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TESTING FOR HIV AND HCV: WHAT'S CURRENT AND WHAT'S COMING

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Testing for HIV and HCV: What's Current and What's Coming

[video transcript]

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Welcome to Physicians' Research Network. I'm Jim Braun, the course director of the monthly meetings of PRN in New York City. Since our beginning in 1990, PRN has been committed to enhancing the skills of our members in the diagnosis, management, and prevention of HIV disease as well as its co-infections and complications.

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We hope this recording of the presentation by Bernard Branson Testing for HIV and HCV: What's Current and What's Coming will be helpful to you in your daily practice and invite you to join us in New York City for our live meetings in the future.

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PRN is a not for profit organization dedicated to peer support and education for physicians, nurse practitioners, and physician assistants and membership is open to all interested clinicians nationwide at our website PRN.org.

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Now allow me to introduce Bernie Branson the former Associate Director for Laboratory Diagnostics in CDC's Division of HIV/AIDS prevention until his retirement in October, 2014 and current Director of Scientific Affairs LLC in Atlanta, Georgia.

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Thanks to everybody for coming tonight. Thanks to Jim for persuading me, twisting my arm to come back and talk to this group again about HIV testing. I had previously been at the CDC as the Associate Director for Laboratory Diagnostics until I retired from there about three years ago. I currently serve as a consultant, as Jim pointed out, to Gilead and I've also been compensated from Siemens Healthcare for doing educational presentations.

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I will be describing principles of HIV tests but also refer to tests by brand names for the purposes of identification and clarity. I don't mean to endorse any specific test by the use of a brand name.

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And some outline is to talk about the basics, how tests operate, go through some of the available HIV tests, the rapid lab and supplemental tests, how the differences between the tests relate to accuracy, a real little bit on the testing sequence for hepatitis C, and then some of the newer information on the effects of therapy and PrEP on HIV antibody test results, and some new information that's just been

accepted on the presentations of acute HIV. So, in terms of evolution of HIV tests, I think most people are familiar with this now.

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First generation tests are the whole viral lysate test that detected IgG antibody. The second generation, or so-called second generation, were EIAs using synthetic peptides but also still detecting IgG antibody. Third generation tests were a big improvement of taking IgM and IgG antibody. And then the most recent approved since 2010, the fourth generation, detecting IgM, IgG antibodies and p24 antigen. And the first thing I want to sort of talk to people about is to stop using the generation term. The CDC has really moved away from it. It's actually somewhat inappropriate at this point in time because generations referred to EIA technology and most of the stuff we're using these days are not EIAs anymore that I will talk about. So, all the tests and all the recommendations and the information from CDC is being updated to specifically talk about what the tests detect as opposed to using the generation term because the generation term is I think led to a lot of misunderstanding.

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But to talk about generations at least with EIAs and how the different tests work, the first and second generation tests basically use those viral lysates or recombinant proteins or synthetic peptides. But the crucial issue is that they bind both IgG and IgM antibodies but the detector uses antihuman IgG, which is why those tests in that category only detect IgG antibodies. In the case of EIAs, they use a color reagent and therefore the result is a color change and the results are expressed in terms of absorbance.

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Pretty much all of the rapid HIV tests that are in current use, the CLIA-waived ones and especially the lateral flow ones, use this anti-IgG technology and so they primarily detect only IgG antibodies.

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The rapid HIV tests of course are designed for results while you wait but CLIA-waived tests require that test use a direct unprocessed specimen, which is either whole blood or oral fluid. Minimal technical skills. The CLIA regulations state there has to be an insignificant likelihood of erroneous results and a fail-safe mechanism to verify that the test is working, which in the case of the rapid test is the control band. And in most of the rapid tests that are CLIA-waived, the control band determines whether or not adequate specimen has been added to the tests.

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The lateral flow tests the way they work basically is first of all they bind they're called immunochromatographic, it's a chromatographic strip. You add the specimen. It migrates across the strip first picking up the conjugate that's colored. If antigen is present, it binds to the antigen at the test line and then it binds over to the control line. But the crucial ingredient here there's just one strip, there's only so much conjugate that's present on there. It does bind to the conjugate first and not to the antigen first, so it's slightly different than the other mechanisms.

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The other one are the flow-through devices, also known as immunoconcentration. And those use microparticles that are mobilized on the little membrane on the test spots. And there can be more than one of them so they're suitable for doing multiplex testing.

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Basically, in the immunoconcentration devices, you pass the specimen through first and the specimen binds to the antigens first and then you pass the detection agent through the conjugate and that then binds to the antigen if that antigen is present and you rinse the rest of it through.

