

Clinical Laboratory COVID-19 Response Call

April 5, 2021

Agenda

- **Welcome**
 - Nancy Anderson, CDC Division of Laboratory Systems (DLS)
- **Emergence of SARS-CoV-2 Variants and Impact on IVD Testing**
 - Richard Creager, NIH RADx Variant Task Force
- **Saliva as a Sample Type for SARS-CoV-2 Detection**
 - Anne Wyllie, Yale University
- **FDA Update**
 - Tim Stenzel, U.S. Food and Drug Administration (FDA)

NANCY ANDERSON: Good afternoon, everybody. I think we're ready to start with today's call. My name is Nancy Anderson. I am a senior advisor for clinical laboratories in CDC's Division of Laboratory Systems Today. I am temporarily taking over for Jasmine Chaitram, who you are used to hearing in this role. She's taking a very well-deserved break. So I will be standing in for her, just for today. On the screen, you'll see the agenda for today's call. We have several topics that are going to be presented as part of the call, and before I start with the agenda, I'm going to cover just a few announcements and some housekeeping items that I know many of you are used to hearing at the start of these calls.

As you may have heard before, or you may know from previous work, we are the CDC division that works to advance laboratory quality and safety, data and biorepository science, and workforce competency across the US clinical laboratory community. We also work closely both with clinical and public health laboratories across the country to support laboratory emergency preparedness and response activities.

Throughout the COVID-19 response, for more than a year now, we've been supporting CDC's Emergency Operations Center by serving as the interface between CDC and the clinical and public health laboratory communities. Some of the tasks, as you've heard about these previous calls, include laboratory biosafety, the regulatory requirements under the Clinical Laboratory Improvement Amendments or CLIA, additional lab quality issues, and the challenges that are associated with implementing laboratory-developed tests.

So these calls are an opportunity for the government agencies to provide you with valuable information for the testing that you're doing. We discuss hot topics, and we also solicit questions from the community about the work the clinical labs are doing in support of the nation's response to the COVID-19 pandemic. Because we do anticipate, and already have, a large number of participants on this call, and we get many questions, we may not be able to directly and immediately answer every issue. However, we do keep note of your questions and feedback, and they're very useful for helping us tailor the content of our future calls.

So, moving on to the housekeeping and reminders page, this talks about a recently published new resource for lab professionals. The [Biological Risk Assessment General Considerations for Laboratories webpage](#), which was just recently launched. From this page you can get basic content, you can see the steps for conducting a risk assessment, and also access a number of relevant resources. So please take advantage, take a look at this page, and share it broadly with your colleagues who may find it useful.

Another page that CDC has recently updated is the [Overview of Testing for SARS-CoV-2 webpage](#). There's new information here, so again, please share this information.

And another page, CDC has now published a new webpage called [Nucleic Acid Amplification Test, or NAATs](#). And here, you can find information on the different types of NAATs that are used to detect SARS-CoV-2.

Also, as a reminder, [CMS recently published a revised FAQ](#) on March 19 that explained how they are temporarily exercising enforcement discretion, under CLIA, to say that labs or facilities can report variant results to public health departments for public health purposes without being CLIA certified, or without conducting that testing in a CLIA certified setting. However, as always, if the results are reported for the purpose of diagnosis, prevention, or treatment of disease of an individual, then the facility does need to be CLIA certified and follow the requirements for the testing.

I also want to give you a little heads up and notification that next week, the Clinical Laboratory Improvement Advisory Committee, which CDC manages on behalf of HHS, will be meeting. A virtual meeting will be available to the public at the website that you see here. One reason that I especially bring this to your attention now is because CLIAC, both at the last meeting in October of 2020, as well as this upcoming meeting, the overall theme of the meeting will be clinical laboratory medicine in the age of COVID-19. So the topics will be relevant to this response and we invite you to join in. The [CLIAC website](#) is available on the slide, and the meeting will be on the 14th and 15th from 11:00 AM to 6:00 PM both days. If you would like to submit a public comment or present a public comment orally during the meeting, you can contact the email address given here (CLIAC@cdc.gov).

One more slide. We'll be sharing slides today. During the call, the speakers will have slides, and we'll post them online along with the audio and transcript by sometime early next week. So you can find all of this information on the DLS Preparedness Portal at the [website listed here](#). This is also where we'll post the slides, audio, and transcript from our previous calls, so you can go back in time and find any of them there.

Just a reminder that we hold these calls every two weeks, and they last for one hour. So the next call after today will be on Monday, April 19th, from 3:00 to 4:00 PM, Eastern Daylight Time. And one more reminder that our training and workforce development branch is always interested to learn more about the education and training gaps that you're experiencing, or training that you need. So we invite you to send your email for this-- your feedback via email at labtrainingneeds@cdc.gov.

