there are some molecular-based tests that have the oropharyngeal swab specimen authorized in their kit.

The last question is, there's a SARS test with the statement below, which I'll read, and then I'll ask the question. So there are some SARS tests. These are, by in large, antigen tests, but sometimes, they can be other tests. So, it's important to look at the FDA labeling and the instructions for use that comes with the kit or online at the FDA website.

So, the statement in some tests says negative results should be treated as presumptive, and confirmation with an FDA-authorized molecular test if necessary for patient management may be performed. So, the two questions are, does this require an automatic reflex to confirmatory molecular test for negatives? In general, no, but it depends. I'll go into that a little bit more.

And the second question is that negatives be reported as presumptive negatives? And yes, that's the labeling. The FDA makes that for valid reasons. Typically, these tests are much less sensitive than, say, the standard high-sensitivity central lab molecular test, and a negative may not be truly negative. It may be a false negative.

So, the language is important, and the patients and clinicians who receive the results should know that.

So, the FDA generally includes this language, as I said. Well, actually, let's back up a little bit.

So, in general, there's no perfect test. Even molecular tests can have a false negative. So, a negative result does not rule out COVID-19, is a phrase that is in many of the authorizations the FDA makes right now. And one single test result should not be used as sole basis for treatment or patient management decisions, including infection control decisions.

So always, clinicians, people taking care of patients directly, laboratorians should have in mind that any result for any tests can be false for a given patient. And it's hard it's hard to manage, but it's just wise to do that because there's no test that's perfect. For tests that have been proven to be lower sensitivities-- this includes all antigen tests but some point-of-care molecular tests-- then the FDA has authorized this test to provide only a presumptive negative result, and we believe that's very important to communicate. I think the recent JAMA article that says that patients do very well with a home test scenario where they get a positive result. They know what to do. If they get a negative result, they don't always know what to do despite the fact that it's presumptive negative, so the FDA is looking at messaging here to see if more robust messaging can help out the home users of these tests.

There's a second part to this question, and there is one other situation where I said maybe. Sometimes, there is a requirement to perform a reflex molecular test, but that's very rare. So just watch out for that. Next part of the question is-- and it's another question about a statement in an FDA authorization that says, the performance of this test has not yet been clinically validated for use in patients without signs and symptoms of respiratory infection. So, we have authorized a number of serial testing situations where

the test can claim for a serial screening claim, but we haven't yet evaluated whether that serial testing screening claim is backed up by data. There's always the postmarked commitment in that situation. We're expecting data to be available in the not-too-distant future based on some NIH studies in this area to look at validating this for many of the tests on the market.

So, the question here is, based on the statement below, if physicians order this test for patients without signs and symptoms of respiratory infection, is the laboratory then responsible for validating the use of test in this population? And the short answer is no if the test is authorized and it allows for this and the lab is using it according to the authorization. And that's right almost on time there, Jasmine. That was a lot of information.

JASMINE CHAITRAM: Yes, it was. Thank you so much as always, Tim, for answering those questions on the call and providing all of that information. We appreciate it.

I am going to wrap this up very quickly because we are right at time, maybe even a little bit over, so just want to thank you all again, thank our speakers for great presentations and answering questions. Thank you all for submitting questions, and we hope that you enjoy these calls and that they are useful to you. And we will see you on the next call on March 7.