HIV Testing Overview

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Learning objectives

• Become familiar with the window periods of HIV diagnostic test methods
• Understand test results, interpretations and next steps of the HIV Diagnostic Testing Algorithm
• Learn about testing challenges, including additional algorithms, testing in the context of antiretrovirals, and self-testing
HIV-1 Viral Markers

Analyte Targets
- RNA
- p24
- IgM Ab
- IgG Ab
- Ab

Days after infection disseminates

X
0
10
20
30
40
50
60
70
80
90


Modified from Sexually Transmitted Diseases44(12):739-746, December 2017. doi: 10.1097/OLQ.0000000000000719
### Window Period: HIV-1 Exposure to Test Reactivity

**Eclipse = HIV-1 exposure to RNA detection**

Estimated duration of eclipse period:
- Median = 11.5 days
- 99th PCTL = 33 days

#### Table: Median and 99th PCTL (Days)

<table>
<thead>
<tr>
<th>Category</th>
<th>Median (IQR; Days)</th>
<th>99th PCTL (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab/Ag laboratory</td>
<td>17.8 (13.0, 23.6)</td>
<td>44.3</td>
</tr>
<tr>
<td>IgG/IgM-sensitive laboratory</td>
<td>23.1 (18.4, 28.8)</td>
<td>49.5</td>
</tr>
<tr>
<td>IgG-sensitive rapid screening</td>
<td>31.1 (26.2, 37.0)</td>
<td>56.7</td>
</tr>
<tr>
<td>IgG-sensitive supplemental</td>
<td>33.4 (28.5, 39.2)</td>
<td>58.2</td>
</tr>
<tr>
<td>Western blot (viral lysate)</td>
<td>36.5 (31.0, 43.2)</td>
<td>64.8</td>
</tr>
</tbody>
</table>

Ab = antibody  
Ag = antigen

doi:10.1093/cid/ciw666
HIV-2: rare in U.S. but important to detect

- U.S. HIV infections 2010-2017
  - HIV-1: 327,502 (99.94%)
  - HIV-2: 198 (0.06%)

- HIV-2 resistant to certain antiretrovirals

- HIV-1 & HIV-2
  - Genomes <50% homologous
  - Antibodies (Abs) cross react
  - Current testing algorithm detects and differentiates HIV-1 & HIV-2 Abs

Adapted from Kuiken, et al. 1999
Los Alamos National Laboratory
From CDC’s Quick Reference Guide, Jan 2018

HIV-1/2 antigen/antibody combination immunoassay

- (+)
- (-)

HIV-1/HIV-2 antibody differentiation immunoassay

- HIV-1 (+), HIV-2 (-)
- HIV-1 (-), HIV-2 (+)
- HIV-1 (+), HIV-2 (+)
- HIV-1 (-) or Indeterminate & HIV-2 (-) or Indeterminate

HIV-1 NAT

- NAT (+)
- NAT (-)

- (+) indicates reactive test results
- (-) indicates negative test results
- NAT: nucleic acid test

