New HIV Testing Algorithms

Douglas Frye, MD, MPH
Chief, HIV Epidemiology
Division of HIV and STD Programs
New HIV Testing Algorithm

• Prior to 2013, California laboratories only allowed to report HIV indicative results from list outlined in MMWR.

• 2011: Clinical and Laboratory Standards Institute (CLSI) recommended new Standard Lab Algorithm
  – Detection of Acute HIV infection
  – Differentiation of HIV1/HIV2
  – Fast turnaround time

• Effective June 26, 2013: Regulation change allows California labs to use any HIV testing algorithm recommended by Centers for Disease Control and Prevention (CDC), Association of Public Health Labs (APHL), CLSI and US HHS.
HIV Diagnosis: New Algorithms and Evolution of HIV Diagnostics

Bernard M. Branson, M.D.
Associate Director for Laboratory Diagnostics
CDC Division of HIV/AIDS Prevention

The findings and conclusions are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention
Limitations of Antibody Testing

- Antibody tests do not detect infection in ~10% of infected persons at highest risk of transmission.

- Western blot confirmation is less sensitive during early infection than many widely used screening tests.

- Antigen/antibody combo tests now FDA-approved can detect most antibody-negative persons during highly infectious acute infection stage.
Evolution of HIV Tests

- **1st generation**: whole viral lysate, detects IgG antibody
- **2nd generation**: synthetic peptides, detects IgG antibody
- **3rd generation**: detect IgM and IgG antibody
- **4th generation**: detects IgM, IgG antibodies, p24 antigen
- "Combi" tests: detect both HIV-1 and HIV-2 antibodies
- Nucleic acid tests: detect HIV RNA
Sequence of Test Positivity Relative to WB (plasma)

166 specimens, 17 Seroconverters - 50 % Positive Cumulative Frequency


Luo et al, J Clin Virol 2013
HIV Infection and Laboratory Markers

Acute HIV Infection vs. Stage 0 HIV Infection

• Acute HIV infection: phase of HIV disease immediately after infection during which the initial burst of viremia in newly infected patients occurs; anti-HIV antibodies are undetectable while HIV RNA or p24 antigen are present.

• “Recent” HIV infection generally is considered the phase up to 6 months after infection during which anti-HIV antibodies are detectable.
  – Stage 0 HIV Infection: up to 180 days after infection.

• “Early HIV infection”: either acute or recent HIV infection.

CDC:  http://www.cdc.gov/mmwr/preview/mmwrhtml/rr6303a1.htm
HIV-2 Infection

- Remains uncommon in U.S., but
  - Does not respond to NNRTIs, some PIs (first line therapy)
  - Undetectable by HIV-1 viral load tests

- Misclassification by HIV-1 Western blot:
  - 54/58 (93%) HIV-2 patients tested had positive HIV-1 WB (NYC)*
  - 97/163 (60%) HIV-2 cases reported had positive HIV-1 WB (CDC)**

- HIV-2 often diagnosed after immunologic deterioration in patient with negative viral load

*Torian et al, Clinical Infectious Disease 2010
**MMWR July 2011
<table>
<thead>
<tr>
<th>Removed</th>
<th>Added</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Detection Tests:</strong></td>
<td></td>
</tr>
<tr>
<td>HIV-1 RNA PCR (QUAL)</td>
<td>HIV-1 RNA/DNA NAAT* (Qualitative)</td>
</tr>
<tr>
<td>HIV-1 PROVIRAL DNA (QUAL)</td>
<td>HIV-2 RNA/DNA NAAT* (Qualitative)</td>
</tr>
</tbody>
</table>

| Viral load tests: | | |
| HIV-1 RNA NASBA | HIV-1 RNA/DNA NAAT* (Quantitative) |
| HIV-1 RNA bDNA | HIV-2 RNA/DNA NAAT*(Quantitative) |
| HIV-1 RNA RT-PCR | |
| HIV-1 RNA | |

| Antibody tests | | |
| | HIV 1/2 Differentiating Test (Multispot) |
| | HIV-2 Western Blot |

*NAAT (also NAT) = Nucleic Acid Amplification Test*
CDC/APHL Proposed New HIV Testing Algorithm
4th generation HIV-1/2 immunoassay

\[ (+) \quad (-) \]
\[ \rightarrow \text{Negative for HIV-1 and HIV-2 antibodies and p24 Ag} \]

HIV-1/HIV-2 antibody differentiation immunoassay

- **HIV-1 (+) HIV-2 (-)**
  - HIV-1 antibodies detected

- **HIV-1 (-) HIV-2 (+)**
  - HIV-2 antibodies detected

- **HIV-1 (+) HIV-2 (+)**
  - HIV antibodies detected
  - **HIV-1 (-) or indeterminate HIV-2 (-)**
    - NAT
      - **NAT (+)**: Acute HIV-1 infection
      - **NAT (-)**: Negative for HIV-1

