Good afternoon. I'm Commander Ibad Khan, and I'm representing the Clinician Outreach and Communication Activity, COCA, with the Emergency Risk Communication Branch at the Centers for Disease Control and Prevention. I would like to welcome you to today's COCA Call: Molecular Approaches for Clinical and Public Health Applications to Detect Influenza and SARS-CoV-2 Viruses. All participants joining us today are in listen-only mode. Next slide please.

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At the conclusion of today's session, participants will be able to accomplish the following: Explain the meaning and potential use cases of Ct values for SARS-CoV-2 testing; discuss the value of SARS-CoV-2 sequencing in public health compared to clinical practice; and describe clinical test ordering and utilization for seasonal influenza in the context of SARS-CoV-2 co-circulation.

After these presentations, there will be a Q&A session. You may submit questions at any time during today's presentation. To ask a question using Zoom, click the Q&A button at the bottom of your screen, then type your questions in the Q&A box. Please note we receive many more questions than we can answer during our webinars. If you're a patient, please refer your questions to your healthcare provider. If you're a member of the media, please contact CDC Media Relations at 404-639-3286 or send an email to media@CDC.gov.

We have introduced self-knowledge checks throughout this presentation. We hope you enjoy these opportunities to assess your understanding of today's session. Please do not type your answers into the Q&A box, as this may disrupt the Q&A portion at the end of the session.

I would now like to welcome our presenters for today's COCA Call. We are pleased to have with us Dr. Manish Patel, who is the Team Lead for the Influenza Prevention and Control Team with CDC's Influenza Division. Dr. John Barnes who is the Team Lead for the Strain Surveillance and Emerging Variants Team as part of CDC's COVID-19 response. And Commander Alison Halpin who's the Taskforce Lead for the Laboratory and Testing Task Force as part of CDC's COVID-19 response. It is now my pleasure to turn it over to Dr. Patel.

Dr. Patel, please proceed.

Thanks, Ibad. Quick mic check.

Dr. Patel, if you can speak a little bit louder. That was a little bit on the low end.

Can you hear me okay? Yes, that's much better. Much better. Thank you.

Thanks very much. So I was going to speak today about the 2021-2022 influenza season, which is now, and testing issues specifically related to influenza in the context, SARS-CoV-2 co-circulation. And my goal was really to give you all very much a high-level overview of the CDC clinical guidance that's available on our websites on issues related to testing for influenza, taking into account SARS-CoV-2 co-circulation with influenza. And I'll basically walk you through these available resources and the hyperlinks on these topics on our CDC website. And you should have those links available in the presentation.

The recommendations in general are categorized by three patient settings. One is outpatients and emergency department patients that are likely to be discharged. Second will be hospitalized patients, and third will be nursing home residents. Keep in mind the different tests and the issues related to the tests themselves will not be covered, though those links are available on the CDC websites. The website gives you all of the more details on those different diagnostic tests that are available and the validity of those tests. And then lastly, I'll focus mostly on influenza and not SARS-CoV-2 itself today in the presentation, as those issues have been covered previously. Next slide.

And so you see in this slide that influenza activity really, as you well know, has a history of unpredictability. You know, last year or last season, really the past 18 months, we have had no influenza activity in the United States, and minimal activity globally in the southern hemisphere or the northern hemisphere. And this really has not happened before since we've had surveillance for influenza. The jury is still out on reasons why that hasn't happened. That said, we do know influenza is going to come back and already has started to reappear in many places in the United States, predominantly in young adults. And recently, CDC has released HANs, Health Alert Notifications, as well as an MMWR to outline the viruses that have been detected recently in the United States. So I think that suffice it to say, it makes sense for us to be prepared and maintain vigilance for influenza. And so that really is the impetus for this presentation, is to provide you some of the recommendations on testing for influenza, as well as show you the links available for the implications for increasing influenza activity in terms of testing and MPIs.

So in terms of monitoring for influenza viruses in the United States, we use laboratory surveillance networks. And what that means is we do surveillance for both influenza and SARS-CoV-2 in the US through various different approaches in two broad buckets. We use public health surveillance networks that are established at the local level within a county, at the state level, and then also at the national level. And then we also have a network of clinical labs where testing is conducted in outpatient or emergency department or hospital settings or nursing home settings. And these clinical test results are submitted to the states and subsequently nationally. And so we use this to monitor for both influenza and SARS-CoV-2. And so I think using these data, we believe that preparing for -- it will allow us to prepare for co-circulation of these two viruses. And I think it's relevant because it helps us mitigate the possible impact on healthcare strain this winter should these viruses continue to co-circulate together. And with

regard to influenza, as you all know, vaccination really is our best tool to reduce healthcare burden. We also have adjunctive treatments and prevention strategies with antivirals and nonpharmaceutical interventions. But at the root of that, we really need a testing plan. Because testing itself can help us identify these viruses specifically in the setting of co-circulation. So for that reason, guiding clinicians towards these testing algorithms is really the primary aim of the presentation today. Next slide.