https://www.cdc.gov/hiv/testing/laboratorytests.html

Acute HIV-1 Infection

Negative for HIV-1
## HIV Ag/Ab Combo IAs

<table>
<thead>
<tr>
<th>Test (Manufacturer)</th>
<th>Yr FDA approved</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architect HIV Ag/Ab Combo (Abbott)</td>
<td>2010</td>
<td>CMIA</td>
</tr>
<tr>
<td>GS HIV Ag/Ab Combo EIA (Bio-Rad)</td>
<td>2011</td>
<td>EIA</td>
</tr>
<tr>
<td>ADVIA Centaur HIV Ag/Ab Combo (Siemens)</td>
<td>2015</td>
<td>CMIA</td>
</tr>
<tr>
<td>Centaur and Atellica instrument platforms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elecsys HIV combi PT (Roche Diagnostics)</td>
<td>2017</td>
<td>ECLIA</td>
</tr>
<tr>
<td>VITROS HIV Combo (Ortho Clinical Diagnostics)</td>
<td>2017</td>
<td>Immunometric</td>
</tr>
<tr>
<td>Alinity i HIV Ag/Ab Combo (Abbott)</td>
<td>2019</td>
<td>CMIA</td>
</tr>
<tr>
<td>LIAISON XL MUREX HIV Ab/Ag HT (Diasorin)</td>
<td>2020</td>
<td>CMIA</td>
</tr>
<tr>
<td>Determine HIV-1/2 Ag/Ab Combo (Abbott)</td>
<td>2013</td>
<td>LF rapid test</td>
</tr>
<tr>
<td>BioPlex 2200 HIV Ag-Ab (Bio-Rad Laboratories)</td>
<td>2015</td>
<td>Multiplex flow</td>
</tr>
<tr>
<td>Elecsys HIV Duo (Roche Diagnostics)</td>
<td>2020</td>
<td>ECLIA</td>
</tr>
</tbody>
</table>

All are designed to detect HIV-1 p24 antigen and IgM/IgG antibodies to HIV-1 and HIV-2

No analyte differentiation

None are designed to detect HIV-2 antigen

Analyte differentiation
Geenius™ HIV1/2 Supplemental Assay

- Intended for **supplemental** use
- Sample: 5ul of serum/plasma
- Detects IgG antibodies
  - Two HIV-2 bands (gp36, gp140)
  - Four HIV-1 bands (p31, gp160, p24, gp41)
  - Internal control band
- Geenius™ Software and Reader

30 minutes to result

**Geenius™ Results and Interpretations**

- Geenius software detects reactivity to individual bands and analyzes the relative strength to produce the **Final Assay Interpretation**

- Package insert: “The **Final Assay Interpretation** should always be reported to the ordering provider”

**APHL recommends that all labs**

1. include the Geenius Final Assay Interpretation, AND
2. exclude Geenius individual HIV-1 and HIV-2 results from the lab report
VioOne™ HIV Profile™ Supplemental Assay

- Intended for supplemental use
- Sample: 160ul of serum/plasma
- ELISA format:
  - Four HIV-1 antigens
  - One HIV-2 antigen
- Microplate washer & reader required

2-3 hrs to result

No viral Ag
HIV-1 p65 \((pol)\)
HIV-1 gp160 \((env, \text{low})\)
HIV-1 gp160 \((env)\)
HIV-1 gp41 \((env, M,O)\)
HIV-1 p24 \((gag)\)
HIV-2 gp36 \((\text{peptide})\)

Manufactured by Avioq, Inc.
### Geenius Final Assay Interpretation vs. VioOne HIV Profile Result Interpretation

<table>
<thead>
<tr>
<th>Geenius Final Assay Interpretation</th>
<th>VioOne HIV Profile Result Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV (Antibody) Negative</td>
<td>HIV Negative</td>
</tr>
<tr>
<td>HIV-1 Indeterminate</td>
<td>HIV-1 Indeterminate</td>
</tr>
<tr>
<td>HIV-2 Indeterminate</td>
<td>No Equivalent</td>
</tr>
<tr>
<td>HIV Indeterminate</td>
<td>No Equivalent</td>
</tr>
<tr>
<td>HIV-1 Positive</td>
<td>HIV-1 Positive</td>
</tr>
<tr>
<td>HIV-2 Positive</td>
<td>HIV-2 Positive</td>
</tr>
<tr>
<td>HIV-2 Positive w/ HIV-1 Cross Reactivity</td>
<td>HIV-2 Positive with Reactivity to HIV-1 Antigens</td>
</tr>
<tr>
<td></td>
<td>HIV-1 gp41 S/CO ≤ HIV-2 gp36 S/CO</td>
</tr>
<tr>
<td>No Equivalent</td>
<td>HIV-1 Positive with Reactivity to HIV-2 Antigen</td>
</tr>
<tr>
<td></td>
<td>HIV-1 gp41 S/CO &gt; HIV-2 gp36 S/CO</td>
</tr>
<tr>
<td>HIV Positive Untypable</td>
<td>No Equivalent</td>
</tr>
</tbody>
</table>