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And of course, the big example of that that's CLIA-waved right now is the INSTI HIV test, which is an immunoconcentration test. And recently evidence was published that the INSTI test detects IgM as well as IgG antibodies in part because of the concentration mechanism. And so that does have some effect on what its window period is I'll show you that data in just a couple of minutes.

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The last technology for rapid HIV tests is the so-called dual path platform. And the dual path you put the specimen in and it goes in one direction and goes and saturates a strip. You might just be able to see very pale blue lines at the top of the strip and then after you've given about five minutes for it to saturate that strip, the conjugate goes on the other direction and crosses the lines there. And if antibodies are present in the specimen, you will get the readout the same way you do in other sort of lateral flow looking tests. The dual path platform has a couple of tests currently FDA-approved.

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One of them is the DPP HIV test, the DPP obviously standing for dual path platform. It is approved for whole blood, for serum, for plasma, and for oral fluids, so it's the second oral fluid HIV test that's available. And the way it works-- I have an arrow here pointing to the little marker on the cotton swab which is used to collect oral fluid specimen. It's the same thing if you use loop for blood but you basically put that into this little vial, which they called a SampleTainer, and you break it off. So, there's some sample remaining in case you have to repeat the tests, unlike the other tests where you put the specimen directly onto the device itself.

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The CLIA-waived rapid tests. This is that list again of the things that I showed you which includes the INSTI test as the flow through test so that most of them, four of them are lateral flow tests, one of them is a dual path platform test, and then the INSTI is the flow through immunoconcentration kind of device.

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In terms of the antigens in RNA, I think everyone here is familiar with the structure of the virus and the antigens that are presented. But the things that we primarily are concerned with with respect to testing

are the surface antigens gp41, gp120, the core p24, and then of course RNA is being used in the nucleic acid amplification tests.

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I don't have an audience response system here but which HIV antibodies do the rapid tests detect? I sort of want to ask maybe for just a show of hands. How many people think they detect p24 antibody? Okay, how about gp41 antibody? How many people think that? Not too many hands. 41 and 120? All three of them, 41, 24, and 120? So, we're all over the map.

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And I want to show you that basically most of them detect only surface antibody. Gp 41 and 120 are in several of the tests but in particular two of them, the Oraquick Advance and the INSTI test have only gp41 antigen in it. And I'll talk with you later about why that's important but I just sort of wanted to bring that up. These are what the rapid tests are actually looking for. Gp 41 is the conserved immunodominant region, so almost all the tests include gp41 but then they include other things depending on the specific design of the tests. They also differ in the size of their blood specimen ranging from 3 microliters here for the Chembio Sure Check all the way up to 50 microliters for the INSTI and Uni-Gold recombigen tests and as I'll talk about later, the determined combo tests also use 50 microliters of O blood. 50 microliters is a hanging drop of blood like from an eyedropper or from your fingers. That's the kind of volume that we're talking about.

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The specimen volumes are a double-edged sword. A large specimen volume contains more specimen, more antibody, and can improve in sensitivity but it can be difficult to obtain by finger-stick. One of the companies was looking at making a home use test and they convened a group of physicians in order to obtain the specimen to perform the home use test. It was a 50 microliter test and not a single physician was able to perform the tests on himself to get the specimen for the device. And so, it is a double-edged sword in terms of the way it works.

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And finally, of course they all have a different time period for what they take to develop the test. In the case of Oraquick it's between 20 and 40 minutes compared to INSTI where the entire test can be completed in about 60 seconds. And so, there's a good deal of variability.

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The difficulty with not waiting the sufficient time period is that you will get sometimes a false negative test if you don't wait the minimum time period. And if you read the test beyond its maximum read time, sometimes the conjugate migrates back and you get a false positive result. So, paying attention to the read times on these tests is really important. And the length of the test procedure obviously also influences throughput, how many people you can test in a short period of time.

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In terms of the EIA third generation or at least the concept here with binding of HIV antibodies both IgG and IgM, in this case it uses HIV antigen conjugated to the color detection reagent as opposed to the anti IgG. But then performs the same way as a regular EIA with a color change in the presence of either IgM or IgG antibodies. And this was again a major advance to be able to detect IgM and I'll talk a little bit later about the potential utility still for third generation EIAs. But most of the third generation EIAs that were licensed used p24 and gp41 and gp120 antigens so they had more different antigen detection as well as a different mechanism of action.

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These were the sort of major ones, Abbott Architect, the Bio-Rad EIA, and the Siemens Advia Centaur all had third generation EIAs. They also now have so-called fourth generation tests, the antigen antibody combo tests. But the only one that wasn't EIA of this group was the Bio-Rad. The Abbott Architect and the Siemens Advia Centaur are all chemiluminescent assays and the new technology is always going toward chemiluminescent assays. And what they use are magnetic microparticles.