How to ask a question - so if you have a clinical laboratory related question you'd like our team to address on a future clinical laboratory COVID-19 response call, you can use the question and answer function, Q&A function, in Zoom during the call, or send an email to dlsinquiries@cdc.gov.

If you submit a question using the Q&A function in Zoom or email, please include your email address so that we can get back to you. If you are a member of the media and have questions, please contact CDC Media Relations at media@cdc.gov. And if you are a patient, please direct any questions to your health care provider.

I'd like to remind everybody that the presentation, the slide deck for the presentations today may contain presentation materials from panelists who are not affiliated with CDC. So the presentation content from the external panelists may not necessarily reflect CDC's official position. Please keep that in mind when you go back and look at the slides that we post on our preparedness portal.

And at this time, I'm going to welcome our first speaker, Dr. Richard Krieger, who is with the NIH RADx Variant Task Force. So Richard, I think you can jump in and take it away.

DR. RICHARD KRIEGER: I just want a quick soundcheck, you can hear me OK, Nancy?

NANCY ANDERSON: Yes. I can still hear you clearly.

DR. RICHARD KRIEGER: OK, thank you. So good afternoon, everybody. I'm going to quickly preview the work that the RADx team has been doing with respect to the SARS-CoV-2 variants. Specifically in their impact on I.B. testing on the programs that are being funded by NIH under RADx. Could I have the next slide, please?

First and foremost, I want to let everyone know that there's a very large group of people working on this program for RADx. You can see the names of those that are associated with RADx on the left. We are partnered with Emory University, who are performing a lot of the lab work for us, and also providing some guidance to us on some of our bioinformatics pipeline.

We're working with some outside universities this is the University of Washington, and I would be remiss in not stating that we are working also closely with many other federal agencies, those listed below in that line, including the CDC, Department of Defense, Department of Energy, the FDA, and other departments within NIH. And it's been a great effort. And we wouldn't be as far along as we are if it weren't for everybody's help here.

Next slide, please. So this program actually started on January 5th, right after the holiday break. I think at that time, everyone was starting to realize that the SARS-CoV-2 virus was mutating and mutating very quickly. And because the RADx effort is really focused on developing a test for the direct detection of the virus, either by nucleic acid amplification technologies or through antigen tests, we were concerned that

these mutations could have an impact on the tests that were being developed. A number of these tests are already on the market; other ones are working their way through development, and clinical studies, and EUA submissions. And we hope to be on the market soon.

So our goal, really, was to come up with a method, or methods, by which we could quickly assess whether those products that are either on the market or being developed would be responsive, would still be able to detect all of the variants that were starting to emerge. And as I mentioned earlier, we are working closely with other federal agencies to put this program together.

Next slide, please. I'm going to go quickly. Again, I think everybody in this call understands that whether there be changes in nucleotides, or which might be nonsense mutations, not a change in amino acids, or those changes in the nucleotide sequence which would result in an amino acid change, whether that be a missed S mutation, or a deletion, or an insertion, could have significant impact on any of the tests that are out there.

Again, this slide just simply summarizes that those changes could end up leading to a loss of binding. That's the binding of a ligand, such as an antibody. Or lead to errors in binding of a primer or a probe. And both of those conditions would ultimately lead to a potential false negative in those tests, whether that be the in nucleic acid amplification or an antigen test. Also, those same changes, depending on where they are in the sequence, could actually reduce the overall binding. So it could actually reduce the affinity of an antibody, for an epitope. Or it could actually decrease the efficiency of an amplification reaction and both of those cases could lead to a loss in sensitivity, or as an example, limit detection of those assays.

So that's what we were worried about and at a high level, and that's the program that we put together over the last three months. And we've concluded from that that, whereas modeling would be great, and modeling is pretty well established in the molecular area, it's not that well established in the antigen area, at the protein level. We wanted to develop a program which combines both modeling studies as well as clinical in vitro lab testing to actually determine whether a specific test would be able to detect a SARS-CoV-2 variant.

Next slide, please. I'm going to go through the next slides pretty quickly. This is actually from the [CDC's site](#) as of last week, it might be able to change, I haven't looked today. But again, we're all well aware of the variants that are happening worldwide and here in the United States. And this is a reflection of what the CDC published last week of the most common variants, the common lineages that are circulating here in the United States.

Next slide. This is a similar cut, this is from a [nextstrain.org](#) data website, where we've now mapped the evolution of the virus from December of 2019. And this is through the end of February of this year. And as you can see in this graph, this is an excellent website for those that want to go there and play with the website. You can do a lot of analysis on this website, and to actually track all the various variants that are circulating around the world. And this particular cut is from North America.