And so could co-infection of influenza and SARS-CoV-2 occur in the same patient? And what are the implications of that? As I mentioned, we really have had minimal activity of influenza in the context of SARS-CoV-2 for the past two years. And so we haven't seen much co-circulation together yet, up until recently. And so we've had very few cases of co-infections of the two viruses in any given patient. However, you know, it is possible to see that, especially when you start seeing both viruses co-circulating together, we will have more cases. Currently because the data are limited, we're not sure what the implications of co-infection would be or the risk factors for patients that might get co-infections, or the potential severity. However, this is something we're going to continue to monitor through our surveillance networks.

Suffice it to say, influenza antivirals can still be used in a setting for infection. In terms of the differences between clinical presentation and some of the epidemiology and transmission of the two viruses, the two viruses are clinically quite different, as you well know. The incubation period for influenza is much shorter. It's about one to three days from the onset of infection to clinical symptoms. For COVID, it can be much longer, anywhere from two up to 14 days. The viral shedding or the period of detection of viral RNA is typically much shorter for influenza than for COVID-19 or SARS-CoV-2 infection. And then of course, loss of taste or smell is quite common with COVID-19 and hasn't been seen that commonly in the past with influenza. Lastly, the timing of onset of the severe disease that we see with COVID is much more delayed with COVID than influenza. COVID typically presents in the second week, eight, nine days after initial infection, whereas influenza tends to present much sooner, within the first few days of infection. Now, that said, at a patient level, it really is clinically challenging to differentiate the two viruses in patients with acute respiratory symptoms. And so what that means is that we really need to rely on more laboratory testing to distinguish those two viruses. Next slide.

And we have several web pages. In this webpage right here, you see the hyperlink on the bottom of this slide. This basically provides you a summary of all of the adapted guidelines for influenza testing in the context of SARS-CoV-2 co-circulation. On the left box in the red, you see the general proposed algorithms for testing. And basically, the testing strategies vary by the three clinical settings -- outpatients and ED, inpatients or nursing home residents. And on the right, you have some more information on the various diagnostic tests that are available. And there's lots of them for influenza. I will not comment on those as I mentioned. It's outside the scope of this presentation. However, the links are very nice and provide you some more up-to-date information that are available for you to review at your leisure. I will walk you through all four of those hyperlinks you see on the left box. Next slide.

And then this slide right here basically gives you the punch line up front on testing. As I mentioned, the general summary of these algorithms is that the testing varies by clinical setting, whether the patients are outpatients or ED patients likely to be discharged home, whether they're hospitalized patients or whether they're nursing home residents. In outpatients or ED patients, testing options could vary. There's a lot more flexibility there. Part of this will depend on local testing availability to those clinicians. So clinicians do have the option to test for SARS-CoV-2 and then just use their clinical judgment for testing of influenza, for diagnosing influenza and treating influenza should the patient require it.

But if testing is available for influenza, which is more and more the situation in recent years, it will help with clinical differentiation of the patient, whether it's SARS-CoV-2 or influenza. And so if testing is available, you could test for influenza. In hospitalized patients and nursing home residents, the recommendation is to test all suspected patients for influenza and for SARS-CoV-2. And the reason is really there are treatment implications, and possibly other infection control implications for these two groups of patients. I think it goes without saying that viral culture and serology are not practically useful for clinical diagnosis of influenza. And you see the reasons outlined here for those two modalities that were used in the past and are still currently used under research settings that are not clinically helpful. Next slide.

In here, you can see a couple of these algorithms at a very high level. First, you see on the left the outpatients and emergency department patients. Actually both of these refer to outpatient clinic or emergency department patients. On the left you see patients who are hospitalized, and on the right you see patients who are not hospitalized. Again, the general difference is that if the patient is hospitalized, the recommendation is to test for both SARS-CoV-2 and influenza. And as I mentioned, the reason to test is that patients benefit from antiviral treatments and there's implications for infection control. Next slide.

