Thank you for joining me for this seminar, where I hope to provide a review of HIV tests, including some new information about test performance, what’s coming next, and what it might mean for your work.
Acknowledgements

- Most of these slides have been presented previously
- All of this work is done collaboratively

- John Brooks, Pollyanna Chavez, Elizabeth DiNenno, Steve Ethridge, Debra Hanson, Silvina Masciotra, S. Michelle Owen, Joseph Prejean, Phil Peters, Bill Switzer, Laura Wesolowski

I have drawn from presentations by my CDC colleagues, including Dr. Michelle Owen (now NCHHSTP Associate Director for Laboratory Science) and Dr. Silvina Masciotra in the Lab Branch and Dr. Laura Wesolowski who is Activity Lead for HIV Diagnostics on our team in BCSB. Our other colleagues listed here all support projects related to HIV testing, and/or work on projects evaluating new HIV tests used in the US.
So what is the window period?
What is the window period for an HIV test?

- 10 days
- 18 days
- 45 days
- 90 days
- All of the above
- None of the above

What do you think or tell people the window period is? Lots of choices up here...

Ok, so, I do teach MPH students and give tests, and this is an example of a bad test question, because I think there are two potential RIGHT answers here...
What is the window period for an HIV test?

- 10 days
- 18 days
- 45 days
- 90 days
- All of the above
- None of the above

All of the above is definitely correct, because the window period of a test varies by test, and different test technologies have different window periods, all of the numeric answers could be right, depending on the type of test, specimen type or an individual clients situation in terms of their route of exposure or immune response
But, if after the test you came into my office and said None of the above could be right too, I would ask you “Well why do you think that?” but I could be convinced... Really the window period is not 1 number, it’s a range, each person is different, so if you have a really vigorous immune response your infection might be detectable by at least one test less than 10 days after infection, and if you started treatment really early there is a possibility that some tests may never indicate you actually have infection because the analyte or biomarker they detect might not be in the body fluid that test is used with...
So, how do we define a tests window period? It is the time between infection and when a diagnostic test can reliably detect infection after an exposure.

The graph describes the different viral markers that appear after HIV infection and shows the early phase of the window period, or eclipse period, where the virus cannot be detected in the blood stream by any diagnostic test.

During this time the virus is replicating in sub-mucosal cells and can not be detected in a blood specimen.

This earliest part of the window period could last from a few days to a few weeks.

After the eclipse period ends, different biomarkers can be detected in the blood, and they all appear at slightly different times after infection.
This means we need to know what the test we are using is looking for in order to know its window period.

We (CDC, Michele Owen et al actually!) coined a term to classify different tests by how they work! CLICK

Many of you are probably familiar with the “generations” terms a 1st generation Western blot, a 4th generation rapid test, et cetera!
Much like other recent uses of the term, after awhile the tests in the supposedly different generations started to look a lot a like, some of the differences are really subtle but important, and sometimes, much like different stories of the Enterprise and her crew - within the same “Generation” different HIV tests are actually quite different...
So we decided to try to reset our thinking about the different types of tests, and focus more specifically on what they are looking for...
But remembering that even within categories defined by biomarkers, unless we say what type of test we are talking about, and what type of specimen we are using, we aren’t going to know the whole story... I will explain more about this in a little bit...
But I want you to take a quick look at this slide, think about how the generations map to the biomarkers that the tests detect and the types of test platforms that can detect that biomarker...

We are running “4th generation” rapid tests on fingerstick specimens, we have “3rd generation” laboratory tests that can produce results in less than an hour but aren’t called rapid tests, two “3rd generation” rapid tests with very different performance, and really the Western blot is the only 1st generation test left, if you can find it!

So look at this carefully, so you can remember how the generations map to the biomarkers detected and types of tests...
And then quickly move on from generations... I know it will be a struggle, but the future of the universe depends on it...

### The window period depends on what the test detects, and how it works!

- This means we have to know what the test we are using detects...