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They're coated with antigens or antibody in the case of the anti p24 antigen. If you add the sample to that, they bind if antibodies or antigen are present in the specimen. You then add a light reagent to that and a wash solution and if it's a positive test, it emits light and so its results are relative light units. And so, as opposed to absorbence from the EIAs, this is the opposite which is it creates a light which is measured. They have a much wider dynamic range and so they have some better sensitivity because of the way they operate. But because we are now talking about the so-called fourth generation tests mostly being chemiluminescent essays, that's one reason for not using the term fourth generation because it's a whole different technology.

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These also operate mostly on random access multiplatform analyzers for HIV testing. So, on the platform there are maybe 40 different assays which include potentially HIV, HCV, could be a pregnancy test for HCG, vitamin D. You just press a button or do a check mark for which test you want. You can do a stat sample without pausing. Most of these platforms develop their results within 30 to 60 minutes and so they're a very quick turnaround time compared with the old EIAs which took about four hours. The antigen and antibody tests obviously allow earlier detection.

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They're suitable for high volume screening in hospitals and health centers. You can get quick turnaround for the test results with these tests. And you can screen for multiple viruses like HIV and HCV from the same sample if you're doing it in a hospital laboratory. I do want to mention the determine combo antigen antibody rapid tests because we get a lot of questions about it. The determine combo was FDA-approved in 2013 and CLIA-waived back in 2015. It has gp41 and gp120 antigens on it so that's the antibody that it detects. It has a separate line for the detection of p24 antigen and then a third line which is a control reagent. This one was CLIA-waived in 2014. As I mentioned, it takes 50 microliters of a

whole blood specimen. A lot of people refer to this a fourth generation test. As I mentioned, in general we don't want to use the term fourth generation but rather antigen antibody combo tests. And the issue here of course is that the performance of this test is very different than the laboratory tests.

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So that in five studies involving 24,000 people with whole blood specimens, the determine combo didn't detect p24 antigen from any of the 33 acute infections that were identified by the laboratory tests or by pooled RNA testing. And in looking at seroconverter panels, I'll show those later. So, eight seroconverters, eight of this group showed a median delay of about six days between the time that determine reacted with plasma and the time that it reacted with whole blood. This problem with detecting antigen with the determine combo is a function of whole blood specimens and so there's less antigen, less plasma, less serum in a whole blood specimen and the test does not operate the same on whole blood as it does on serum or plasma. It's only waived for use with whole blood so for point of cure use it does not have good sensitivity for p24 antigen. As a rapid test in the laboratory with serum or plasma, it's not quite as good as the laboratory tests but it's performance p24 antigen is a lot better and I'll show you how it compares with the other tests in just a minute.

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Finally, the most recently approved test was the Bio-Rad Bioplex HIV antigen combo assay. It uses a bead conjugate to different antigens HIV 1 and HIV 2 and its claim to fame is that when you get a result, it tells you whether it was P24 antigen or HIV 1 antibodies or HIV 2 antibodies that are present that caused reactive tests. The company wanted them to market this is a fifth generation assay and that's when everybody got over the generation stuff and said okay, enough of that stuff. And that's partly I think the biggest stimulus for the movement away from generations.

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In terms of lab testing, the drawbacks of lab testing of course is that you have to draw blood. There's a need for a phlebotomy. But with the antigen antibody combo tests, there are also some problems with handling specimens. So that for a clinic or for a place that's at a distance, you have to separate the serum or plasma from the red cells and the specimen is only stable at room temperature for a short period of time so transport can be a problem. The Abbott Architect is three days, the Bioplex is four days, the Bio-Rad combo EIA is for only two days at room temperature so that basically if you were shipping specimens unless you're put it and keeping them cold it's problematic. The Siemans Advia Centaur, another antigen antibody only allows for 24 hour stability at room temperature so that you have to be careful with specimen handling otherwise you lose sensitivity for p24 antigen. Probably a lot of folks have seen this before.

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It's been used a lot looking at the laboratory markers as a schematic diagram. Infection happens here at point zero. HIV RNA is the first thing to appear an estimated 10 to 14 days after infection. Subsequent to that, p24 antigen appears approximately five days later. It increases until antibodies begin to appear. It then binds with antibodies and the antigen antibody complex makes it undetectable. So, it's not exactly

like p24 antigen goes away but you just can't detect it anymore without doing some kind of dissociation step. In terms of antibody development, it begins at around 21 days with IgM antibodies and then somewhere around 42 days is when the IgG antibodies develop. So, what we're looking at here in terms of most rapid tests have a sensitivity or an interval from infection to detection of somewhere between 45 and 50 days. Most laboratory antibody tests are sensitive for IgM and therefore 21-22 days from the time of infection and the antigen antibody test of course at around 15 days from the time of infection. But the difference between rapid tests in general is between four to eight weeks or between antibody tests and so that's where we have to make choices and know what is the exact tests in fact that we're using.