And I think everyone on these calls knows that variants are not homogeneous. Variants are really families of related mutations. A lot of the nomenclature that we're using here, which is the Pango nomenclature, are basing the names of these mutations, specifically on changes in the spike protein.

And as you can see on the next slide, this is also from the Nextstrain website. We are seeing mutations both at nucleotide level and the amino acid level in every part of the genome of the virus. So it's not just the spike gene that's changed, every gene indeed is showing a significant number of mutations. And this is what has us, I guess, the word was concerned, that these changes could impact the nucleic acid and a protein-based test.

A lot of the protein-based tests are targeting two proteins, the spike protein and the nucleic acid protein. And you can see in the upper panel that there are significant changes in these variants in both of those proteins. And on the nucleotide level, for the nucleic acid amplification tests, a lot of the assays on the market and assays on the developing are targeting multiple genes. Or if 1A or 1B are common targets, as is the nucleocapsid gene and the envelope gene. But indeed, you can see from here that every gene is indeed impacted.

So next slide. So what we did is, this is a summary of the comprehensive program that we put together. It combines both a bioinformatics platform, which is known as ROSALIND, that's being provided by a commercial third-party vendor, and we're also tying that to actual clinical in vitro or wet-lab testing. What ROSALIND is able to do, in the upper right, where it says sequence databases, ROSALIND is pulling in sequence databases from GISAID, also from NCBI GenBank and it also has capability of bone and sequences from other sequence providers, and such as we are networked with NCBI on their curated database that is coming from the CDC Surveillance Program as well.

So we're actually using this database to actually do bioinformatic modeling, both on the amino acid level and the nucleic acid level, with the various tests. Each of the companies that are participating, RADx can introduce their target epitopes, if they know them. They can enter in their primaries and their probe sequences, regardless of what type of technology they're using. Whether that be PCR, LAMP, FISH, or CRISPR. And the ROSALIND system will use various bioinformatic modeling to identify what sequences may, potentially, cause an impact to their assay.

On the amino acid level, on the protein level, we are doing some modeling that we put together with the help of some scientists at Emory. Those models are not as well-developed as the nucleic acid technologies, so they might be more prone to errors. We're also using the ROSALIND database, and we're also tracking very closely to what the CDC is pulling out, and we're working to actually acquire samples from the labs participating in both the CDC surveillance, as well as other reference labs from around the US, to acquire clinical samples and to create a variance biobank.

So, again, ROSALIND is helping us to identify variants of concern and variants of interest. And then we're reaching out to those labs that are participating in that surveillance program, and we're actually acquiring

those actual clinical samples to create that biobank. We're now using that biobank, testing has actually started today, to actually do in vitro clinical testing, or wet-lab testing at Emory. We're also going to send these panels to some of the companies that cannot send their products to Emory. So the manufacturers themselves. And we also are contracting an independent third-party site to do testing of our diagnostic products.

All those results, all these panels are being set up in a blinded fashion. So the manufacturers do not know what they're going to be testing. Each of the specimens are being prepared, in a series of dilutions, to go from relatively low CT to a relatively high CT, so we can determine whether the tests either can detect the virus or not. Or perhaps, if it's not a true LOD method that we're setting up, it's an LOD suggested method, or almost like a range-finding method, where we can see whether there's a difference in sensitivity as compared to the Wuhan strain that started this out or the Washington strain, and that's the two most common strains that most of these tests were used for their development. So this is a comprehensive program that's actually up and running right now. ROSALIND is live, and web testing is live as of today.

Next slide, please. And again, as I mentioned, we're working extremely closely with the CDC and the CDC Surveillance Program. We are contracting with many of the laboratories that are participating in the surveillance program for the acquisition of these clinical samples.

Next slide, please. So, where are we at so far today? Our bioinformatics program on ROSALIND is up and running. That program is open to other federal agencies. The FDA is using that as we speak, as other parts of NIH, and we have opened that up to other government agencies. And of course, it's also being used for ourselves to identify variants. And it's been used by RADx companies to participate in their own analysis for the tests that they have as part of RADx. They also can actually use that for other tests that they're developing independent of RADx.

We are then, as I mentioned, sourcing samples from the CDC-funded sequence laboratories and other laboratories. Right now, as of today, we have over 800 samples. CDC has recently mentioned-- --Emory, and we assume will be sending out panels to all the other companies that cannot send their products to Emory for their own testing.