### Generations?

- Biomarkers

<table>
<thead>
<tr>
<th>Biomarkers detected</th>
<th>Type of Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>p24 antigen, IgM and IgG antibodies</td>
<td>Lab test performed on plasma</td>
</tr>
<tr>
<td></td>
<td>Rapid test performed on plasma</td>
</tr>
<tr>
<td></td>
<td>Rapid test performed on blood or oral fluid</td>
</tr>
<tr>
<td>IgM and IgG antibodies</td>
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<td></td>
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</tr>
</tbody>
</table>
So, back in this universe again, the window period of an HIV test is defined as the time between infection and when a diagnostic test can reliably detect infection.

The graph describes the different viral markers that appear after HIV infection and shows the early phase of the window period, or eclipse period, where the virus cannot be detected in the blood stream by any diagnostic test.

As well as the fact that in a relative way detection of some viral markers will happen sooner (RNA) and some viral markers show up later (IgG) after infection. But again, these are sort of average curves, and you’ll notice that I didn’t put numbers on either the X or Y axes… That’s because some tests might be able to, for example detect IgG at lower levels and therefore earlier than those that require higher levels of analyte to give a reactive result.
Why is the window period of the test you are using important?

As you all know, HIV testing is a key component of HIV prevention and knowing when an HIV test can accurately detect an infection is important for patient care.

The CDC recommendation for when to get tested following an exposure and if the initial test is negative is highlighted with blue text and underlining in the slide. This currently appears in the FAQ on the HIV testing Basics page on our website... Last night an aids.gov blog referred everyone to this page as part of “updated information” for the upcoming HIV testing day!!

But we KNOW that Window periods for newer HIV tests are shorter than 3 months, and, being able to rule out infection as soon as possible is important to counselors and patients
Now that we have talked a little about the window period I want to walk you through some new information that came out recently.
This paper was published in the January 1 issue of Clinical infectious diseases...
So a couple slides ago, I said that we KNOW the window period for newer tests is shorter than 3 months... How do we know that? CDC’s HIV Laboratory Branch started evaluating the performance of FDA-approved assays over a decade ago as new tests came to market. In 2008 they published a method to measure the emergence of HIV test reactivity relative to the first positive HIV-1 western blot result. They showed that newer test types were more sensitive than western blot which was used as a confirmatory test – This result was one of the main motivations for updating the Diagnostic algorithm to use NATs instead of Western blot when the CDC guidelines were updated in 2014...
So, beginning in 2008 we published a sort of timeline of when different HIV tests detect infection...
Note that the times on here are relative to when Western blot would detect infection, and are all negative.
So this shows that Aptima detects infection 26 days before Western blot, but when does Western blot detect infection?

This is useful for saying in a relative way how early each test detects infection, but it’s not useful to a clinician or counselor when someone says – “I had sex without a condom last week and the guy just told me he was HIV-positive”
Another important point here is that at some point we stopped using the entire figure in favor of the timeline version...

In Dr. Owen’s 2008 paper she showed not only the timeline (part b) but also the entire distribution - Part A here,

For example Aptima (CLICK) (called Procleix in the original paper) is the first line here, and while the Median is
26 days before Western blot
The 99th percentile is much later, with overlap with the distribution of time to reactivity for the antibody only tests on this graph...
So our objective in the analysis published in January was to update and improve upon the 2008 analysis by developing estimates of the distributions of time from infection to detection for each of the 20 FDA-approved HIV tests that are currently available.
The process by which we arrived at these estimates is summarized here.

We used results from the laboratory testing of serial specimens collected early in infection with all FDA-approved HIV tests. And Defined what our statistician called the inter-test reactivity interval (ITRI) - the period of the time between Aptima detection of HIV infection and the detection of HIV using each of the other tests.

We then use four statistical models to estimate the distribution of the ITRI And combine them for an overall estimate.

We also simulated the time from infection to Aptima detection i.e., the eclipse period.

