4th Generation Testing: What Does It All Mean?

Caroline Campbell
VDH HIV Testing Coordinator
Kendra Weindling
STI/HIV Prevention Program Supervisor
Richmond City Health District
We’ll Be Discussing...

• 4\textsuperscript{th} Generation Conventional Testing
  
  • What it is
  
  • How to interpret results
  
  • How to counsel clients regarding results
Testing Has Evolved...

• That’s a good thing!

• What’s still the same?
  • Conventional blood draw and rapid test options still exist...
What’s Changed?

• 4\textsuperscript{th} generation testing identifies infection \textit{sooner} than earlier tests

• Multispot is no longer in production

• Geenius is an automated test that produces additional results not previously available with Multispot

• We now have a broader algorithm to guide medical management decisions
How Is Infection Identified?

• IgM antibodies: produced soon after exposure to an infection
  • Usually present about 3 weeks after infection

• IgG antibodies: a later response to infection; a long term response to infection
  • Usually present about 6 weeks after infection
What Is p24 Antigen?

- Viral protein that makes up most of HIV-1 viral core
- Serum concentrations of p24 antigen high in the first few weeks after infection & appear again in end stage HIV infection
- Tests sensitive to p24 antigen useful for diagnosing very early infection (i.e., acute) when antibody levels are below antibody-based assay detection limits
How Is Acute Infection Identified?

- Identification of HIV RNA in the absence of antibodies

- HIV-1 RNA is first detectable approximately 10 days after an individual becomes infected

- Detectable by HIV-RNA Nucleic Acid Amplification (NAT) test
HIV Testing
Through the “Generations”

Screening Tests
EIA (Enzyme Immunoassay)

• 1st Generation EIA (1985)
  • Detects HIV-1 IgG Antibody

• 2nd Generation EIA (1991)
  • Detects HIV-1 IgG Antibody

• 3rd Generation EIA (1992)
  • Detects HIV-1/HIV-2 IgM & IgG Antibodies

• 4th Generation EIA (2010)
  • Detects HIV-1/HIV-2 IgM, IgG Antibodies PLUS p24 Antigen, however does NOT distinguish which test component of the assay is positive

Old Algorithm
Confirmation of HIV-1 by Western Blot Assay

New Algorithm
Identification of HIV-1 & HIV-2 by Differentiation Assay (Geenius)
“Window Period” is the time frame between initial exposure and the time it can be reliably detected. Window Period aka Eclipse Phase.
HIV 1/2 Ag/Ab screening immunoassay

Repeatedly reactive screening immunoassay

HIV-1/2 antibody differentiation immunoassay (Genius replacing Multispot)

HIV-1 (+) HIV-2 (-)  
HIV-1 (-) HIV-2 (+)  
HIV-2 (+) with HIV-1 cross reactivity  
HIV Positive untypable (undifferentiated)

Result is positive for HIV; give patient result and appropriate counseling, link patient to care

Negative

HIV-1 indeterminate  
HIV-2 indeterminate  
HIV indeterminate

HIV-1 NAT

NAT is required; do not give results

Result is negative for HIV; give patient result
HIV-1 indeterminate
HIV-2 indeterminate
HIV indeterminate

Negative

HIV-1 NAT

Not detected
Result is negative for HIV; give patient result

Detected
Result indicates acute HIV-1 infection; give patient result and link to care

HIV-1 NAT

HIV-1 indeterminate

Not detected
Result is negative for HIV; give patient result

Detected

HIV-1 NAT

HIV-2 indeterminate

Not detected

HIV-1 NAT

HIV indeterminate

HIV-1 NAT

Not detected

HIV-1 negative; HIV-2 inconclusive; repeat algorithm in 2-4 weeks
*OR may be able to run different validated HIV-2 test
Here’s Where It Starts...

HIV 1/2 Ag/Ab screening immunoassay

Repeatedly reactive screening immunoassay

HIV-1/2 antibody differentiation immunoassay (Geenius replacing Multispot)

Negative for HIV-1 and HIV-2 antibodies and p24-Ag

Result is negative for HIV; give patient result
...And Here’s Where Antibodies Are Detected...

HIV-1/2 antibody differentiation immunoassay (Genius replacing Multispot)

- HIV-1 (+) HIV-2 (-)
- HIV-1 (-) HIV-2 (+)
- HIV-2 (+) with HIV-1 cross-reactivity
- HIV Positive undetermined (undifferentiated)

Result is positive for HIV; give patient result and appropriate counseling, link patient to care
...And Here’s Where More Testing Is Needed...
Possible NAT Results...