Next slide, please. This is just, I'm not going to go through this. This is a complicated slide. This is a summary of the bioinformatics output that's coming out of ROSALIND, where an individual company, or RADx, because we can actually see all of the data as well. In the upper left here, you can add your manufacturing. Right now we're focusing on the United States data, although we eventually will be bringing in worldwide data. This database is currently updated weekly from GISAID and GenBank, but it will soon be updating on a daily basis.

And then, on the lower right, each company could look at their individual genes, their individual primaries and probes, and see what variants they might have an issue with. Notes are known as incidents that are reported around the world. So again, it's a lot more complicated, a lot more complete than this particular

slide, but the ROSALIND offers a wide range of capabilities for the companies to determine if their products are actually potentially having an issue with variants, or to help them actually design new products that will actually minimize their impact by variants.

And then my last slide just summarizes what we're doing. So, again, the virus is continuing to mutate. We're going to be using this program, it's not a one-and-done program. We're actually using this to identify new variants, and to collect those variants, and to continue to do wet testing for as long as the pandemic is continuing. As I mentioned earlier, we're going to work closely with the CDC and other federal agencies to actually collect real time field data. And with that, I'll bring it to an end. And maybe we'll take questions later, I think at the end of this, or during the Q&A and I'll try to get back to you. Thank you.

NANCY ANDERSON: Thank you so much Richard. Yeah, I'm scrolling through the questions that we have now, and I don't see any that I think are specifically for you. So if people have questions, please put them into the Q&A box and we will direct them to you, or we will use them for a future call. But thank you so much for being with us today and with that we will move on to our next speaker, Dr. Anne Wyllie, who is going to be talking with us about saliva as a sample type for SARS-CoV-2 detection. Thank you, Anne, for joining us. And you can keep me posted, and I will take care of the slides for you.

DR. ANNE WYLLIE: Thank you very much and thank you for having me. So indeed, I'd like to share some of the work that we've been doing for our small research team has been doing on validating and optimizing saliva as a sample type for SARS-CoV-2 detection over the past year.

Next slide, please. So, very early on in the pandemic, like many others, we had all of the same difficulties with nasopharyngeal swabs, many of which we still see today. They are difficult to obtain, they do require trained health care professionals, we do get testing aversion in response to the prospect of nasopharyngeal swab, and of course, they carry risk to the health care workers if the patients are to sneeze or cough onto their health care worker.

Next. And so, while we were collecting these swabs very early on, we also just so happened to be collecting saliva. And as these samples were coming in, we were testing them all, both the swabs and saliva for SARS-CoV-2 RNA, and we saw very early on that we were getting promising results. We saw comparable, if not better sometimes, detection of SARS-CoV-2 RNA in the saliva. So this is the paper that we published quite early last year, as an NADM, showing that success that we had with saliva mixed. And so, based on this, we continued with our research into validating saliva as a sample to help overcome some of the challenges that we were seeing with the swabs.

Next slide, please. Next. So, first and foremost, saliva tests must be designed for saliva. Saliva is the collection of oral fluid; it is not sputum and it is not mucus. We try to discourage any sniffing or coughing prior to the sample collection. That also decreases the risk of respiratory droplets. But if not properly collected, saliva can be difficult to work with.

Next. Since saliva is not a traditional diagnostic sample type, methods for swabs don't necessarily work on saliva. We did look at 54 RT-qPCR-based studies comparing saliva and swabs, and we actually saw that in 69% of those studies saliva had greater, or similar, sensitivity to swabs. Saliva also detected an additional 10% of positive cases.

Next. So this sort of suggests that maybe some discrepancies that we see in these comparison studies are actually due to poor samples or poor methods.

Next slide, please. So, good quality saliva samples can be collected. We've really refined our protocol of having people be patient, think about their favorite food, don't sniff or cough, and don't spit, with the idea that spit can create a lot of aerosols as well. Next.

So we had 30 study participants. They had four devices each. A funnel, a bowl to transfer pipette, the pipette tip, short straw, and we observed them and then unobserved collection, so all cameras switched off, microphones switched off. They had instructions, they hadn't been given any verbal instructions, and they had to collect saliva for us.

Next. And, based on this, we saw-- on the left, we had the participant feedback. We asked a whole array of participant feedback. But overall, we found that participants felt as though they didn't need help while collecting their saliva, and they felt pretty confident that they had collected their sample properly. In the lab, when these samples we received, they were all pretty much of sufficient volume, they were easy to pipette, they were true saliva, free from food particles, and they were not discovered. And importantly, they were all mostly in the expected range that would have for human RNase P as our sample quality control.

Next. So, with this in mind, and thinking about mass testing strategies, we developed SalivaDirect, which is an extraction-free and dualplex RT-qPCR.