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Looking at an example of this, this is on a large set of specimens that we actually first tested with rapid tests back in the early 2000s. We compared it to a regular IgG EIA. And what you see in terms of rapid tests on the left side and the false negative column here on the right is compared to an IgG EIA, we had a relatively small number of false negatives. The largest number of those was with the oral fluid test, fewer with the same test when used on blood. When you compared it to an IgM EIA, those same specimens, we tested them retrospectively, you can see that the number of actual false negatives really went up substantially.

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And then when those were all tested with RNA, we saw that there were almost three times as many false negatives on those tests so it all depends on what the comparator is. And for me that's troubling because when you look at the package inserts for most of these tests and they talk about sensitivity and specificity, the sensitivity is compared to the IgG EIA. So, when they talk about 95 percent or 98 percent or 99 percent sensitivity, it really is this as a comparator rather than the more sensitive comparators that we now are routinely testing for.

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At CDC we tried to take a look at how all of these tests compared with each other using seroconversion panels and these are the results of all of the FDA-approved tests. And so, this was basically how many days before the Western blot turned positive. We didn't know when people got infected so we compared them all to Western Blot positivity. This is obviously hard to interpret and so we basically drew a line across the middle and said all right when can all the tests detect half of the specimens on these seroconversion panels that are positive? So, this is a 50 percent cumulative frequency that we're talking about compared to Western Blot positivity and this is the way the tests compare with each other. The APTIMA qualitative RNA tests picked up infection 50 percent cumulative frequency about 26 days, nearly a month, before the Western Blot turned positive. The first generation EIA, the Vironostika IgG EIA that was the comparator for the rapid tests before identified infection about two days after the Western Blot turned positive. The lateral flow tests, CLIA-waived ones, SureCheck, STAT-PAK, Unigold, OraQuick between one and five days before the Western Blot turned positive. The flowthrough tests, Multi-Spot, Reveal, and also the DPP slightly sooner. Six or seven days before the Western Blot turned positive. The INSTI test, as I mentioned, picks up IgM antibody and so it's around 9 days before the

Western blot positivity. The laboratory IgM sensitive tests around two weeks before the Western blot turned positive. The antigen antibody combo tests around three weeks before the Western blot turned positive. These are all again with plasma specimens on seroconversion panels. In addition, the Determine antigen antibody combo test on plasma, as you see here, picked up infection about two weeks before the Western blot turned positive, right about the same as the IgM sensitive laboratory tests and the antibody component of the Determine combo was really quite sensitive. And then the Bio-Plex was the same as the other antigen antibody combo tests at around three weeks before the Western blot turns positive. I keep emphasizing that this was done with plasma and I do that for a couple of reasons. One is that in an oral fluid comparison, compared to an EIA, the oral fluid detected infection 40 days later, 40 days after the Western blot turned positive. So, the window period with oral fluid testing is about six weeks longer than it is with blood testing for people in the same kinds of interval.

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In the Bangkok tenofovir study, in addition they identified delayed detection in oral fluid of patients who were taking PrEP. So that in this study, the participants who were receiving tenofovir who got infected while on PrEP took longer to develop a reactive oral fluid test, the OraQuick, about six months longer than the participants receiving placebo who had turned positive in about two weeks. I think there's a couple of potential explanations for this that I'll go into a little while, at least speculate about somewhat. But again, I think oral fluid especially in doing followup testing for people on PrEP is really ill-advised. Now, in addition to oral fluid, this is where the Determine combo stands when you use it on whole blood. So, as you see here, it detects infection about eight days before the Western blot turns positive or really right in the middle of the other rapid tests. And so its sensitivity when you use it on whole blood is very comparable to other rapid HIV tests. It is not comparable to the laboratory-based antigen antibody combo tests. INSTI tests on whole blood, however, basically has the same sensitivity, about nine days before. So, it does not suffer the disadvantage that the Determine combo does when you use it as a point of care kind of test. And then the Multispot supplemental test and the Geenius test used as a supplemental test is pretty equivalent to sensitivity for the Western blot. The difficulty of course in all these comparisons is that what we're looking at here is comparing them against the Western blot and what everybody really wants to know is the other side of this question is what's the window period? And that's pretty hard to figure out because you don't know when somebody got infected. I'll be talking a little later about some other data from this where we'll be getting more information. But there's a study going on in Thailand where they're doing finger-stick RNA testing twice a week on high-risk individuals and so they pick them up very quickly from the time of infection. They will begin learning a lot more about this early interval.