**NAT**: nucleic acid test (e.g., RNA)
FDA-approved 4\textsuperscript{th} Generation Tests

- **Abbott Architect 4\textsuperscript{th} Gen Ag/Ab Combo Assay**
  - Chemiluminescent immunoassay that detects p24 antigen and HIV antibody
  - \textit{results in 29 minutes}

- **Bio-Rad GS HIV Combo Ag/Ab EIA**
  - 3\textsuperscript{rd} generation Ab format plus p24 antigen

- **Determine Combo Rapid HIV 1/2 Ag/Ab Test**
  - Distinguishes Ag from Ab
FDA-approved HIV-1/HIV-2 Antibody Differentiation Assay
Interpretation for Diagnostic Testing Algorithm that Differentiates HIV-1 and HIV-2 Antibodies:

Nonreactive

Only the Procedural Control Spot shows purple color development. The 3 Test Spots show no color development. Test result is interpreted as negative for HIV-1 and HIV-2 antibodies. Additional testing is recommended, including HIV nucleic acid testing (NAT).

Reactive

**HIV-1 POSITIVE:**
The Procedural Control Spot shows purple color development and **both** the recombinant HIV-1 Spot and the HIV-1 Peptide Spot show purple color development. Test result is interpreted as Positive for HIV-1 antibodies

**HIV-2 POSITIVE**
The Procedural Control Spot shows purple color development. The HIV-2 Peptide Spot shows purple color development. Test result is interpreted as Positive for HIV-2 antibodies

**HIV POSITIVE (Undifferentiated):**
The Procedural Control Spot shows purple color development. The HIV-2 Peptide Spot shows purple color development as well as one or both HIV-1 Spots. In this case, the specimen may be tested by additional methods which allow for differentiation between HIV-1 and HIV-2. See dialutional procedure which follows.

Indeterminate

**HIV-1 INDETERMINATE:** The Procedural Control Spot shows purple color development and either the recombinant HIV-1 Spot or the HIV-1 Peptide Spot shows purple color development, but not both HIV-1 Spots. Test result is interpreted as Indeterminate for HIV-1 antibodies and testing for HIV nucleic acid is recommended.
Nucleic Acid Test (NAT) for Diagnosis

- APTIMA HIV-1 qualitative RNA assay is only NAT FDA-approved for diagnosis
- Clinicians can order HIV-1 viral load tests, but labs cannot use them as a reflex part of the algorithm
- Any NAT, including Viral Load, or p24 positive result is reportable and sufficient for a reportable case in eHARS
## Guidance for Reporting Results from HIV Testing Algorithm

<table>
<thead>
<tr>
<th>1st test: 4th Gen Ab/Ag</th>
<th>2nd test: HIV-1/2 Ab Different’n IA</th>
<th>3rd test: HIV-1 NAT</th>
<th>Overall Interpretation</th>
<th>Reporting to LAC DPH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonreactive</td>
<td>NA</td>
<td>NA</td>
<td>No evidence of HIV infection</td>
<td>NOT required</td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV-1 (+), HIV-2 (+)</td>
<td>NA</td>
<td>Evidence of HIV-1 and/or HIV-2 infection</td>
<td>Report 1st, 2nd test results</td>
</tr>
<tr>
<td></td>
<td>HIV-1 (+), HIV-2 (-)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIV-1 (-), HIV-2 (+)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HIV-1/2 (+) undifferentiated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV-1/2 (-) or indeterminate</td>
<td>Detected</td>
<td>Acute HIV-1 infection</td>
<td>Report 1st, 2nd, 3rd test results</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reactive</td>
<td>HIV-1/2 (-) or indeterminate</td>
<td>Not detected</td>
<td>No evidence of HIV infection</td>
<td>NOT required</td>
</tr>
</tbody>
</table>

Note: Table Adapted from CDC, NY State and City of SF HIV Surveillance information.
CDC Website

- Below is the link to the CDC HIV testing website, which includes links to several documents pertaining to the new testing algorithm.
  - [http://www.cdc.gov/hiv/testing/lab/guidelines/index.html](http://www.cdc.gov/hiv/testing/lab/guidelines/index.html)
Algorithm 1 – Ag/Ab Combination

HIV-1/ HIV-2 Ag/Ab * immunoassay

Results

Positive (run duplicate)

