HIV Testing Technology and the Latest Algorithm

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HIV Testing has changed over time

- **1985**: Blood Banks
- **1987**: HIV-1 WB
- **1989**: P24 Ag test
- **1993**: Patients with high risk
- **1995**: WB HIV Testing algorithm
- **2001**: All pregnant women
- **2006**: More public and private health care settings
- **2010**: Waived Rapid IA
- **2012**: 4th Gen Combo IA

Voluntary screening in all health care settings
**1989 HIV Algorithm**

**T1: HIV-1 EIA**
- **Non-reactive:** Report as HIV Neg.
- **Reactive**
  - **Negative:** Report as HIV Neg.
  - **Indeterminate:** Report as Indeterminate
  - **Positive:** Report as HIV-1 Pos.

**T2: Western blot (WB) or immunofluorescence assay (IFA)**
New HIV testing strategies/algorithms

- 1989 algorithm remained largely unchanged for over 30 years
- Learned more about HIV-1 and HIV-2 disease
  - Disease progression
  - Virus dynamics
  - Window Period
- Advancements in technology and molecular tests
Evolution of the HIV Immunoassays

• Designed for sensitivity and use as screening tests

• Reactive result considered “preliminary positive”
  – Supplemental testing needed to confirm

• IAs have become more sensitive with each new generation
  – 1st gen EIA: HIV-1 viral lysates; indirect IA using labeled antihuman IgG
    • Required significant dilution to overcome non-specific reactions with cellular protein contaminants
Evolution of the HIV Immunoassays

• 1991---2\textsuperscript{nd} gen IA: Improved sens & spec
  – HIV-1/HIV-2
  – Synthetic peptide or recomb Ag w/wo viral lysates
  – IgG

• 1992---3\textsuperscript{rd} gen IA: HIV-1/HIV-2
  – synthetic peptide or recomb Ag
  – IgM + IgG Ab
  – Ag sandwich format; lower specimen dilution; increased sensitivity
Evolution of the HIV Immunoassays

• 2010---4\textsuperscript{th} gen IA
  – Similar to 3\textsuperscript{rd} gen w/ addition of p24 Ag detection

• 5\textsuperscript{th} gen multiplex flow IA:
  – differentiates HIV-1 Ag, HIV-1 and HIV-2 Ab (Bio-Rad)
### 4th Generation HIV Ag/Ab Combo Assays

<table>
<thead>
<tr>
<th>Test Name (Manufacturer)</th>
<th>Instrument</th>
<th>Specimen Types</th>
<th>Result Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbott ARCHITECT HIV Ag/Ab Combo (Abbott Diagnostics)</td>
<td>Fully automated, random access (i2000SR)</td>
<td>Serum, Plasma</td>
<td>Nonreactive, Reactive</td>
</tr>
<tr>
<td>GS HIV Combo Ag/Ab EIA (Bio-Rad Laboratories)</td>
<td>Manual or semi-automated instrument (Evolis)</td>
<td>Serum, Plasma</td>
<td>Nonreactive, Reactive</td>
</tr>
<tr>
<td>ADVIA Centaur HIV Ag/Ab Combo (Siemens Healthcare Diag.)</td>
<td>Fully automated, random access (Centaur/Centaur XP)</td>
<td>Serum</td>
<td>Nonreactive, Reactive</td>
</tr>
</tbody>
</table>
4th Generation Ag/Ab Test

- 3 FDA-approved kits available
  - ARCHITECT HIV Ag/Ab Combo (Abbott)
  - GS HIV Ag/Ab Combo EIA (Bio-Rad)
  - ADVIA Centaur Ag/Ab CIA

- Detect HIV-1 p24 Ag, HIV-1 and HIV-2 antibodies

- Reactive result:
  - Doesn’t distinguish between Ag and Ab
  - Preliminary positive
  - Supplemental testing required
5th Generation Ag/Ab Test

• BioPlex 2200 HIV Ag/Ab Test
  – Detects and differentiates HIV-1 Ag, HIV-1 Ab, and HIV-2 Ab in serum or plasma
  – Early infection detection similar to other laboratory Ag/Ab tests
  – High sensitivity and specificity
  – Further data needed on performance in the algorithm
Changes that impact HIV testing strategies/algorithms