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But these data from the seroconversion panels were re-analyzed and looking at the window periods, there are a bunch of different window periods. The old one was a seroconversion window period between infection and the Western blot. Now we have Eclipse Period, which is the time when nothing is detectable after infection. When infection is detected by virus before antibody, that's the period of acute infection from a laboratory definition. When IgM becomes detectable, we then have the period of

recent infection until IgG becomes detectable and the antigen disappears. And then of course we have longstanding infection and that whole time period. And the real challenge with figuring out window period was figuring out how long the Eclipse Period is because you don't know when somebody got infected. You can't really detect that.

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People have done some Weible modeling. This was published in CID and last year. I have a reference in another slide. Looking at the eclipse period and what you can see, this is what the range basically is of different eclipse period that it goes anywhere from around five days out to about 30 days depending on the individual but the average is between seven and ten days from the time of infection before RNA becomes detectable.

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And using that with the other data that we had from the seroconversion panels, this is the table of the estimated window period of the different kinds of tests. So that for the antigen antibody lab tests, the median value for the window period is 18 days. For the IgG IgM sensitive antibody laboratory test, about 23 days. You can see that for the IgG sensitive rapid screening test we're out at around 31 days, not very much different from the supplemental tests or the Western blot, around 36 days. So that the shortest is around 18 days and most of the tests we're using at point of care are about 30 days in median window period. Now fortunately, they also calculated here the 99th percentile. And for me that was really important because the question that everybody wants to know is when can I be sure? After this last exposure, when can I be sure that I wasn't infected. So, this basically tells you what the outside range is, the 99th percentile, for all these tests. It's about 44 days for the antigen antibody test and it ranges out to what we used to say, a little over two months for the Western blot when you could have your certainty in that. These data both give us what our median is for the different tests as well as the outside range based on the seroconversion panels and hopefully we'll get more data to support this from these other studies.

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Now, this is I think an update of that slide that I showed you before looking at when different things develop to see that the RNA and the antibodies are the same there. But what they marked is what the threshold is for the second generation test-- they didn't get the memo about not saying generations-- so, that's when the IgG sensitive tests were there and sensitive. And then we have down there with the IgM antibody or potentially antibody antigen combination tests were sensitive down at the lower lines so they put those thresholds on here. And the thing that really I want to show you is this is what has happened in people who were started on ART during their acute HIV. So, this is their pattern of antibody response. So, you see the RNA went down very quickly there, the red line from that. But as you see, in most of these cases in people who were started during acute HIV infection on therapy, their antibody level never got high enough to be detected on these anti IgG tests. Most of their antibody response was really, really considerably attenuated. In particular, the gp41, which is the envelope there on the bottom, you can see is that it barely comes up to being detected by the IgM IgG antibody tests. And that's the gp41 that's in all of the tests that they use and in the case of the OraQuick and the INSTI test,

it's the only antigen that's there. It's the only antibody that would be detected. So, this is now I think raising some concerns about what happens when we start early therapy. This is the p24, which does seem to be a little bit less affected by the antibody attenuation effects of early therapy.

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Now we look at basically the study where they looked at second generation tests and third generation tests and fourth generation tests in people at different stages after treatment. So, this is up out to 24 weeks after treatment. In the little bar that's hatched over there, those are people who are identified as only NAT positive. They're p24 negative and IgM negative. And you can see that on the second generation tests, 60 percent of them did not have a detectable antibody at 24 weeks after the time of infection. But importantly, looking here which is sort of worrisome to me on the fourth generation assays, it also didn't detect very well on that and 17 percent of all people who had been started on treatment during acute infection were undetectable by fourth generation assays at 24 weeks after the time of infection compared to only 4 percent of people who were undetectable at 24 weeks by the third generation immunoassay. And I think the reason for that is that the third generation assay detected p24 antibody. The fourth generation test, because it detects p24 antigen, can't detect p24 antibody because it uses p24 antibody to detect the antigen. And so, I think it's got to do with which antigen is present but overall the real concern is here is that looking at the second generation tests were negative about 33 percent of people who had been started on early therapy and 17 percent of people were negative overall on fourth generation tests. And so, I think that trying to demonstrate positivity for someone who's been started on effective therapy and achieves viral suppression is going to be a challenge to prove whether or not somebody is positive, especially when they've been getting therapy someplace else and they come to see you as they go along. This is actually a worthwhile paper to look at. NCID in 2016, they staged people-- I'm not going to go through all of it by the different fiebig stages in terms of what was present, whether it was just NAT that was detectable when serotherapy was started, whether it was NAT and p24 antigen that was present, whether it was NAT, p24 antigen, and IgM. But you can see for all the people started early during therapy, there was an attenuation in the antibody response. For those of you who haven't seen it, there was a paper on that I'm not sure-- it was published before print, I'm not sure if it's out yet in AIDS about doing empiric therapy for acute HIV infection identified in patients in the emergency department so that LAC USC when people come into the ED, they're screened with a fourth gen antigen antibody test. I keep saying gen, sorry. But they're screened with the antigen antibody test and if that is positive, they're interviewed by the ID staff and if they have risk factors, they start on ART before any of the other test results come back. And so, we may start seeing more people who don't develop full antibody responses.