And then the second web link you see up on the bottom right of this slide, you click on that, you will come to this page. And this algorithm here basically helps you drill down on the patients. I'm sorry, one second. It helps you drill down on the patients by hospitalization status, and an algorithm for testing. On the left box over there, you have the different steps, including specimen collection, that process for SARS-CoV-2 and influenza testing and then algorithm for treatments with antivirals. On the right slide--side, you have patients if they're not hospitalized, an algorithm for SARS-CoV-2 testing and then influenza testing and treatment. Next slide.

And then the basic summary of those--that webpage for outpatients and ED patients who are likely to be discharged is that for influenza, these patients, again, clinicians have flexibility in testing. And testing is only recommended if it changes clinical management. And this might be in various different forms, such as it might reduce further diagnostic testing, X-rays, antibiotic treatments, and it might also help guide antiviral treatment. If testing is available, it is a nice thing to do, and it does help guide clinical treatment. The assays that could be used here could be single-plexes or multiplexes. If it is a single-plex assay, then you would probably need to collect two different specimens, one for SARS-CoV-2 and one for influenza. If rapid influenza molecular assay is not available in outpatient settings, it is okay to use a rapid antigen assay for

influenza. However, keep in mind the sensitivity for those assays are lower. So rapid influenza molecular assays are the preferred assays if they are available. Next slide.

And then similar to the page for outpatients and ED, this page with the hyperlink on the bottom takes you the testing guidance for hospitalized patients. Next slide.

And here's the general summary of that webpage. There are four specific details the webpage covers. First, among these hospitalized patients, as I mentioned, the recommendation is to test all suspected patients for influenza to help guide antiviral treatment, help reduce antibiotic usage and also help with infection control measures. Clinicians here in the hospital setting should use multiplex or single-plex assays, but they should be molecular assays. Antigen assays, rapid antigen assays are not as useful to hospitalized patients because the sensitivity is much lower, and largely they have fallen out of favor. For immunocompromised patients, multiplex assay, you know, with a broader panel of respiratory pathogens is typically recommended. Next slide.

And then lastly, the fourth webpage -- the hyperlink again is on the bottom -- takes you to the testing considerations for nursing home residents. And each one of these web pages gives you more details than I'm presenting here. But essentially, the guidance for nursing home residents is quite similar to inpatients, hospital patients. For influenza, same thing as for hospitalized patients, the preferred assay is a rapid influenza nucleic acid detection assay or molecular assays. And then if they're not available, rapid antigen assays are allowed, however keep in mind sensitivity is lower for those latter assays. Next slide.

And here's the general details presented -- overview of the details presented on those webpages. First and foremost for nursing home residents, health departments should be notified for both SARS-CoV-2 and influenza infections in either residents or healthcare personnel working within the nursing homes. And then with regard to testing, as I mentioned, the recommendations are exactly the same as the hospitalized patients. And basically, if patients are positive for influenza, they should be treated with antivirals. I will not go through all the details because they're listed out there, and they're the same as the ones I just covered for hospitalized patients. Next slide.

So in summary, testing for both influenza and SARS-CoV-2 is recommended in all patients who have acute respiratory illness in hospital or nursing home set settings, nursing home residents, or outpatients or ED patients who are likely to be discharged home. As I mentioned, influenza testing can really depend on the clinical judgment, and it affects clinical management. For example, it could be used to reduce further diagnostic testing or to guide antiviral treatment, or perhaps even reduce unnecessary antibiotic use. The rapid molecular assays, they're becoming more widely available, are preferred for influenza because of the lower sensitivity of the antigen assays. And then lastly, keep in mind, we are just seeing an uptick of influenza activity nationally. And this is really some of the first influence activity in the context of SARS-CoV-2, co-circulation, and so we're not sure what that's going to look like in terms of healthcare burden or co-infections. And so we will continue to monitor this and reassess and provide updated

guidance on testing or treatment, should that arise as the season progresses. Flexibility does exist to modify all of this locally as needed, depending on the activity and the burden. And the guidelines itself might also evolve as well as the slightly different data at the state level, depending on what's happening locally. Next slide.

So that brings us to the knowledge check here. I'm going to read the question and the answers real quick. What influenza assays are not recommended for diagnosis of influenza infection in hospitalized patients with acute respiratory illness? A, viral culture. B, antigen assays. C, serology. D, A and B only. And E, all of the above. I'll give you a second. Next slide.

And the correct answer here is E, all of the above. Viral culture, as I mentioned, is not practical or sensitive for detecting influenza viruses. Antigen assays, they have lower sensitivity compared to RT-PCR. And then serology assays require both acute and convalescent sera four weeks after the initial blood draw, which is not practical for diagnosing acute infection. Next slide.