Next. We developed that in mind with the lower access to testing, to make it less invasive, less expensive, less prone to supply chain issues, to really help improve access and especially thinking about mass testing strategies going forward.

Next. This was developed on our principles, that we found saliva is stable without preservatives, next. So, this means that no expensive collection devices are required. You can take raw saliva in a simple laboratory plastic tube, and we tested these samples fresh or after seven days at 4 degrees Celsius, room temperature for seven days, 30 degrees Celsius for seven days, 40 degrees Celsius for 72 hours, and we also put them through the FDA summer and winter profiles, and we found that you see a little bit of fluctuation in the CT values there, but ultimately, these raw saliva samples are stable without expensive preservatives, allowing simple laboratory plastic tubes to be used, which can keep the cost prices down when doing mass testing strategies.

Next. We were also very keen on keeping as much sensitivity as possible while taking out that RNA extraction, so we started developing this a year ago when, obviously, RNA extraction, PCR, is still the gold standard most sensitive approach. We were terrified of false negatives. So we made sure that when we were developing this assay, we kept that level of sensitivity. SalivaDirect has the registered sensitivity as the same as the CDC's RNA extraction PCR assay, so we're very pleased with that.

Next. We were also keen to keep the sensitivity when dualplexing the PCR. We had been doing N1, N2, and RP, all from the CDC, in singleplex, but getting them into that dualplex, which is important for time, and also for saving on resources. And again, we maintain that level of sensitivity.

Next. Importantly, we validated multiple windows, reagents, PCR instruments, at every single step of the way. This is just the substate from initial validation, but we did this to make sure that labs could use their existing supply chains. They could use whatever they had, some of the better relationships that they have with their existing suppliers. Especially the PCR instrumentation, we didn't want labs to be having to buy anything new. And importantly, we also wanted to keep competition between the suppliers so that we wouldn't see price hikes in response to any of our methodology.

Next. What's been really interesting to see in this is that we developed a universal RT-qPCR protocol. So we have master mixes from NEB, Bio-Rad, Thermo Fisher, we've got the new Quantibio one, and regardless of the master mix that we have, regardless of the instrumentation you have, we've found that we can have a universal PCR protocol across all of those instruments and mixes, again, just to keep things simple. So if one of your PCR mixes runs out, you can still use this same PCR protocol without having to worry about updating and make it reagent specific.

Next. One of our most recent developments that we've had, which is also very exciting, is that we actually took that standard universal protocol, the same sort of master mixes that we were using and expanded that for the 384-well format as well. So we have now validated this assay in 96-well format instruments, and 384-well instruments. It has been a fabulous collaborative effort. I was unaware at first that it could potentially not be able to do 20 microliter reaction mixes in the 384-well format, that many labs jumped to help us out and validated this half mix that we can use to high throughput testing.

Next. And this is some of our validation from the 384-well format work. So right on the left, you have the list of the different 384-well format PCR instruments, and then mixed with that are the different PCR kits. And these are the results from our LOD finding studies, and again, I mean this is just the finding studies, so we only do the three replicates, but you can see that for most of these combinations, we are between that 1.5 and 6 copies per microliter, so roughly around the 3 copies per microliter, with this universal protocol, with any sort of combination of these different reagents and instruments.

Next. We've also continued to evolve our protocols. So this has been in response to a number of the labs that we've worked with, who have been wanting to do heat pre-treatment. When receiving their samples in labs they prefer to heat them. So we saw what was commonly being used or asked for. We evaluated 95 degrees for five minutes, 95 degrees for 30 minutes, and 65 degrees for 15 minutes, and we trialed

this with the standard SalivaDirect protocol, but also going straight into PCR as well. So we've heard that this can make the samples safer to handle, but we've also heard that this can improve the viscosity of the saliva for easier pipetting next.

And these are also our LOD-finding results. This is actually going down to those 20 replicates in the different combinations. And again, we were hitting around that three to six copies per microliter later with these different steps, with and without the normal SalivaDirect protocol.

Next slide, please. We have also validated how this works in asymptomatic individuals for large-scale screening. This is just a validation study, and we only, fortunately, this was done with a group that were doing very, very well with their bubble, so we had very few positive samples come back, but we were collecting saliva to test by SalivaDirect. And that was being compared to appeared anterior nares and oropharyngeal swab, tested by crystal bio-reference. So we did have a high positive agreement, but ultimately, we tested almost 4,000 paired samples. We saw a very, very low and invalid rate, being negative for RP, and an incredibly low false-positive rate with this.