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In terms of PrEP, we know that it requires an HIV-negative test at baseline and it's recommended to be repeated every three months. Because of the oral fluid difficulties that I pointed out, the serum or blood based HIV test is the most appropriate choice on that. And this is just the data from the study. 436 who were negative by both oral fluid and serum EIA at baseline. Two of the serum-- the oral fluid negatives at baseline actually were already positive on their serum EIA. And then there was this big delay in the people who got infected in the positivity on their oral fluid tests, so it was not terribly useful for that

kind of monitoring. But I've been called on consult now and I think and this is the first of three cases related to people taking PrEP.

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In this study, this was a 25-year-old MSM in an HIV-discordant relationship. He'd been started on PrEP. He said he was perfectly adherent. He had two other sex partners in addition to his primary partner. His primary partner was virally suppressed. His antigen antibody test was repeatedly negative until May, 2016 at which time he had a positive antigen antibody test and negative multispot antigen antibody differentiation assay. His RNA was less than 20 but reported as signal detected. They repeated that two weeks later and four weeks later and the results were exactly the same. Reactive antigen antibody test negative, HIV 1 HIV 2 antibody test and HIV RNA less than 20 but signal detected. So, they had called me and asked what they should do and one of the questions was should they just take him off all the therapy and see what happened, see if the virus went up. And I recommended that they try to get a western blot on the person.

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And they did that and his western blot had bands at 160 and 55, at 31 and 24, and the one that was missing was gp41. And the multispot test actually is also a gp41 only test. So, this is an example of potentially PrEP having that same effect. And I was thinking back, way back in 2003, we had reported that people who were tested with OraQuick and started an early and effective therapy, and this was 2003 so early therapy then had a different definition than it does now, but they did have waning titers and a proportion of them had absent gp41 titers. I wonder whether some of the people in that Bangkok study was also because it was gp41 and not just because it was oral fluid that they had delayed detection with using that test. In this particular patient, they ultimately were able to get a DNA genotype. He had multiple drug resistance including to TDF and FTC and his virus was not related to that, it was a virally suppressed partner. So that this was a case basis of a PrEP failure acquiring multiple drug resistant strains.

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For that reason, I say the antigens matter, the OraQuick Advance, INSTI, and the Multispot all use only gp41, which might be affected by PrEP, which might be affected by early therapy. Laboratory antigen antibody complex test also use only gp41 in a detection system and so as I showed you before, these rapid tests and the fourth gen tests may miss a lot of people who don't develop antibodies when they started an early therapy or potentially on PrEP.

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This caution I'm going to say is that it does diminish with early effective therapy. That's old evidence and that is responsible for the warning in some of the rapid test package inserts that people on long term therapy may test false negative and then now these delayed seroconversions among patients infected while on PrEP as well as patients on early therapy.

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This is the current algorithm for HIV 1 HIV 2 testing. You start with a combo immunoassay, the antigen antibody assay and follow that up at the current time with an HIV 1 HIV 2 antibody differentiation assay. If that test is positive for HIV 1 antibodies or for HIV 2 antibodies, that's the end of the story. Sometimes it's positive for both HIV 1 and HIV 2 antibodies and the question is what to do at that point in time if the person is positive for both. At the current time, the recommendation is if you're negative on an antibody test, you want to do an RNA assay but also if you're positive for both, you go on to do an HIV 1 RNA assay. And if the HSV 1 RNA assay is reactive in the presence of antibodies for both HIV 1 and HIV 2, you can tell that patient they're infected with HIV 1. You don't need to investigate HIV 2. HIV 2 infection is extremely uncommon. HIV 2 cross reactivity is very common with HIV 1. And if a person is positive for HIV 1 RNA with cross reacting antibodies, you don't need to do a further workup for HIV 2, which obviously not very easy to get HIV 2 RNA testing.

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This differentiation assay, that's the second test, has at the time these were written and conceived, it was the Multispot assay. The Multispot, as I mentioned to you, had a recombinant and a peptide but only gp41 that was on it. It was withdrawn from the market in July of 2016. New York bought enough to last I think another year, so they were using it for a while after that. But it's been replaced by the Geenius assay, the Geenius HIV 1 HIV 2 assay, which was approved in October of 2014.

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The Geenius is-- this is the whole assembly. It uses a laptop and a reader and a cartridge. It is sort of like an HIV 1 HIV 2 rapid western blot test.