Finally we developed window period estimates by combining ITRI results with simulated data for the eclipse period to get a distribution for the window period for each test.
I’ll skip over the statistical details, but do want to point out that, because we only had data on 25 seroconverters and 222 specimens we used simulations from four different models to come up with average curves – and this adds uncertainty and variability to our estimates that were not captured in prior estimates of test window periods.

**Statistical methods**

- 10,000 simulations of the eclipse period were added to the simulations for each of the four models
- We then averaged over the four different models
- And then averaged by test type
The window period depends on what the test detects, and how it works!

- This means we have to know what the test we are using detects...

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Biomarkers detected | Type of Test
---|---
p24 antigen, IgM and IgG antibodies | Lab test performed on plasma
| Rapid test performed on plasma
| Rapid test performed on blood or oral fluid
IgM and IgG antibodies | Lab test performed on plasma
| Rapid test performed on plasma
| Rapid test performed on blood or oral fluid
IgG antibodies | Lab test performed on plasma
| Rapid test performed on plasma
| Rapid test performed on blood or oral fluid

So the next few slides are graphs and tables from the results of the paper, and in it we officially swear off use of the generations terms... So I want to remind you of this slide, where we saw the types of biomarkers and tests that detect them, grouped in this new, but hopefully still simple way...
Here are the curves for each test type...
Showing the entire, average distribution
In the paper we report selected percentiles from each distribution, and interpret them
For example
we take the median time as the earliest timepoint when each test category could reliably detect infection
And the 99th percentile as the time when a negative result can be reliably considered to be a true negative
This table summarizes the results for the different categories of tests

<table>
<thead>
<tr>
<th>Category (No. of Inclusive Tests)</th>
<th>Median (Interquartile Range; Days)</th>
<th>99th Percentile (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody/antigen laboratory (4)</td>
<td>17.8 (13.0, 23.6)</td>
<td>44.3</td>
</tr>
<tr>
<td>IgG/IgM-sensitive laboratory (3)</td>
<td>23.1 (18.4, 28.8)</td>
<td>49.5</td>
</tr>
<tr>
<td>IgG-sensitive rapid screening (6)</td>
<td>31.1 (26.2, 37.0)</td>
<td>56.7</td>
</tr>
<tr>
<td>IgG-sensitive supplemental (2)</td>
<td>33.4 (28.5, 39.2)</td>
<td>58.2</td>
</tr>
<tr>
<td>Western blot (viral lysate) (1)</td>
<td>36.5 (31.0, 43.2)</td>
<td>64.8</td>
</tr>
</tbody>
</table>
The medians, or the earliest time a test can reliably detect infection, range from 18 days for Antigen/antibody combination tests to 37 days for western blot.
Likewise the 99th percentile, or the time after exposure when a negative result can be trusted, ranged from 45 days for antigen/antibody combination tests to 65 days for Western blot.
Understanding the window period of HIV tests is important when counseling patients about when to test and the need for appropriate retesting.

Some individuals test after a recent high-risk behavior or after an occupational exposure to contaminated body fluids. In these cases, the exposure may have occurred more recently than the window period of the test available, or really, if it was in the last 10-14 days, too recently for infection to be detected by any test.

In this situation, an initial test should still be conducted to rule out prior HIV infection; however, if the result of this test is negative, the provider should retest the client after the test’s window period is over.

Until now we have generally said that providers and doctors should wait 90 days, this number appears in many places, and 90 days is much longer than the 45 days suggested by our data for laboratory-based antigen/antibody tests.
Poll: What specimens do your sites test with/train others to use?

- If you use more than one type or train on more than one type, pick the one you do most often:
  - Don’t know
  - Oral fluid
  - Fingerstick blood
  - Whole blood from a tube filled from a vein
  - Spin a tube of blood to get plasma/serum

I ran this slide as a poll in a webinar for CBB grantees two weeks ago...
None of the sites participating in the call reported that their sites or those that they train routinely use plasma or serum, they all used one of these other specimens...
So now I am going to be the bearer of bad news, because I am going to tell you about some areas where we don’t have much information yet...
So, why did I ask what specimen type sites typically use? We have already covered the idea that different tests detect different things, but they also do it in different ways. Not only does what you are detecting – Antigen, IgM antibody or IgG antibody make a difference, but how you detect that analyte matters too...