Here you see a series of references that you could revert to. And then next slide.

That brings me to the end of the presentation. Thank you for your attention, and please feel free to reach out to me, should there be any questions. And thank you for all your efforts during the pandemic. Thanks.

Thank you very much. Next slide, please.

Now I'd like to turn it over to Dr. Barnes. Dr. Barnes, please proceed.

Hi. Thank you for having me today. Today I'm going to talk about a number that has been widely used and talked about in the SARS Coronavirus outbreak and pandemic and some of the considerations that you have to think about when -- about really talking about cycle threshold numbers and and where we may be inducing error into our process. Next slide, please.

So I put this slide in there to to really kind of go through where we are when we're doing a test, where we may pick up variability and where we may actually have implicit bias. And there are certain areas in which we have -- have potential for both. There's a lot of steps -- we think we're ordering like a test order for PCR or something is relatively simple, but there's a lot of steps involved in the actual testing procedure. And some of these things can actually drive bias in the sample sets that we are looking at, that we may utilize Ct values on. And then others may actually induce quite a bit of variability that may not be apparent when this testing is is done. And really you can see that through many, many, many steps in the pathway. There are individuals that are different in our testing parameters. So whether we're testing symptomatic people or asymptomatic people, vaccinated people, or whatever, these may bias some of our results. Specimen quality -- the quality of specimen, the type of specimen that we take, specimen storage and transport. Reverse -- technical things like reverse transcription efficiency,

platform, and test that we're using. Assay performance interpretation, all the way through to really do the RT-PCR dynamics in this cycle threshold. Next slide.

So Ct values. Ct values are are are a value that we get -- if you look at the bottom panel of this of this graph that we see in the bottom, they're a value we get through a setting of a threshold line, this red line that you see through that panel, that is essentially the -- where we start to get divergence from the background fluorescence of a particular PCR amplification. And this can absolutely be related to genome copies. What we're basically doing is amplifying a small piece of that genome, and we are amplifying it up in a very, very specific way that can be related to genome copies. And in fact, one of the things that my laboratory does is actually manufacture and develop diagnostic tests. And so when we go through a process like this, we actually look at that as, look at our ability to relate to genome copies as one of the reason -- one of the factors that we use to tell how well that test is actually working. So if you see the top panel of this synthetic RNA that we have, that we're utilizing to make this panel, we know we have a certain amount of that RNA, and a certain amount of total copies per reaction. And our Ct values roughly move in a threefold manner, which means that we have three -- roughly a jump in three Ct per log change in nucleic acid concentration. And this is something that we want to maintain.

The slope of this line should be good and true, even when we get down to a very low, low level. And this is actually indicative of a good test. I will say that this isn't the -- isn't a requirement, though. And so this should always be kept in mind. But when we're running it in these ways, we're doing a lot of controls around this. We're using the same instrument, the same run conditions and assay, the same operator, quality, material, analysis and everything like that. And it makes it this this relationship very, very standard. And we uh -- but what often happens is there are assumptions made to the Ct data that this test maintains this linear relationship in all cases. And then the the assay site that we're using -- utilizing, meaning the pieces of DNA that we're actually amplifying, there is no mutation in that that may change our ability to efficiently amplify that particular target. Next slide.

So one of the things that uh many people do not know when they're looking at Ct settings and how they may impact -- Ct -- threshold settings and how they may impact Ct value is that the threshold line that I was showing you back on the on the red line in the previous graph and now in a green line here, can in some tests can actually be set by the person running the test, by the operator. And what you can see as in this curve, this amplification curve that shows as this PCR is being amplified from a signal of from a detection, that depending on where you set that threshold line, you get very, very different Ct values. Those Ct values, if you go back to that same rule of roughly three Ct equals a log change in nucleic acid concentration, it basically gives you a range of 0. 2 logs of difference. So if you were talking about 100, you go up to 1,000, to 10,000 copies, at the top, you could only detect at 10,000 copies, or you could detect at 100 copies at the bottom. And so this this really shows that there's variability just in the way that it can be set on a purely arbitrary setting. This is not the case for all tests, but it is the case -- these are are are considerations when you're actually looking at utilizing these values. Next slide.