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It's got bands on it. For HIV 2, it has gp36 and gp140. For HIV 1, it has p31, gp160, p24, and gp41.

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The way this test is done, this is another dual path platform test. So, you can use either whole blood or serum or plasma. You put it on at the bottom port and let it migrate up to it.

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You then follow that with five drops of buffer in the second well to migrate across the test.

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And then after 15 minutes, you put the cassette into a reader for an automated interpretation. And when you look at the readout, it takes a picture of the test result.

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It tells you which bands are present on the test and it gives you an interpretation of the test. This reader actually is really quite sensitive. It's got ten levels of intensity. The first five of which are not visible to

the naked eye. And so, although in the European Union, they sell the tests without making you buy the reader, in the US we don't have that choice. The FDA requires that you use it only with a reader.

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And the problem with it is that there are eight different results that came out so that for those of you haven't started receiving these results, the big advantage of the new algorithm was it did away with a bunch of indeterminates and guess what? They're back. So that the easy ones are HIV negative, HIV 1 positive, or HIV 2 positive. So, those are three possible results that it comes out with. But there are three kinds of indeterminates, HIV 1 indeterminate, HIV 2 indeterminate, and HIV indeterminate, which means there are both stripes for HIV 1 and HIV 2 there but not enough to meet a diagnostic criterion. And then there are two more categories of cross reactivity or untypable or undifferentiated. So, I think this is going to create a lot of headaches for folks in terms of doing it. In my personal opinion, especially since I don't work for CDC anymore, I think what we ought to be doing is going from a positive first test to a viral load because we're going to want a viral load on all these people because 99.8 percent of them are going to have a reactive viral load and we won't waste time with another antibody test. But none of the viral load tests are approved for diagnosis. Only the qualitative RNA test is approved for diagnosis. Now, hepatitis. Roche just got a hepatitis C test approved, a viral load test for diagnosis. But that of course because it's the second test in a row because there's enough of a market for it. So, if we made RNA the second test, then I think the viral load test would also get approvals for HIV but I can't wait for it. And that's what I'm sort of saying here is that we're going back to indeterminates and difficulty with interpreting antibody tests. I think nucleic acid tests are going to play a much bigger role in our testing and interpretation in the future.

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In terms of acute HIV, what I wanted to talk about here is a paper that was just accepted.

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We basically had six emergency departments across the country who were doing antigen antibody screening of all their patients coming through for different time periods with the antigen antibody tests. But basically, overall they performed a substantial number of tests. It was 214,000 overall. They had about between 1 and 2 percent of those tests were reactive. Of those reactive tests, about 25 percent overall and in some places more were new HIV infections but 14 percent of the people with new HIV infections in the ED had acute HIV infection. So, they were antibody negative. They would not have been detected without an antigen antibody test.

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And in these people, we looked at their chart to look at what their reason for visit was when they came in and compared the people with acute HIV and the people with established HIV. And what you see is that the people with acute HIV mostly came in for symptoms associated with a viral illness. They came in because of the symptoms of having HIV infection: fever, viral syndrome, gastrointestinal. It appeared people with established HIV who came in for more usual emergency department stuff, musculoskeletal problems, for pulmonary problems, for people requesting the test.

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In terms of signs and symptoms that were present at the time, 75 percent of the people with acute HIV had fever. 67 percent had gastrointestinal complaints and very often abdominal pain. 57 percent of them had malaise. It was actually interesting, the least common things were skin rash, sweats, and lymphadenopathy, which are the stuff that we always heard about that was commonplace with acute HIV infection. Only 7 percent of people had no symptoms or signs. Most of them had a lot. So, 61 percent of people had fever and at least three other symptoms or signs. So, the people are pretty sick when they came in with acute HIV infection or at least the ones who were seeking care in an emergency room probably for those symptoms.

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In terms of HCV infection, I'm not going to talk much about HCV tonight but the schema is very much the same for HIV. You start out with the HCV antibody test. If it is non-reactive, you consider the person to not be HCV-infected. If the antibody test is reactive, you go on to do an RNA test in order to identify people who have current HCV infection or active HCV infection. If that RNA test is negative, the person either has resolved hepatitis or had a false positive initial test and hepatitis hasn't gone through the root to say what we should do to rule out false positives in that circumstance. You're probably less familiar with the HCV genotype but this is sort of what it looks like with the different components of it.

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All of the HCV tests look for IgG antibodies. The version 1.0 only detected for the NS4a antigen had a sensitivity of about 80 percent, window period of around 15 weeks. The more recent one, the 2.0, detects core antigen, NS3, NS4a. That's what the protease inhibitors, boceprevir, telaprevir, and ledipasvir work on. That's what they inhibit. And then the most recent ones, the third generation so-called HCV tests, detect a lot more core NS3, NS4a, NS5b, but 99 percent sensitivity, window period 10 weeks and that additional antigen, the NS5b, that's the one that the new polymerase inhibitors work on, the sofosbuvir in the dasabuvir. So that we're now looking at the antibody diagnosis of hepatitis C being pretty accurate at least for screening for people. It probably, however, doesn't make the most sense because the thing we really would like to know if it's present or not is hepatitis C core because the core would be the think that indicates the presence of current active infection.