Some tests can be used with 4 different types of specimens Oral fluid, fingerstick blood, whole blood from a tube, or plasma

On here I have listed them from left to right in terms of how much analyte (either antigen or antibody) would be expected to be in the specimen type...

Oral fluid has much less antibody than blood... But there are different types of blood too

Prince Harry here was tested with a fingerstick blood test... fingerstick blood is mostly the same as blood drawn from a vein, but blood drawn from a vein (click)
Is a mix of different things (click)
This last tube has been spun in a centrifuge to separate the different components – most HIV antigen and antibodies remain in the plasma... So if you are testing the same volume of plasma and whole blood the concentration of antigen or antibody will be much higher in plasma.
The updated window period data I described was limited to testing of plasma or serum samples that require processing of blood tubes in the laboratory.

In that context they DO justify the recommendations in the 2014 laboratory testing algorithm, to start with a screening test that detects both antigen and antibody.

But as we saw in that polling question, and I assume you’ll agree, many CDC-funded sites that you work with or train use rapid tests – in the paper we have data on plasma for these tests, but in the field they often use blood from a fingerstick blood or even fluid from the mouth.

Other studies have found delays of several more days to several weeks when testing these other body fluids.

We do get some information from our analysis on plasma - The plasma window period distributions are the low end of what might be expected with other specimen types.

Window periods for fingerstick and oral fluid rapid tests are more than 45 days but might be less than 90 days.

If you want to rule out infection sooner, we would recommend that you follow a negative rapid test with laboratory testing according to the 2014 algorithm.

While we don’t yet have information on window periods for fingerstick or oral fluid,
Outline

- An overview of the “HIV test window period”
  - What it is
  - What new information CDC has and how it might affect your work
  - Where we don’t have new information yet
  - What’s coming next
- Summary
- Resources

But we do have plans to collect the information we need – my next few slides will describe those plans...
The reason we don’t have data on window periods for fingerstick or oral fluid is that these specimen types can’t adequately be evaluated in the lab with stored specimens. They need to be evaluated the same way you use them in the tests, on unprocessed specimens.

Read
Here are our study objectives

Click
Over the entire project (6 years as proposed), we will compile repository of matched plasma, Dried blood spots and oral fluid specimens from HIV-infected and uninfected individuals that will be useful for years to come. The slide shows the overall project goals for each specimen set.

We are in the first study cycle now: in which we expect ~125 established positive specimens, 10+ seroconversion panels and up to 900 HIV-negative specimens to be collected. Up to 4 additional but optional 1 year study cycles will be conducted if funds are available.
DETECT objectives

1. Evaluate test sensitivity and specificity:
   - ~600 established positive specimens
   - ~50-60 seroconverters
   - Nearly 6000 HIV-negative specimens

2. Evaluate seroconversion sensitivity via serial follow-up for whole blood, oral fluid and plasma

3. Evaluate risk characteristics of those identified in seroconversion

4. Assess transmission risks for seroconverters compared to participants with newly diagnosed and previously diagnosed infection

We will have closely spaced seroconversion panels with results on fingerstick and oral fluid specimens
And we will have paired behavioral data to identify possible exposures (to improve information on timing of seroconversion on each test) as well as