And likewise, Ct values on the same amount of starting material can vary differently based on the test and based on the assay performance. So the -- if we look at -- my lab produced a a multiplex test called Flu SC2 Multiplex, and if we look at that target and then we look at a commercial assay that we have, and that has two different targets, you can see what I mean by this. If we utilize a standard amount of material and drop again through a dilution series, you can see that you get very different values between the multiplex target that we have testing for the SARS-Coronavirus-2 and the commercial targets for both N and RdRp. And what is also you can might be able to see in this is that the distri -- the difference between the jumps in those targets are quite different. Whereas we have a roughly three Ct jump between every on the multiplex target, 23 to 27, 23 and a half to 27 as we go on -- we have over over six Ct jumps between the commercial end target. And then the RdRp target seems to almost fall off a cliff. Meaning that what you have there is nonlinearity in the way that the actual target is progressing through a defined number of copies per reaction. Meaning that it is very, very hard then to correlate the amount of Ct to the amount of genome copies actually detected. Next slide.

So self-knowledge check. Which of the following factors can change assay performance and induce variability in Ct values of a molecular test? A, specimen site of collection. B, specimen quality. C, enzyme used in assay. D, lab or technician preference for setting threshold line. Or E, all of the above. Next slide.

The actual correct answer is E, all of the above. The reason is because all of these factors can have a very profound effect on the perceived sensitivity of a molecular assay, and can serve as sources of variability in Ct values. Next slide.

So, viral mutations within a probe or primer region can impact Ct value quite a bit. And as we have a situation like we have with influenza or SARS, where the virus moves end-to-end very quickly and mutates very quickly, these are not -- we tend to try to put -- good good assays tend to try to be put in biologically constrained areas. So they don't actually move very many times, or we don't pick up mutations very often. But, but they can occur. You can actually get mutations that occur in primers and probes of these individual assays. And those can affect the efficiency of that assay into actually producing a Ct value or a result. It does not mean that those are less likely to necessarily be positive or negative on an individual patient, but it can have that effect. And it could actually cause what is called a delay in the Ct value actually coming up.

And if you look at this, this is a particular mutation that we found in uh between two different probes, both in the nucleocapsid region in the SARS-Coronavirus. So if we look at the nucleo -- first probe for the nucleocapsid, N1, there is no mutation. And so, and the Ct values of these two targets usually run really, really close together, basically right on top of each other. So they should be roughly equivalent. And that when you see the number of mutations that we have in the first three samples as being one, basically, we don't get much discernible change between the N1 Ct and the N2 Ct. But when we look at a second mutation that would be introduced in the N2 target, induced by the red and the blue arrow, then we can actually see that we start to

affect the sensitivity of the overall assay. That we are getting that as a less efficient amplification and detection, and therefore a delay in that Ct. So you can see a battle log worth of difference, or three Ct change, again, or a log worth of difference between the number of potential genome copies detected by that assay with that mutation. This is just an example, and just a fairly minor example, but others can happen and have much more detrimental effects. Next slide.

So besides working and just looking at the individual things that can happen with a Ct value and the actual ability of that Ct value to detect genome copies, we also need to look at the use of Ct values to try to actually -- try to actually look at infectiousness of a patient and/or transmissibility. Often, because Ct values can be correlated to the number of genome copies detected in an individual, we try to make this jump in which we utilize the number of genome copies of the virus there to estimate viral load, and then therefore, assume infectiousness or assume transmissibility. And this can have a lot of problems.

In this particular study, which is done from Dutch healthcare workers, there were two populations in which they were kind of looking through. One was a very much unvaccinated population. And they were testing these people between January and April of 2020. And this is really when the -- when we had basically Alpha going through, or the first kind of variant of the Coronavirus. So when we were -- excuse me -- we have Wuhan and Alpha going through. So when we had -- when the first of these things, we only had this this D614G population, if you will. The vaccinated people really were looking at a wave on which we had the Delta Coronavirus going, basically a much more infectious -- known much more infectious virus. And what they found was even though they have a very, very close correlation between the two values -- Ct values on the same on these populations, that those from Delta ended up being uh having a much less replication-competent virus. And this -- so even though these populations with the Ct value, as you see right here, we didn't get actually good viral particles from that. And those viruses were not as infectious, even though this virus was -- were assumed to be similar through the Ct values of those two populations. Next slide.

Likewise, this is a a study that we've done by Ben Joe in the lab, in which, if we look at and compare RNA copies, which is what we detect with a Ct value on that nucleic acid amplification test, and determine with a standard curve and infectivity under conditions, we can see that we have the same number of RNA copies left at four degrees or room temperature or 37 degrees. Those are very, very similar at day three, day seven and only start to diverge at the 37-degree mark at day 14 and 21. But infectious virus titer held at infectious virus, actual viral particles there -- if we hold those at the same -- to the same levels with seven, if you look at the blue arrow here, you can see at day three, you get vast divergence of those viruses held at room temperature and at 37 degrees than you do the RNA copies.