**Sources:**
- [https://www.fda.gov/media/130312/download](https://www.fda.gov/media/130312/download)
- [https://www.fda.gov/media/143115/download](https://www.fda.gov/media/143115/download)
HIV-1 RNA diagnostic testing

Step 3

- Before 2020, only one RNA assay FDA approved for aiding in diagnosis
  - Aptima HIV-1 RNA Qualitative assay (Gen-Probe/Hologic)
- Since 2020
  - Aptima HIV-1 RNA Quant Dx (Hologic): Qual/Quant
  - Alinity m HIV-1 AMP Kit (Abbott): Qual/Quant
  - Cobas HIV-1/HIV-2 Qualitative (Roche): Qual only
    - Differentiates HIV-1 and HIV-2
- New assays: Automated, faster, less prone to error
When do you need additional HIV-2 testing?

- If specimen is repeatedly HIV-2 or HIV indeterminate and HIV-1 NAT negative
  - Refer sample for testing with Cobas HIV-1/HIV-2 Qualitative assay or a validated HIV-2 supplemental test (Ab or NAT) or
  - Recommend repeating algorithm with a new specimen in 2 to 4 weeks
- HIV-2 qualitative NAT is available to public health labs enrolled in APHL HIV NAT Reference Centers Project

HIV Reporting Language

- Updated January 2019
- Addresses:
  - CDC Technical Update on the use of the Determine™ HIV 1/2 Ag/Ab Combo assay
  - CDC’s January 2018 update to their Quick Reference Guide
  - Clarification and updated recommendations for reporting Geenius™ test results

[Link to document]