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We used to be that we did arrive a RIBA, the recombinant immunoblot assay. That's no longer commercially available. People used to use signal to cutoff ratios for hepatitis C antibody test. That's also no longer recommended to do that. So, it's basically a positive negative kind of thing.

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In terms of HCV core antigen, about 50 percent of the HCV antibody positive people who get tested never get an RNA test result. So, we don't know whether they're infected. The core antigen test is actually approved in Europe for testing. You perform it on the same specimen as the antibody test, which makes it very efficient. It does require an initial step to disassociate the antigen antibody complexes but we've been encouraging people to bring the core antigen test to the U.S. because that

would be the ideal screening test to find people with chronic HCV infection. And certainly in places like corrections where everybody's antibody positive, you'd like to screen for the core antigen in the first place in order to look for the people who truly had active hepatitis.

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Interpretation in terms of lab testing for HCV. A negative antibody test means the person is uninfected. If you have a suspicion of acute HCV infection, you would repeat the test in six months because of the window period being long. If the antibody test and the RNA test is positive, the person is considered to have active infection. If it's positive antibody test and negative RNA test, they're considered to have resolved infection or maybe a false positive antibody test. And the best thing to do in that situation is use a different antibody test because if both antibody tests are positive, it really increases the likelihood that it's resolved hepatitis C infection as opposed to a false positive test.

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On the horizon, there's a couple of things that are coming.

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First of all, the lateral flow multiplex tests. This is in clinical trials right now, the DPP HIV-Syphilis test which is actually very useful in a couple of places. One thing this company markets outside the United States and isn't currently planning to bring the United States is a syphilis screening confirm test so it's sort of like getting an RPR and a treponemal test on the same person in order to look at it. We're trying to encourage them, again, to bring that to US.

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The RNA tests, the qualitative or quantitative? I already mentioned that it's only the qualitative tests that are approved for diagnosis but it's the viral load tests that we all want the result from. The laboratory has to follow the package insert. It can only do the tests that the FDA says is approved for diagnosis. But as a clinician, you can order whatever you want. And so you can order a viral load test, which a lot of people have done, in order to resolve folks who have a positive antigen antibody test and a non-reactive antibody test afterwards. And again, we're hoping to encourage places to move in that direction.

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In particular because we're now coming into the era of point of care nucleic acid tests. This one is pretty far along. It's the gene Xpert test. It's approved in the European Union for both viral load and for a qualitative test. For a viral load, it takes one milliliter of plasma, gives results in 90 minutes. Limited detection down at 32 copies per milliliter and the qualitative test has a limited detection, about 200 copies per milliliter. This gene Xpert platform is already in a whole lot of hospitals. They use it for testing for MRSA, for TB resistance, for things like that. So, I think that it comes either with a single standing unit like this or one that's got 64 of them in a big platform and all the cartridges for the different tests are interchangeable so you can test for any one of those nucleic acid tests using the same device.

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The viral load test for HCV was also approved in the European Union and is being moved along in the United States. It does work for genotype 1 through 6. Again, produces results in about an hour and a half.

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This is a point of care nucleic acid test made by a Alere which is marketed outside the U.S. It takes a 25 microliter whole blood specimen and they claim it does an HIV 1 or HIV 2 viral load in about 60 minutes. I'm a little skeptical about HIV 2 because HIV 2 viral loads are very low. Usually you have you cellular tests on that. Again, it's approved by the European Union. They have not yet indicated their intention to come to the U.S.

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And finally, that group is the LIAT, the lab in a tube test, which was acquired by Roche and they're working on improving it. They had been in use before, at least outside the United States. The LIAT lab in a tube test is already FDA cleared for influenza A and B and for strep and the strep test is already CLIA-waived because so easy to do. So, these nucleic acid tests I think begin to offer us the prospect of doing point of care Nucleic Acid Testing, RNA testing on people and it will solve some of these antibody test result controversies that we've had.

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In summary, the HIV and HCV tests continue to evolve. The highly sensitive lab assays for HIV can now deliver results in less than 60 minutes making it feasible to use for emergency department testing. The point of care multiplex tests for HIV, syphilis, and HCV are under evaluation. And I think that the point of care viral load tests are probably around the corner. We'll begin seeing some of those pretty shortly. So, the advances will hopefully keep pace with our advances in treatment.

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Thank you very much.

[Video End]