- **Negative**
  - HIV-1 NAT
    - **Not detected**: Result is negative for HIV; give patient result
    - **Detected**
      - Result indicates acute HIV-1 infection; give patient result and link to care

- **HIV-1 indeterminate**
  - HIV-2 indeterminate
  - HIV indeterminate
...And What About Indeterminates?

Diagram:
- HIV-1 indeterminate
  - HIV-1 NAT
    - Not detected
      - Result is negative for HIV; give patient result
- HIV-2 indeterminate
  - HIV-1 NAT
    - Not detected
    - HIV-1 negative; HIV-2 inconclusive; repeat algorithm in 2-4 weeks
      - *OR may be able to run different validated HIV-2 test
Goal of the HIV Laboratory Testing Algorithm

• According to the Association of Public Health Laboratories, the goal is “to maximize the identification of new, previously undiagnosed HIV infections”.
Lab Result: Negative

<table>
<thead>
<tr>
<th>Sample Information</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SAMPLE ID: 1674406</td>
<td></td>
</tr>
<tr>
<td>CLINICIAN: PARPART,FAYE</td>
<td></td>
</tr>
<tr>
<td>CLINICIAN PHONE:</td>
<td></td>
</tr>
<tr>
<td>SOURCE OF SPECIMEN: BLOOD VENOUS</td>
<td></td>
</tr>
<tr>
<td>SOURCE TYPE:</td>
<td></td>
</tr>
<tr>
<td>PROGRAM: CLINIC</td>
<td></td>
</tr>
<tr>
<td>SUBPROGRAM: VD</td>
<td></td>
</tr>
<tr>
<td>DATE COLLECTED: 01/20/2017</td>
<td></td>
</tr>
<tr>
<td>DATE SUBMITTED: 01/20/2017</td>
<td></td>
</tr>
<tr>
<td>DATE RECEIVED: 01/21/2017</td>
<td></td>
</tr>
<tr>
<td>SPECIMEN SITE:</td>
<td></td>
</tr>
<tr>
<td>SITE TYPE: STD</td>
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<tr>
<td>VDH OUTBREAK #:</td>
<td></td>
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<tr>
<td>MEDICAL RECORD #:</td>
<td></td>
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<tr>
<td>GRANT CODE:</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>TEST RESULTS</th>
<th>HIV Combo Ag/Ab EIA</th>
</tr>
</thead>
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<tr>
<td>Test</td>
<td>Results</td>
</tr>
<tr>
<td>HIV 1+2 Ab+HIV1 p24 Ag</td>
<td>Non-reactive - Negative for the presence of detectable HIV-1 p24 antigen, HIV-1 antibodies (Groups M and O) and HIV-2 antibodies by EIA.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Information Provided by Submitter</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Questions</td>
<td>Responses</td>
</tr>
<tr>
<td>Has a rapid test been performed?</td>
<td>NO</td>
</tr>
<tr>
<td>Who is the attending Clinician?</td>
<td>PARPART,FAYE</td>
</tr>
</tbody>
</table>
Counseling messages

• Negative screening test:
  • HIV not detected, return for re-testing in one month if there is concern of having been exposed to the infection within the past month

• Positive screening test:
  • We will not know because we do not get these back without the Geenius
  • There would be nothing we could tell the patient at this point because we do not have a completed algorithm
Lab Result: Positive
## FINAL REPORT

### Printed By
RICHMOND CITY HEALTH DISTRICT
500 N 5th Street
Richmond, VA 23219
HEALTH DEPT SITE CODE: 7634E

### Submitted By
RICHMOND CITY HEALTH DISTRICT
109 Governor Street
Richmond, VA 23219
PHONE: 804-884-7200 x 2

### Sample Information
- **SAMPLE ID:** 1/2160Z
- **CLINICIAN:** PARPART FAY
- **CLINICIAN PHONE:**
- **SOURCE OF SPECIMEN:** BLOOD VENOUS
- **SOURCE TYPE:**
- **PROGRAM:** CLINIC
- **SUBPROGRAM:** VD

### Test Results

<table>
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<tbody>
<tr>
<td>Genius HIV-1/HIV-2</td>
<td>HIV-1 Positive - The test result is POSITIVE for HIV-1 by the Geenius HIV1/2 Supplemental Assay using an immunochemistry method. A positive assay result confirms the presence of antibodies to HIV-1.</td>
</tr>
<tr>
<td>HIV 1+2 Ab-HIV1 p24 Ag</td>
<td>Reactive - Repeatedly reactive for the presence of HIV-1 p24 antigen, HIV-1 antibodies (Groups M and C) and/or HIV-2 antibodies by EIA.</td>
</tr>
</tbody>
</table>
Counseling messages—Positive for HIV 1 or HIV 2

• Your HIV test is positive, which means you have HIV infection. It’s important that you see a doctor for a medical evaluation. I can help you set that up.