Next slide, please. We also looked at the CT values that we got from symptomatic and asymptomatic individuals. And when you put them side-by-side, we actually find that whether someone's symptomatic or asymptomatic, those main CT values do not really differ. So just because you're asymptomatic doesn't necessarily mean that you're going to have a low viral load.

Next slide, please. So that was a lot to rush through, I really know, I really thank you for your time and just wanted to leave just a full disclaimer, disclosure. We make no profit from SalivaDirect, no licensee fees, no commissions, no royalties. We simply are able to authorize CLIA certified labs to use this protocol, but really the main thing behind SalivaDirect that we did was to help reduce testing costs for the community, to help reduce the implementation time that labs need to use saliva. We continue to do that validation work for you. But, importantly, to increase the number of tests available in the community and to increase the number of affordable tests in the community. So thank you very much for your time.

NANCY ANDERSON: Thank you so much, Dr. Wyllie. We do have several questions here, and I think we have some time. First, I'll ask, there were a couple of questions that I think were specific to your study. Can you go back up, just-- there we go. The first ones were, what volume of saliva do you need? And how long are the samples stable for use in PCR?

DR. ANNE WYLLIE: So we only use 50 microliters for this assay. We do - generally we ask that people give 200 to 500 microliters, just so you can exclude bubbles, just in case we need to revisit the sample for any reason, but you do just need 50 microliters. We always freeze our lysates. So the samples remain stable as we store them. Raw saliva itself can remain stable at a variety of conditions for at least seven days. And then once we do the workup, we get that lysate. We generally try to test that as soon as possible, but we do actually freeze ours at -80 degrees Celsius, and it can survive a number of freeze thaws with robust N1 detection as well.

NANCY ANDERSON: OK. And one more minor question, does pH variation cause difficulty?

DR. ANNE WYLLIE: So we don't see that in-- the only thing that we've found to inhibit, so you can you get RP negative, you've seen something inhibiting the reaction. So far, the thing that we've identified so far are blood and mucus. And that's when you particularly have, maybe, a severely ill patient. But again, this is why we very much stress that you need just that good, normal saliva. Take a swallow, let the normal saliva flow back into the mouth. That is what you want to test, that's what's easier to pipette. And with that, we've had saliva of every single color come through, from various foods and drinks that people have eaten, but we still always get that RP detection. So far, we've only identified blood and mucus as inhibitory.

NANCY ANDERSON: OK great. Now here's another one that's a little bit related to that. I understand the need to not have actual sputum in the sample, but can you comment on the sensitivity, discrepancy between saliva without coughing and saliva with coughing in the IDSA grade summary?

DR. ANNE WYLLIE: I think that really depends on the stage of infection that one person is at. Has that got down into the lungs? Do you want to bring that up? Can you potentially collect more signal that way? If it's a severely ill patient, I think early on in the picture you probably won't see that so much. There's really, so the study that I touched on where we compared all the different PCR studies, that's actually in press at the moment.

So that table, we have a table comparing all of this coming out soon. There are actually other studies that also use coughing or not. And there's also a lot of discrepancy between coughing, so I don't really necessarily think it's even always just the coughing, that in the end, the method that is used to extract the RNA, even if extractionless, but just the fact that you need a robust-- whatever sample you collect, however you collect it, it needs to be the right method for obtaining the right amount of that RNA for detection, and that's what's more important.

NANCY ANDERSON: Great, thank you. Now I think I'll ask you a couple that are related to antigen testing. There was one, a general question, can you use saliva for antigen testing? And then there's one more here that says, going forward do you see the standard SalivaDirect protocol being used as a comparator for those who are developing saliva-based antigen assays instead of having them go up against NP as the comparator?

DR. ANNE WYLLIE: So, that is actually a great question for the FDA, and this is a question that we struggle with ourselves at times in how we can do that. I mean, to some extent, I mean, there are a number of EUA saliva tests out there at the moment. And indeed, I mean, if these tests have EUA, they're getting robust detection with saliva, I think personally, my own personal opinion, that it could be fair to have this as a comparative. But of course, that guidance does obviously have to come down from above. And of course, getting the nasopharyngeal swabs can be difficult.

There is also the risk with nasopharyngeal samples that you can detect historic cases. So you can have that prolonged shedding of RNA from a nasopharyngeal sample. So you're not even testing an active infection, which can make that an unfair comparison to make when you're trying to capture an active infection if that also makes sense. So that is something that I think is a very interesting topic for further conversation.

NANCY ANDERSON: Good, good. Well we'll use that to transition to the update but let me just squeeze in one more quick one. Did you include pediatric specimens in your SalivaDirect study?