What this basically tells you is that although you would have a very, very similar Ct at day -- at four degrees and 37 degree at day seven, you would have 100,000 fewer infectious viral particles at 37 degrees. So you cannot necessarily utilize -- you cannot utilize Ct to -- as a measure of infectiousness. A similar phenomenon was also identified by this preprint by Eyre et

al., and the impact of SARS-CoV-2 vaccination on Alpha and Delta transmission. They observed that viral loads determined by Ct were not representative of viral loads at transmission. Next slide.

So Ct's can be used at estimating genome copies. A standard can be used to actually help you improve the correlation between Ct and genome copies. NIBSC, which is the National Institute for Biological Standards and Control manufactures such a standard, and these can be used to help standardize assays between two different assays, and to each other. Standard curves for these should be run regularly. And these really should be run on a prospective basis. It's not something that you can now run a standard curve and claim all of your good data in the past, that you know how many genome copies you necessarily detected. That's probably not the best practice. It does not eliminate all the caveats associated with this, though. And these still cannot be linked to infectiousness or transmiss -- transmissibility without something like additional data. An example would be culture. Next slide.

So how can Ct values be used? They can be used prospectively in a quantitative assay. And there are ways to do that. I use a molecular standard with standard curves, monitoring of reproducibility of how per plate, per instrument, per operator, et cetera. These really should be used in conjunction with sequencing so that you actually look at the viral -- piece of target of amplification that you're utilizing to make sure that you don't have systematic changes in the assay site. And they can also be used as with other confirmatory lab data like culture that helps your confidence in the use of Ct values. Or they can be used in groups, as an estimate of viral load. The same assay really should be used for this to compare this, or you should use a comparison standard. And standardization improves of populations improves the correlation, sample type, symptom onset, asymptomatic, or symptomatic. And as you can see, I put an arrow here and really kind of saying that the top end of this slide is really the most the best use of these this data. And the bottom is really the kind of the not quite as good. But Ct values, again, should never be used as an estimate of infectiousness without additional supporting data. Next slide.

So the takeaways, Ct values are not a definitive measure of infectiousness. Ct values can correlate with genome copy. The studies that are designed prospectively to minimize variability, and for instance can be strengthened by applying a standard and a standard curve, especially at smaller sample sizes. Ct values can be used to compare data from populations or groups to infer general assumptions on viral load. They can be used -- Ct comparisons from the same test or standardized for references are preferable in this method. Language used here should be more suggestive and not definitive. Typical -- also, typical diagnostic and clinical reporting of Ct values are very difficult to administer and interpret. One number without a lot of background on how that number was actually derived is really, really hard to understand. Substantial technical barriers in diagnostic labs, in the major diagnostic labs, to actually getting these numbers out in any, in any real way. Assay kit result capture is positive generally for these labs, there's generally positive, negative, inconclusive, or invalid. And actually getting Ct values is not necessarily easy. And then also these labs a lot of the time use multiple assays which can

introduce significant variability, and the values can be generally greatly overly interpreted. Next slide. Thank you for your attention.

Thank you very much, Dr. Barnes. Next slide, please.

Now, I would like to turn it over to Commander Halpin. Commander Halpin, please proceed.

Thank you very much. Hello, everyone. Thank you for joining today. Next slide, please.

So in the past few years, the pandemic has really only further demonstrated the value that sequencing and sequence data are critical factors driving our ability to track, monitor, and analyze pathogens, including SARS-CoV-2. Based on the system that we've set up, you want to target something that is both representative and sensitive. And based on the system that we have set up across the country, both CDC, investments across the nation, as well as other academic and institutions who are working really hard to advance and improve our sequencing capacity, we estimate that there's a very high probability -- probably as much as 95% -- that our national baseline surveillance system would be able to detect something circulating at very, very low levels in the population. Something as low as even. 05 or. 03 percent. Next slide, please.

So why do we do genomic surveillance sequencing for public health purposes? Sequencing as a public health surveillance tool allows us to do population-level molecular epidemiology. And what does that mean? That means we can detect, track, and analyze any pathogens circulating in the population at a very granular level. We can watch over time as the proportions of certain variants change. And beyond variants, each of which has a particular constellation of mutations or genetic changes, we can zoom in on specific mutations of interest as well.