Read 3 and 4
So this project is ongoing, at least through the end of this fiscal year. We hope, however, that it continues for several more years because we are excited about PROJECT DETECT but
these are all new nucleic acid or viral load tests under development – some of which we hope to evaluate in future years in PROJECT DETECT and other studies...
Hologic received approval for their Quantitative HIV viral load test for use on their Panther instrument—a big automated platform that can produce results in 1.5 hours... just recently. While it is too soon for us to have much data, their trial data suggest it performs similarly to other viral loads. It does not currently have a diagnostic claim for use in either diagnosis of acute infection or as an aid in diagnosis in the CDC/APHL algorithm...
Several other companies have developed new NAT technologies in response to the global need for direct viral detection and quantification in settings with limited laboratory resources... These are all more portable and may even be eligible for CLIA-waiver. The rate limiting step in gaining access to this technology in the US is completion of clinical trials for FDA approval, including the specific and different trial requirements for qualitative diagnostic claims compared to quantitative claims for viral load. For example, would a test that can detect an HIV viral load of 1000 copies from 50 microliters of fingerstick whole blood be “good enough” for both diagnosis and monitoring? This is a question that remains unanswered but that needs to be considered in the context of another question - Where in an HIV diagnostic algorithm would we want to use such a test...
This is both a heads up on things that are coming next and a bit of a sales pitch

We have thusfar evaluated 3 tests with LB, at least 2 more coming to the lab in 2017

But this was on stored plasma, the POC NATs are intended to be run on fingerstick specimens – We plan to evaluate these in DETECT, assuming we get funding to continue the project

These studies will evaluate test performance, but not use cases or best practices for POC NAT

We do have access to these tests and test manufacturers now, and want to start thinking about and demonstrating how best to integrate them into our HIV prevention and care activities!
Right now we have more questions than answers, and we need the help of people like you to think about these issues...

Where do YOU think a POC NAT could have impact?

### POC NAT – Open questions

<table>
<thead>
<tr>
<th>1. In what settings would a POC NAT be most useful?</th>
<th>2. How could it have impact?</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) PrEP clinics</td>
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</tr>
<tr>
<td>i. Initiation</td>
<td>i. Faster time to first dose</td>
</tr>
<tr>
<td>ii. Monitoring</td>
<td>ii. Diagnose infection on PrEP</td>
</tr>
<tr>
<td>b) In the context of HIV care</td>
<td>b) In the context of HIV care</td>
</tr>
<tr>
<td>i. Initiation</td>
<td>i. Same day VL at first visit</td>
</tr>
<tr>
<td>ii. Reengagement</td>
<td>ii. Change the message</td>
</tr>
<tr>
<td>iii. Monitoring</td>
<td>iii. Streamlining follow-up</td>
</tr>
<tr>
<td>c) Laboratories</td>
<td>c) Laboratories</td>
</tr>
<tr>
<td>i. Simplifying algorithm</td>
<td>i. 2nd test in algorithm</td>
</tr>
</tbody>
</table>
I know I went through this fast, and some of it can get too technical, so I want to quickly summarize what I tried to say.
Summary

- New data from BCSB, Lab Branch and QSDMB suggest that the window period for laboratory-based antigen/antibody tests ends by 45 days after exposure in most people.
- We do not yet have enough data to say when the window period would end for rapid tests performed on blood or oral fluid.
  - But we are trying to update that information as well.
- New tests are coming and, with your help, we hope to evaluate their impact on HIV prevention soon.
I want to end by pointing you to resources for more information...
Resources – Current and Future

- www.cdc.gov/hiv/testing
  - https://www.cdc.gov/hiv/testing/nonclinical/index.html
  - https://www.cdc.gov/hiv/testing/laboratorytests.html
  - https://www.cdc.gov/hiv/testing/clinical/index.html

- For those of you able to attend USCA this year we are also developing a 2 hour seminar

Most all of you are probably familiar with these pages... As I pointed out earlier, the main source for finding our “current” information on HIV tests are the CDC HIV/testing web pages, where we have information about testing in non-clinical and clinical settings, as well as our recommendations for laboratory testing.

All of these pages are in the process of being updated, so check back often

Finally, we will be developing a 2 hour seminar on the updated window period and testing information for USCA this year, I hope many of you will be able to attend this expanded version of this presentation...
Feel free to email me at the address above if you have more questions...