• Evolving technology
  – Availability of rapid tests
  – Increased sensitivity of screening assays
    • Western blot and IFA now much less sensitive than screening assays which they are intended to “confirm”
Western blot (WB)

- Designed for *specificity*
- Must meet specific criteria for a positive interpretation
- Indeterminate results occur for a variety of reasons
  - Early infection, late infection, HIV-2 infection, other
- Western blot technology has not advanced
  - Newer immunoassays are more sensitive than WB
- Lack of sensitivity can lead to false negative and inconclusive results
Sequence of HIV Assay Reactivity During Early HIV Infection Relative to Western Blot*

*Assay sensitivity above is based on frozen plasma only. Whole-blood and oral fluid has not been characterized for early infection.
**Current data suggests that the Gen-Probe Aptima can detect HIV-1 RNA ~5-28 days after infection.

HIV Test Strategy Workgroups

- Two workgroups were formed in October 2006
  - Focused on strategies for both POC and Laboratory settings

- HIV Diagnostics Conference in December 2007
  - Data was presented to substantiate various algorithms

- Status Report started fall 2008
  - APHL/CDC Steering Committee produced a report of the current status of the proposed algorithms
• 2010 HIV Diagnostics Conf.
  – Announced consideration of Multispot as supplemental assay

• 2012 HIV Diagnostics Conf.
  – Draft recommendations for a new HIV testing algorithm

• 2014 HIV Diagnostics Conf.
  – CDC publishes new HIV testing algorithm
Representation on Laboratory and POC Workgroups

- Association of Public Health Laboratories
- American Clinical Laboratory Association
- American Society of Microbiology
- Blood Banks
- College of American Pathologists
- US Centers for Disease Control and Prevention
- Commercial Laboratories
- US Department of Defense
- US Food and Drug Administration
- HIV Program Staff from NASTAD and Public health departments
What are we looking for from these new testing strategies?

- Resolution of indeterminates
- Ability to confirm HIV-2 infections
- Increased detection of acute infection
- Assays that can be used as screening or confirmatory/supplemental tests and as part of multi-test algorithms
- Guidance for laboratory confirmation of POC rapid tests
Laboratory Algorithms
New CDC Recommendations for HIV Testing in Laboratories

Step 1: “Fourth generation” HIV test
- Detecting HIV sooner
  - Detects HIV in the blood earlier than previously recommended antibody tests by identifying the HIV-1 p24 antigen, a viral protein which appears in the blood sooner than antibodies.
  - Positive
    - Diagnosis: HIV-negative
  - Negative
    - Diagnosis: Acute HIV-1 Infection

Step 2: HIV-1/HIV-2 antibody differentiation immunoassay
- Diagnosing HIV-1 vs. HIV-2
  - Produces results faster than the previously recommended Western Blot.
  - Distinguishes between HIV-1 and HIV-2, which the previously recommended Western Blot cannot do – this distinction can have important treatment implications for a patient.
  - Negative or Indeterminate
  - Positive
    - Diagnosis: HIV Infection
    - Interpret Test Results as HIV-1 or HIV-2

Step 3: Nucleic Acid Test (NAT)
- Acute HIV-1 infection or “false positive”?
  - Ensures accurate detection of early infection or indicates a false positive from the fourth generation test.
  - Negative
    - Diagnosis: HIV-negative
  - Positive
    - Diagnosis: Acute HIV-1 Infection

This graphic is designed to illustrate key concepts of the new testing approach in laboratories. For more detail, please see the full guidelines here: http://www.cdc.gov/hiv/pdf/HIVtestingAlgorithmRecommendation-Final.pdf.