Negative for HIV-1 and HIV-2 and p24 antigen

H-1 /2 Ab Differentiation Immunoassay

H-1 (+)  
H-2 (-)  
Pos for HIV-1

H-1 (-)  
H-2 (+)  
Pos for HIV-2

H-1 (+)  
H-2 (+)  
Pos for HIV**

H-1 (-)  
H-2 (-)

Nucleic Acid Test (NAT)

Positive for HIV-1 RNA

Negative for HIV-1 RNA

*Antigen/antibody immunoassays (4th generation assays):
  • ARCHITECT HIV Ag/Ab combo assay (Abbott)
  • GS HIV Combo Ag/Ab EIA (Bio-Rad)

** Need further testing to differentiate HIV-1 from dual infection
Algorithm 2: Antibody test with supplemental tests

HIV-1/HIV-2 Ab results

HIV-1 NAT
- Positive for HIV-1 Abs and HIV-1 RNA
- Negative for HIV-1 RNA and inconclusive for HIV 1 / 2 Ab

HIV-1 WB, HIV IFA
- Positive for HIV-1 Abs

HIV-1/HIV-2 Ab Immunoassay
- Positive (run duplicate)
- Negative for HIV-1 and HIV-2 Ab
Algorithm 2a: Antibody test with supplemental tests

HIV-1/HIV-2 Ab results

- Positive (run duplicate)
- Negative for HIV-1 and HIV-2 Ab

HIV-1 WB, HIV IFA, Line IA

- Positive for HIV-1 Abs
- Negative for HIV-1 WB and inconclusive for HIV 1 / 2 Ab
- Indeterminate

HIV-1 NAT

- Positive for Abs and HIV-1 RNA
- Positive for Abs and HIV-1 RNA
Algorithm 3: Presumptive Diagnosis with sequential Antibody tests

HIV-1/ HIV-2 Ab immunoassay

Positive

Negative for HIV-1 and HIV-2 Abs

First immunoassay results

Positive

Presumptive positive for HIV-1 or HIV-2 Ab, required additional testing

Negative

Inconclusive, additional testing needed

Second immunoassay results (different type from first)
Algorithm 4: Antibody test with initial oral specimen

HIV-1 or HIV-1/HIV-2 Ab immunoassay (oral fluid)

Positive

Negative for HIV-1 and HIV-2 Abs

Oral HIV-1/HIV-2 Ab results

Positive

Negative for HIV-1 and HIV-2 Abs

Different HIV-1/HIV-2 Ab immunoassay (blood)

Positive

Presumptive pos. for HIV-1 or HIV-2, additional testing needed

Negative

HIV-1/HIV-2 Ab immunoassay (blood for 1st immunoassay)

Positive

Inconclusive, additional testing needed

Negative

Negative for HIV-1 and HIV-2 Ab
Using Rapid HIV Testing Algorithms to Improve the Accuracy of HIV Testing, Receipt of Test Results, and Linkage to Care

- Delaney et al, CROI 2011
Intervention

- Rapid test algorithm
  - Clients with a preliminary-positive test have blood drawn for standard (offsite) confirmatory testing
  - Up to 2 additional rapid blood tests
  - 2 positive rapid tests = same day referral for HIV care

- Los Angeles: 4 sites  San Francisco: 5 sites
Comparison

- Rapid test with laboratory confirmation
  - Clients with a preliminary-positive test had blood drawn for standard offsite confirmatory testing
  - Appointment scheduled (usually for 7 days later) to receive confirmatory test results
  - Referral if confirmatory test positive

- Los Angeles: 12 sites; San Francisco: 11 sites
## Results

<table>
<thead>
<tr>
<th></th>
<th>Intervention Sites</th>
<th>Comparison Sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>False-positive rapid test</td>
<td>37 14.8%</td>
<td>124 13.6%</td>
</tr>
<tr>
<td>Confirmed positive</td>
<td>213 85.2%</td>
<td>791 86.4%</td>
</tr>
<tr>
<td>Positive on multiple rapid tests</td>
<td>213*100.0%</td>
<td></td>
</tr>
<tr>
<td>Received results</td>
<td>250 100.0%</td>
<td>430 47.0%</td>
</tr>
</tbody>
</table>

*Includes one client who tested (false) negative on the 2nd test before testing positive on a third rapid test.
Conclusions

- **PPV:**
  - rapid test algorithm 100%;
  - single rapid test 85%
- **Engaged in care <90 days:**
  - 67% of clients who received referral
  - 50% of clients who did not return for confirmatory results or receive referral
- **Referral to care after reactive rapid test is essential**
CONTACT INFORMATION

Douglas Frye, MD, MPH
Chief HIV Epidemiology/DHSP
Ph. 213.351.8190