DR. ANNE WYLLIE: We haven't-- in this data, we do not have pediatrics. But I work closely with, we are doing some childcare studies at the moment, so we are actually collecting saliva samples from infants using transfer bulb pipette to sort of try and get saliva out of their mouth. We have two-and-three-year-olds who are, more the three-year-olds who are very capable of providing a raw saliva sample, so we are testing infant populations as part of studies. I'm not sure if that will be taken into the EUA any time soon, but it is possible.

NANCY ANDERSON: OK. Well thank you so much. As you can see, there's a lot of questions about this, and a lot of interest. We will turn it over, at this point to the FDA. But if you have time and want to scroll through and see any other questions that you can answer, feel free to do that in the Q&A box. But we really appreciate the presentation and the information that you shared with us.

DR. ANNE WYLLIE: I'd be happy to take those questions. And thank you again for having me.

NANCY ANDERSON: Thank you. And so, at this time, we are going to turn it over to Tim Stenzel, who is generally with us every call to answer the FDA-related questions, is not available today. So we have Sara Brenner stepping in to take his place, and we will let Sara-- I'll move on to the FDA slides, and we will let Sara proceed with answering the questions or presenting any updates she would like to. Thank you.

SARA BRENNER: Great. Thanks so much, Nancy. Just double checking you can hear me because I did change my audio.

NANCY ANDERSON: Yes. You sound just fine.

SARA BRENNER: Perfect. So yes, Tim send his regards. I'm the Chief Medical Officer for In Vitro Diagnostics at FDA, and I've been detailed, also, to HHS on the national response to the diagnostics heavily for a year now. Crazy.

So I'm just going to run through questions that came in from last week and provide some office-cleared answers. I anticipate there will be a lot of questions potentially coming in today about some of the newer releases from FDA, including the serial training pathway, and so on and so forth. I'll do the best I can, but of course can take any questions back to the office to present on next Monday.

So what came in last week? I'll just run them through. Now that the BioFire respiratory panel 2.1 has gone through the De Novo pathway, will all new tests for SARS-CoV-2 have to go through the 510(K) process, or will the new EUAs still be accepted? I think Tim addressed this in previous calls, that we are still receiving and reviewing EUA requests for SARS-CoV-2 tests. That'll probably be the case for quite some time, including multi-analyte tests that includes SARS-COV-2. So we don't see an end to doing that for as far out as we can see.

Next, are commercial tests assessing neutralizing antibodies for COVID in the pipeline, and what can you share regarding protective titers of such antibodies? There is one authorized test to detect neutralizing antibodies, and we are interested in working with developers of additional neutralizing antibody tests. So if you're one of those, bring them in, and as always, we encourage you to engage early and often with FDA.

How is the FDA regulating reporting for OTC COVID-19 tests? For example, the Cue Health Point of Care, which is now a fully over-the-counter PCR test. So this is sort of a complex question, not entirely in the lane of FDA but across the federal response, including HHS. FDA itself is not solely responsible for regulating the responsibility of reporting to public health authorities, but FDA does work with USG partners who do. So that would include other sister agencies and HHS all the way up to the Department level and there's high level policy at the White House level that also dictates the secretary level and the White House level that dictates reporting requirements.

So, while FDA has not issued its own standalone sort of requirement as part of the authorization process for at-home tests, we do strongly encourage it, and we actively work with test developers to bring EUA's in on plans to implement reporting solutions. And when I get to the end of the questions, I'll drop a link in the chat or the Q&A to [diagnostic data and reporting information on HHS's website](#), which includes frequently asked questions and also includes a table mandatory minimum core data elements for all COVID-19 diagnostic tests. That's broken down by federal, state requirements, and also the source of the data for lab-based and non-lab-based tests. Over-the-counter tests would be, of course, non-lab-based tests.

I will point out, since the example was asked specifically here about Cue, that Cue did go to market with a data capture harmonization, and automation, and wireless transmission to public health authorities capability. They're one of what I would consider a gold-standard diagnostic company that already integrated those capabilities into their diagnostic workflow at the launch of the product.

Next question. Is there an EUA approved home dried blood spot DBS collection kit? There is not a standalone DBS collection kit authorized for use without SARS-CoV-2 serology tests at this time. Next question. Can you help us understand the difference between direct-to-consumer claims versus over-the-counter claims for an EUA at-home collection kit for SARS-CoV-2 testing at a reference lab? So OTC, or over-the-counter indicates that no prescription is needed. You could walk up and purchase a test at will from a pharmacy or other vendor. We tend to refer to non-prescription home collection kits, where the sample is sent a lab for processing, as direct-to-consumer to distinguish them from the over-the-

counter at-home tests where there's no lab involvement. Because the latter is what most people think of when they hear OTC.