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

www.cdc.gov/nchhstp/newsroom

JUNE 2014
Algorithm with 5th Generation Test

HIV-1/2 antigen/antibody combination immunoassay

(+): HIV-1Ab, HIV-2 Ab, HIV Ab, HIV-p24

(-): Negative for HIV-1 and HIV-2 antibodies and p24 antigen

HIV-1/HIV-2 antibody differentiation immunoassay

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

HIV-1 (-) HIV-2 (+)
HIV-2 antibodies detected or HIV-2 w/ cross reactivity

HIV-1 (+) HIV-2 (-) or indeterminate
HIV antibodies detected

NAT

NAT (+): Acute HIV-1 infection
NAT (-): Negative for HIV-1

*Test for HIV-2 if Geenius HIV or HIV-2 IND OR Bioplex HIV-2 + and Geenius HIV-2 NEG
Advantages of the New Algorithm

• 4th Gen Ag/Ab more sensitive and specific
• Allows detection of acute HIV infection
• Eliminates expensive, labor intensive, and problematic WB
• Allows detection of HIV-2 infection
• Geenius HIV-1/HIV-2 is simple, rapid, and less expensive than WB
HIV-1 NAT

- Only one HIV-1 RNA test FDA-approved for use as an aid in the diagnosis of HIV-1 infection
  - APTIMA® HIV-1 RNA Qualitative Assay (Gen-Probe)
- Several HIV-1 quantitative tests (viral load)
  - FDA approval is for patient monitoring, i.e. assess prognosis, monitor effects of therapy
  - Not intended as a diagnostic test to confirm the presence of HIV-1 infection
- WSLH using proviral DNA RT-PCR
HIV-1/HIV-2 Discriminatory Immunoassay

- Multispot HIV-1/HIV-2 Rapid Test (Bio-Rad)
- FDA-approved:
  - To detect & differentiate antibodies
  - Suitable for multi-test algorithms
  - Current package insert does not specify use as a supplemental test

- Single-use rapid test
- Results in <30 min
- Not CLIA waived
- Serum or plasma
Geenius HIV 1/2 Supplemental Assay
Geenius HIV ½ Supplemental Assay

- FDA approved Oct 2014
- Replaces Multispot
- Differentiates HIV-1 and HIV-2 antibodies
- 30 minutes
Geenius

Protein A colloidal gold detection

Test Antigens (lines, left to right)
1. HIV-2 gp36 (env peptide)
2. HIV-2 gp140*
3. HIV-1 p31 (pol peptide)
4. HIV-1 gp160 (env recomb prot)
5. HIV-1 p24 (core recomb prot)
6. HIV-1 gp41 (env peptides)
7. Control (protein A)

*Multimer of HIV-2 gp36 env peptide
# Geenius™ Compared to Multispot

<table>
<thead>
<tr>
<th>Multispot</th>
<th>Geenius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rapid testing (2004) or Supplemental (2013)</td>
<td>Supplemental use only (confirmation)</td>
</tr>
<tr>
<td>Manual reading and interpretation</td>
<td>Geenius Reader and automatic interpretation on Geenius Software</td>
</tr>
<tr>
<td>Manual result entry into LIS/LIMS</td>
<td>Bi-directional connection to LIS/LIMS</td>
</tr>
<tr>
<td>Manual labeling</td>
<td>Full traceability; barcode identification</td>
</tr>
<tr>
<td>Dilution protocol in PI to resolve cross-reactivity</td>
<td>No Equivalent</td>
</tr>
<tr>
<td>Serum or plasma</td>
<td>Serum, plasma, fingerstick or venous whole blood</td>
</tr>
</tbody>
</table>
# Geenius™ and Multispot Results

<table>
<thead>
<tr>
<th>Multispot Results</th>
<th>Geenius Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonreactive</td>
<td>Nonreactive</td>
</tr>
<tr>
<td>Reactive: HIV-1 positive</td>
<td>Reactive: HIV-1 positive</td>
</tr>
<tr>
<td>Reactive: HIV-2 positive</td>
<td>Reactive: HIV-2 positive</td>
</tr>
<tr>
<td>No Equivalent</td>
<td>Reactive: HIV-2 positive with HIV-1 cross-reactivity</td>
</tr>
<tr>
<td>Reactive: HIV positive (undifferentiated)</td>
<td>Reactive: HIV positive untypable (undifferentiated)</td>
</tr>
<tr>
<td>Indeterminate: HIV-1 indeterminate</td>
<td>Indeterminate: HIV-1 indeterminate</td>
</tr>
<tr>
<td>No Equivalent</td>
<td>Indeterminate: HIV-2 indeterminate</td>
</tr>
<tr>
<td>No Equivalent</td>
<td>Indeterminate: HIV indeterminate</td>
</tr>
</tbody>
</table>
Geenius Results-What They Mean

• HIV-2 positive with HIV-1 cross-reactivity
  – Antibody to HIV-2 confirmed
  – HIV-1 reactivity does not meet criteria to be considered positive
    • only one HIV-1 envelope band detected
    • Gp160 or gp41
    • Indicative of HIV-1 cross-reactivity
  – Final Interpretation Reported
    • HIV-2 Positive
  – Patient should be referred to care for HIV-2 infection
What results are seen with HIV-2 Ab positive specimens?