And finally, another strength of the genomic surveillance system and approach is that it focuses on collecting and sequencing primary specimens that are SARS-CoV-2 positive that can be selected for culture. And building a comprehensive repository of cultured viruses serves as a really important resource for the scientific community at large. And this -- these individuals, these laboratories, they're working really aggressively to characterize these specimens and these viral isolates as quickly as they can with regard to natural immunity, the impact on natural immunity, vaccines, therapeutics and diagnostics. For example, shortly after Omicron was reported to the World Health Organization in late November, CDC turned on enhanced surveillance through its national SARS-CoV-2 strain surveillance system. And the enhanced surveillance is really meant to target strains of interest or variants of interest.

In this case, we were targeting a mutation in the Omicron lineage as a screen, which allowed us to prioritize specimens for sequencing to confirm that if a specimen was indeed Omicron or not. And if it was indeed Omicron, then moving forward towards subsequent isolation. And states rapidly provided us specimens that fit this description, allowing CDC to start this process. And then once we're able to start this process, anything that's isolated can be shared with

partners who are working to phenotypically characterize SARS-CoV-2 variants, and it can also be used for phenotypic characterization in-house at CDC as well. Next slide, please.

I'm sure many of you have seen the CDC COVID data tracker. And this is actually a relatively old screenshot, but I wanted to pick something that wasn't all Delta all the time. And you can see on the left panel how Delta was really successful at edging out the other variants that were circulating across the country at the time. You see from week to week the changes that were happening with Alpha in the teal, and Gamma in the olive green, shrinking proportions in the sequence data week over week over week until it became virtually all Delta, that burnt orange color. And it's been that way ever since. However, we are watching closely to see how these proportions will change with the introductions of Omicron into the United States in the coming weeks and months. Next slide, please.

Now, genomic sequencing in general is still not what we would call rapid. Certain approaches, many approaches can require days to weeks to complete from specimen collection, to shipping, all the way through sequencing and analysis. Therefore, the results are not available fast enough to direct patient-level therapeutic choices. However, as I mentioned, we can use public health's genomic surveillance to monitor specific changes or mutations in the sequence data, including those mutations that are indicative of therapeutic resistance for treatments or preventative purposes. This includes both the monoclonal antibodies and the small amount of molecular antivirals that are available. Our sequencing surveillance system can provide information at the regional and perhaps even at the state level to help guide appropriate distribution of therapeutics, based on the prevalence of specific mutations that are associated with resistance to therapeutics used in COVID prevention and treatment. And we've included a few links to additional information on therapeutics themselves and how to order and administer them, if that is something you're interested in. Next slide, please.

Okay, so just to make sure you've been following along, our self-knowledge test check is that genomic sequencing should be ordered for persons diagnosed with SARS-CoV-2 infection for the following reasons: A, to determine which monoclonal antibody might be appropriate. B, to determine which small molecule antiviral might be appropriate. C, to inform recommendations for the length of isolation. D, to assess the need for high-level care. E, A, B and D. Or F, none of the above. Next slide, please.

And the answer, of course, is F, none of the above. Next slide please.

And the reason this is F, as I mentioned, the time required between specimen collection and availability of sequence data obviates the benefit of genomic sequencing for diagnostic purposes or clinical management at the patient level. We just aren't there yet in many cases in terms of speed. Furthermore, the results of genomic sequencing of SARS-CoV-2 are not typically CLIA-validated or authorized by FDA, meaning they're not meant to be used for -- on human samples in terms of patient management. They're not meant to diagnose, prevent, or treat disease or assess human health. If you're interested in more information about that, there's some information at the bottom of the slide footnote. CDC and other public health laboratories

across the country and globally are performing genomic sequencing for the following purposes. Surveillance, as we've discussed at length in this presentation. Investigations, and this includes, for example, outbreaks or superspreader events. And of course research purposes. Methods for near real-time characterization of variants are under investigation, and hopefully as the science continues to advance, we will see improvements in this area. Next slide please.

Thank you very much for your time and attention.

Thank you very much. Presenters, I would like to thank you for providing our audience with this timely information. We will now go into our Q&A session. Please remember that in order to ask a question using Zoom, click on the Q&A button at the bottom of your screen, then type your question. So our first question asks, are you aware of either the existence of or the development of any testing kits that test for both SARS-CoV-2 and influenza simultaneously?

Yeah.

Do you want to take that?

Sure, sure. Yes, there are several out there. There are actually a couple of rapid tests even that do SARS-Coronavirus-2 and influenza. And there are nucleic acid tests that are available for SARS-Coronavirus, flu, and RSV as well. Like I said in my presentation, we actually created a B-influenza and SARS test.

Thank you very much. Our next question asks, is there Ct value data available for the Omicron variant? And if not, do you have an anticipated timeframe having the data available and analyzed similar to the others?