<table>
<thead>
<tr>
<th>Test Sequence</th>
<th>Laboratory Algorithm Interpretation</th>
<th>Interpretation for Provider Use</th>
<th>Further Actions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1</td>
<td>Step 2</td>
<td>Step 3</td>
<td></td>
</tr>
<tr>
<td>HIV-1/HIV-2 Ag/Ab IA</td>
<td>HIV-1/HIV-2 Antibody Differentiation IA</td>
<td>HIV-1 NAT</td>
<td></td>
</tr>
<tr>
<td>Nonreactive</td>
<td>n/a</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>HIV-1 antigen and HIV-1/HIV-2 antibodies were not detected. No laboratory evidence of HIV infection.</td>
<td>HIV negative</td>
<td>If recent HIV exposure is suspected or reported, conduct HIV-1 NAT or request a new specimen and repeat the algorithm according to CDC Guidelines.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV-1 Positive</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Positive for HIV-1 antibodies. Laboratory evidence of HIV-1 infection is present.</td>
<td>HIV-1 Positive</td>
<td>Link patient to HIV medical care and provide appropriate prevention counseling.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV-2 Positive</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Positive for HIV-2 antibodies. Laboratory evidence of HIV-2 infection is present.</td>
<td>HIV-2 Positive</td>
<td>Link patient to HIV medical care and provide appropriate prevention counseling.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV-2 Positive with HIV-1 Cross reactivity</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Positive for HIV-2 antibodies. Laboratory evidence of HIV-2 infection is present.</td>
<td>HIV-2 Positive. This result is distinct from HIV positive untypable (undifferentiated).</td>
<td>Link patient to HIV medical care and provide appropriate prevention counseling.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV Positive untypable (undifferentiated)</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Positive for HIV-1 and HIV-2 antibodies. Laboratory evidence of HIV-1 and/or HIV-2 infection is present.</td>
<td>HIV Positive</td>
<td>Link patient to HIV medical care and provide appropriate prevention counseling. Provider may consider additional testing for HIV-1 RNA or DNA and HIV-2 RNA or DNA to verify or rule out HIV-1/HIV-2 dual infection. Request additional specimen if original specimen volume is insufficient.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV-1 indeterminate or, HIV-2 indeterminate or, HIV indeterminate</td>
<td>Detected</td>
<td></td>
</tr>
<tr>
<td>Positive for HIV-1. Laboratory evidence of HIV-1 infection consistent with an acute HIV-1 infection.</td>
<td>Acute HIV-1 Positive</td>
<td>Link patient to HIV medical care and provide appropriate prevention counseling immediately to expedite prevention practices.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV-1 indeterminate</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>HIV-1 antibodies were not confirmed and HIV-1 RNA was not detected.</td>
<td>HIV Negative</td>
<td>If recent HIV exposure is suspected or reported, request a new specimen and repeat the algorithm according to CDC guidance.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV-2 indeterminate</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>HIV antibodies were not confirmed and HIV-1 RNA was not detected. HIV-2 inconclusive.</td>
<td>HIV-1 Negative, HIV-2 inconclusive</td>
<td>Refer sample for testing with a different validated supplemental HIV-2 test (antibody test or NAT) if available. Alternatively, redraw and repeat algorithm in 2-4 weeks to assess HIV-2 infection.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV indeterminate</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>HIV-1 antibodies were not confirmed and HIV-1 RNA was not detected. HIV-2 inconclusive.</td>
<td>HIV-1 Negative, HIV-2 inconclusive</td>
<td>Refer sample for testing with a different validated supplemental HIV-2 test (antibody test or NAT) if available. Alternatively, redraw and repeat algorithm in 2-4 weeks to assess HIV-2 infection.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV Antibody Negative</td>
<td>Detected</td>
<td></td>
</tr>
<tr>
<td>Positive for HIV-1. Laboratory evidence of HIV-1 infection consistent with an acute HIV-1 infection.</td>
<td>Acute HIV-1 Positive</td>
<td>Link patient to HIV medical care and provide appropriate prevention counseling immediately to expedite prevention practices.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV Antibody Negative</td>
<td>Not detected</td>
<td></td>
</tr>
<tr>
<td>HIV antibodies were not confirmed and HIV-1 RNA was not detected.</td>
<td>HIV Negative</td>
<td>If recent HIV exposure is suspected or reported, request a new specimen and repeat the algorithm according to CDC guidance.</td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV Antibody Negative or Indeterminate</td>
<td>Invalid or not performed</td>
<td></td>
</tr>
<tr>
<td>Inconclusive</td>
<td>Inconclusive</td>
<td>Request an additional specimen and repeat the algorithm. Ensure HIV-1 NAT is performed, if indicated by results of HIV-1/HIV-2 Ag/Ab IA and HIV-1/HIV-2 Ab differentiation IA.</td>
<td></td>
</tr>
</tbody>
</table>

Challenge #1: Use of NAT as Step 2 of an HIV Testing Algorithm

• Three tests currently FDA approved for ‘aiding in diagnosis’
  – Aptima HIV-1 RNA Quant Dx (Hologic): Qual/Quant
  – Alinity m HIV-1 AMP Kit (Abbott): Qual/Quant
  – Cobas HIV-1/HIV-2 Qualitative (Roche): Qual only HIV-1/HIV-2

• Would it be more efficient to use RNA test as Step 2 instead of HIV-1/HIV-2 differentiation immunoassay?
Alternative use of an HIV-1 NAT at the Second Step of an HIV Diagnostic Algorithm