Last question, people have been using EUA authorized PCR antigen tests for screening throughout the last year without enforcement. Does FDA now intend to enforce EUAs for screening? Here's a little clarification on this question. Screening claims have always been regulated by FDA. We have authorized screening claims when supporting data was submitted. For tests authorized for individuals suspected of COVID-19, health care providers should always prescribe them for screening at the health care provider's discretion. However, the test developer could not market them for screening without having that claim authorized.

And this still remains the case. So the new supplemental template that I referenced at the beginning opens a new approach, the new pathway, to get a serial screening claim, so that you could market it as such and we do have a post-authorization validation of the asymptomatic serial screening requirement for that claim.

And those were the questions that came in, and I'll turn it back over to you, Nancy and thanks for showing some of our links. I'm also going to drop, as I mentioned, one in the chat having to do with [diagnostic data elements](#), capture reporting at the federal and state level, based on whether the data is flowing from a lab-based diagnostic or a non-lab-based diagnostic.

NANCY ANDERSON: OK. Thank you Sarah I am going to ask a question that is very similar to one that you already answered, and I do believe Tim has answered it before, but perhaps someone new is on the call and didn't hear the answer. It is a question about EUAs moving to full FDA approval like the BioFire test. And can you clarify whether this will be required or recommended for all EUAs at some point, or will the EUAs permanently remain in place?

SARA BRENNER: So I'm not sure exactly how Tim answered that previously. I mean typically EUAs stay in place until a public health emergency is rescinded at the secretary level, which of course is the department above FDA, so it's not purely FDA's decision. Whether or not folks who are holding EUAs want to go ahead and apply for the full approval process, the full approval pathway, would be up to them. Certainly, they might feel that it's in their best interest to do so, or others might not. So I don't think that FDA is going to really issue a formal statement, or encouragement, et cetera, with regards to what folks do. But I can go back and check. We could be surprised, but that would be my hunch.

NANCY ANDERSON: OK. Thank you. Let me see if there are any others for FDA here. Let's see, this one I'm not sure if you have an answer for. There are several sequencing instruments on the market. Variant sequencing is not yet approved for clinical use. Do you foresee variant sequencing will be approved for clinical use in 2021? And what are your thoughts about moving the testing into the clinical laboratory?

SARA BRENNER: Yeah, I'm not sure how to answer that one or what the agency's official stance on that is. Certainly, we're open to changes, and recommendations, and movements to incorporate new technology, especially when the data suggests that clinical care could be advanced in that way. So I would say FDA would be open to that, but I'm not aware of a formal position or stance the agency has at this time.

NANCY ANDERSON: OK. I'm not really seeing anything else that seems to be specific for FDA, so I think we will let you off the hook for today. Appreciate your stepping in for Tim. And let me take one more scroll to the bottom. I don't think there is anything else there that is directly asking a question of FDA. So if we get something, you know we will send it on to you, and you can answer it on the next call. But we appreciate your being here today and look forward to either you or Tim being back on the next call.

SARA BRENNER: Great, thanks so much, Nancy.

NANCY ANDERSON: Thanks, Sara. And with that, let me make sure I didn't miss-- oh, here was another FDA slide that we-- some people may be interested and need this information. Here are some of the social media websites for information from CDC. So that is bringing us to the close of the call for today. Again, thank you all for being here, for listening.

We hope the information that we have provided is helpful to you. We do encourage you, if you haven't already done so, to opt-in to the CDC Laboratory Outreach Communication System, or LOCS, to get ongoing lab communication that we put out related to the COVID-19 response. So if you need to sign up for that, please send us a message at locs@cdc.gov, or you can click the [link](#) for that in the chat box. As we mentioned before, we will be posting the slides, the transcript, and the audio to the website by early next week. So for any of these resources, please visit our [Preparedness Portal](#).

Another reminder that we hope you will be able to join us on our next call on Monday, April 19th, at 3:00 PM. We will have the CDC Director, Dr. Rochelle Walensky, who will be giving the opening remarks on that call, so we hope you'll be able to join us and encourage your clinical lab partners and colleagues to join us for that call.

And once again, if today, or at any time in the future, you have questions to submit for consideration for this call, or that you need an answer to, please use the Q&A function in the Zoom or email us at dlsinquiries@cdc.gov.

And if you do submit a question, again, please make sure to include your email address so that we have a way to get back in touch with you. And I think that is it for today. Again, thank you again for joining us. Thank you for all of the good work that you continue to do. We are grateful for you and the important work you are doing in the COVID-19 pandemic response. And we look forward to talking with you again in another couple of weeks. So have a good afternoon, everybody.