• Your HIV test is positive for HIV-2, which is more common in certain parts of the world (such as West Africa). It’s important that you see a doctor, and I can help you make an appointment. It’s also very important that you tell the doctor that your test is positive for HIV-2, as that will affect decisions regarding your medical care.
*New Results*

- HIV-2 reactive with HIV-1 cross reactivity: This is considered HIV-2 positive
  - Link patient to care

- HIV positive-untypable (undifferentiated): Positive for HIV-1 and HIV-2 antibodies; evidence of HIV-1 and/or HIV-2 infection is present
  - Link patient to care; provider may consider additional testing to verify or rule out dual infection
Lab Result: Algorithm Incomplete!!

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<tbody>
<tr>
<td>HIV-1/2 Ab+HIV-1 p24 Ag</td>
<td>Reactively reactive for the presence of HIV-1 p24 antigen, HIV-1 antibodies (Groups M and O) and/or HIV-2 antibodies by EIA.</td>
</tr>
<tr>
<td>Geenius HIV-1/HIV-2</td>
<td>HIV Negative - No serological evidence of HIV-1 and HIV-2 by the Geenius HIV-2 Supplemental Assay using an immunochromatographic method. A negative or indeterminate result does not preclude the possibility of exposure to HIV or infection with HIV. The sample will be sent for nucleic acid amplification testing (NAAT).</td>
</tr>
</tbody>
</table>
Lab Result: Algorithm Incomplete!!
Counseling messages

• Your HIV results are not back yet; we need to schedule another time for you to return. In the meantime, it’s important for you to take precautions to protect yourself and others – use condoms for vaginal, oral and anal sex, and if you inject drugs, don’t share needles or works with anyone.

• This is one reason it is important to tell the patients that it takes 2 weeks to get results
Lab slip: Negative NAT
Counseling messages

• HIV not detected, return for re-testing in one month if there is concern of having been exposed to the infection within the past month
Lab slip: Positive NAT

| Bloodborne Viruses Laboratory | 120 New Scotland Avenue  
| Specimen Id: RIL1607500012 | Albany, New York 12208  
| Specimen Type: Serum | Phone: (518) 474-2163  
| Qualitative HIV-1 RNA (TMA): | Fax: (518) 473-0008  
| HIV-1 RNA Detected | The APTIMA HIV-1 Qualitative Assay is an FDA-approved nucleic acid assay using transcription-mediated amplification (TMA) for the detection of HIV-1 in human plasma and serum.  

END OF REPORT
Counseling messages

• Your HIV test is positive, and shows you likely got HIV recently. Based on your history and this test result, it is important that you see a doctor very soon. It is important that you know that HIV is very easily transmitted at this stage. I’d like to assist you with a referral to a doctor.

• This indicates likely acute infection. Link patient to care or to a patient navigator
  • An expedited visit should be scheduled
What If Geenius Is Indeterminate and NAT Is Not Detected?

• If Geenius is indeterminate for HIV-1 and NAT is not detected, the final result is negative.

• If Geenius is indeterminate for HIV or indeterminate for HIV-2, and NAT is not detected, the result is HIV-1 negative, HIV-2 inconclusive.
  • Redraw in 2-4 weeks and repeat algorithm
  • *OR may be able to run different validated HIV-2 test--stay tuned for more information
4th Generation HIV Algorithm

Benefits

• Detects IgM & IgG Antibodies; p24 Antigen
• 4th gen IA detects HIV-1 approximately 1 week earlier than 3rd gen assay
• Addition of HIV-1 RNA NAT helps detect false positive results & increases sensitivity to detect acute HIV infection
• Geenius assay differentiates between HIV-1 and HIV-2
4th Generation HIV Algorithm

Benefits (con’t.)

• Once linked to care & on ARV therapy, can result in better health outcomes for the patient

• Reduces likelihood of spreading infection
  • Individuals are less likely to spread infection if they are aware they are infected
  • Once on ARV therapy, greater chance of achieving undetectable viral load
Why Does Understanding the WHOLE Algorithm Matter?

- If NOT understood...

- Could lead to misinterpretation of results by health care providers

- Unnecessary additional testing may be ordered

- Could lead to delay in accessing care
Questions?

• Got it?
• ...or starting to get it?
• GOOD!!!

• Caroline Campbell
  • 804-864-7978 or Caroline.Campbell@vdh.virginia.gov

• Kendra Weindling
  • 804-482-8007 or Kendra.Weindling@vdh.virginia.gov