- Includes dual claim Qual/Quant NATs

HIV-1/2 antigen/antibody combination immunoassay

(+) → HIV-1 diagnostic NAT

HIV-1 (+) HIV-1 RNA detected

(-) → HIV RNA not detected

HIV-1/ HIV-2 antibody differentiation immunoassay

HIV-1 (+) HIV-2 (-) HIV-1 antibody detected

HIV-1 (-) HIV-2 (+) HIV-2 antibody detected

HIV-1 (+) HIV-2 (+) HIV antibody detected

HIV-1 Indeterminate OR HIV-2 Indeterminate

HIV-1 (-) AND HIV-2 (-)

HIV antibody not detected

(+) indicates reactive test results
(-) indicates negative test results
NAT: nucleic acid test

*To include dual claim qualitative/quantitative NATs;
1Additional testing should be considered

Presented at: 2022 Advancing HIV, STI and Viral Hepatitis Testing Conference (Mar 29 – Apr 1, 2022) by Dr. Jeff Johnson (CDC)
Alternative use of an HIV Differentiation NAT at the Second Step of an HIV Diagnostic Algorithm

Presented at: 2022 Advancing HIV, STI and Viral Hepatitis Testing Conference (Mar 29 –Apr 1, 2022) by Dr. Jeff Johnson (CDC)
Benefits & Limitations of NAT as Step 2

Potential Benefits:
- Could reduce TAT
- Could allow viral load at diagnosis

Potential Limitations:
- Will not distinguish acute infection
- If NAT is negative, need guidance on interpretation and next steps
- More stringent specimen handling requirements

Sources:
Challenge #2: Diagnostic testing in the context of antiretrovirals (ARV)

Early Antiretroviral Treatment
- Start treatment as early as possible after diagnosis
- Reduces viral reservoir, reduces transmission and improves disease prognosis

Pre-exposure Prophylaxis (PrEP)
- Daily/bi-monthly treatment to reduce risk of acquiring HIV
- Recommended for HIV-negative people at high risk
- Oral and injectable forms

https://www.cdc.gov/hiv/clinicians/prevention/prescribe-prep.html
Diagnostic testing with antiretrovirals (ARV)

• If someone becomes infected and continues on PrEP or has early antiretroviral treatment…
  – Emergence of HIV markers (RNA, Ag, Ab) may be delayed
  – Virus levels may be near limit of detection
  – Waffling between pos and neg results over successive samples

• Performance data for FDA-approved tests did not include people on PrEP

### Challenge #3: Self-Testing for HIV

<table>
<thead>
<tr>
<th>Category 1</th>
<th>Category 2</th>
<th>Category 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specimen Collection Setting</td>
<td>Non-Clinical/Home</td>
<td>At a Collection Facility</td>
</tr>
<tr>
<td>Specimen Testing Setting</td>
<td>Non-Clinical/Home</td>
<td>Clinical Laboratory</td>
</tr>
<tr>
<td>Examples</td>
<td>OraQuick In-Home HIV Test (FDA-Approved)</td>
<td>Some commercial labs offer direct to consumer testing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(e.g. Let’s Get Checked, My Lab Box)</td>
</tr>
<tr>
<td></td>
<td>Any box/kit sent to a consumer for collection</td>
<td>Some commercial labs offer direct to consumer testing</td>
</tr>
<tr>
<td></td>
<td>(e.g. Let’s Get Checked, My Lab Box)</td>
<td>(e.g. Quest)</td>
</tr>
</tbody>
</table>
Considerations for Self-Testing

- May help people get tested and learn their status
- Oral fluid and DBS testing less sensitive than plasma/serum
  - May be less appropriate for people on PrEP
- One FDA-approved test (OraQuick), rest are ‘lab-developed’
- Consumers may not understand results or test limitations
- Verifying specimen suitability and identity of source patient may be difficult
Final Points

• More test options are available for each step of the recommended algorithm
• An alternative diagnostic algorithm with a NAT as Step 2 has been proposed
• Early ART and PrEP may delay HIV markers and cause false negative or fluctuating results
• Self-testing for HIV will allow more people to access testing but may be less sensitive and difficult for consumers to interpret
Questions?