So that's a really good question. And there were several in here about Ct values and use of those. And this is exactly what we're trying to discourage a bit. The tests that we have in large part, and there may be actually a -- I haven't checked in a little while, but there may be actually a test that is approved for -- that is actually approved for actually looking at the number of genomes or quantitative method. But most of the tests that we actually have out there are not quantitative. They are just for a positive or negative result. And doing that can come with a lot of different a lot of different problems. So I have not seen any data like that yet, but I wanted to make sure that we covered that.

Thank you very much. Our next question asks, do you anticipate that genomic sequencing will be used in acute clinical care in the near future, if the methods that you were discussing for near real-time characterization methods are available and authorized in time?

This is Alison. That's a great question. I think there is great promise in the sequencing technology. I think it's also important to remember that one of the key components is that there needs to be a defined use for clinical care. You know, knowing the variant that a patient is harboring or infected with may or may not impact their, you know, infection prevention

decisions being made with regard to that. And some of the mutations may impact treatment in the future. But I think part of it is recognizing that it's really important that we are very confident in the performance of the test before it's used for patient care.

Thank you very much. Our next question is specific to a patient population. I know, Dr. Patel, you talked about outpatient clinics, emergency departments, hospitals and nursing homes. The question asks, do you have recommendations similarly for incarcerated populations? Would you consider them similar to nursing homes or would you have different or varied recommendations for incarcerated populations?

That's an excellent question. So recently, CDC issued a HAN, which I'm sure we can add as a link if it's not already accessible to participants. And in the past, there are 2018 CID guidelines that are posted in the reference list, which consider long-term care facilities and nursing homes as institutions. Prisons -- there's no specific guidance on prisons or other congregate settings. However, in the context of SARS-CoV-2, I think there's a lot of flexibility for considering those institutions -- those congregant settings as institutions. So I think the HAN does layout that flexibility for purposes of testing, purposes of treatment with antivirals and possibly prophylaxis with the two antivirals that are currently available, oseltamivir and baloxavir. So that's addressed in the HAN released by CDC on December -- November 14th. [Note: This HAN was disseminated on November 24, 2021]. Thank you.

Thank you, Dr. Patel. And for our audience who are interested in looking at the HAN, you can direct your browsers to emergency.CDC.gov/HAN, and you'll be able to find the HAN in question in the archives.

Okay, we have time for one last question. And our question states, in light of co-circulation with SARS-CoV-2, does CDC have different or updated antiviral recommendations for influenza? Or do those recommend -- recommendations stay unchanged?

I'll take that question also. It's very similar to the previous question. And the HAN itself does address those. I think there is more flexibility that is necessary. And CDC recognizes that. There are no specific guidelines or recommendations that are made specifically to co-circulation SARS-CoV-2. So two things. One, in the setting of co-infections, antivirals for influenza can be used if there's no contraindications or limitations or restrictions for use. The use of antivirals baloxavir or oseltamivir could certainly help mitigate localized outbreaks with treatment and/or prophylaxis. And that can help reduce healthcare strain in the context of co-circulation of two viruses this winter. So there is a lot of flexibility, and the HAN covers those issues, but no specific guidelines or recommendations that are changing for influenza and antiviral use.

Thank you very much. This concludes today's presentation. I want to take a moment to thank the presenters for sharing their time and expertise with us. All continuing education for COCA Calls are issued online through the CDC training and continuing education online system at https://TCEOLS.cdc.gov. Those who participate in today's live COCA Call and wish to receive continuing education, please complete the online evaluation by January 10, 2022 with the

course code WC2922-120921 The access code is COCA 120921. Those who will participate in the on-demand activity and wish to receive continuing education should complete the online evaluation between January 11, 2022 and January 11, 2024, and use course code WD2922-120921. Again, the access code is COCA 120921. Continuing education certificates can be printed immediately upon completing your online evaluation.

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Continue to visit emergency.CDC.gov/COCA to get more details about upcoming COCA Calls as we intend to host more COCA Calls to keep you informed of the latest guidance and updates on COVID-19. We invite you to subscribe to receive announcements for future COCA Calls by visiting emergency.CDC.gov/COCA. You will also receive other COCA products to help keep you informed about emerging and existing public health topics. Keep up with new research and scientific studies about COVID-19 by signing up to receive CDC's weekly COVID-19 Science Update email by visiting the webpage on this slide. We also invite you to stay connected with COCA by liking and following us on Facebook at Facebook.com/CDCClinicianOutreachandCommunicationActivity.

Again, thank you for joining us for today's COCA Call, and have a great day.