- Package Insert Data for 200 specimens
  - HIV-2 Positive for 38.5% (77/200)
  - HIV-2 with HIV-1 cross-reactivity for 54% (108/200)
  - HIV Undifferentiated for 6% (12/200)
  - HIV indeterminate for 1.5% (3/200)
Geenius Results-What They Mean

- HIV-2 Indeterminate
  - Repeat before reporting
  - If repeat is “HIV-negative, report as such
  - If repeat is “HIV-1positive” or HIV-2 positive”, report as such
  - If repeat is HIV-2 indeterminate, report as HIV-2 indeterminate
    - Result may indicate acute HIV-1 infection
    - HIV-1 NAT should be performed
Geenius Results-What They Mean

- HIV-2 Indeterminate (con’t)
  - If HIV-1 NAT is negative
    - Refer specimen for testing with a different HIV-2 test
    - Or repeat testing in 2-4 weeks
Geenius Results—What They Mean

• **HIV Indeterminate**
  – Bands present for both HIV-1 and HIV-2, but positive criteria not met for either
  – Repeat before reporting
  – If repeat is “HIV-negative, report as such
  – If repeat is “HIV-1 positive” or HIV-2 positive”, report as such
  – If repeat is HIV indeterminate, report as HIV indeterminate
    • Result may indicate acute HIV-1 infection
    • HIV-1 NAT should be performed
Geenius Results—What They Mean

• HIV indeterminate
  – Perform HIV-1 NAT
    • If NAT pos, refer patient to care for HIV-1
    • If NAT neg, refer specimen for HIV-2 testing with NAT or different antibody test
    • or repeat testing in 2-4 weeks
Geenius Results-What They Mean

• HIV-1 indeterminate
  – Same testing sequence as for HIV indeterminate with except additional HIV-2 testing is not necessary
Geenius Results-What They Mean

- HIV Positive Untypable (undifferentiated)
  - Antibodies to HIV-1 and HIV-2 confirmed in the specimen
  - May occur in an HIV-2 positive sample with significant cross-reactivity to HIV-1
  - Or co-infection with both HIV-1 and HIV-2
  - Repeat to confirm before reporting
  - Interpret similar manner as “HIV indeterminate”
Geenius Results - What They Mean

• HIV Positive Untypable (undifferentiated)
  – Perform HIV-1 NAT
    • If NAT pos, refer patient to care for HIV-1
    • If NAT neg, refer specimen for HIV-2 testing with NAT or different antibody test
      repeat testing in 2-4 weeks
# Geenius Results Reporting

<table>
<thead>
<tr>
<th>HIV-1 result</th>
<th>HIV-2 result</th>
<th>Geenius Assay Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>HIV Negative</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Negative</td>
<td>HIV-1 Indeterminate</td>
</tr>
<tr>
<td>Negative</td>
<td>Indeterminate</td>
<td>HIV-2 Indeterminate</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Indeterminate</td>
<td>HIV Indeterminate</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>HIV-1 Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>Indeterminate</td>
<td>HIV-1 Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>HIV-2 Positive</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Positive</td>
<td>HIV-2 Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>HIV-2 Positive with HIV-1 cross-reactivity: Antibody to HIV-2 confirmed in the sample. HIV-1 positivity (with only one HIV-1 envelope band, gp160 or gp41), is due to cross-reactivity and precludes confirmation of HIV-1.*</td>
</tr>
<tr>
<td>Positive</td>
<td>Positive</td>
<td>HIV POSITIVE Undifferentiated: Antibodies to HIV-1 and HIV-2 confirmed in the sample. This may occur in an HIV-2 positive sample with significant cross-reactivity to HIV-1, or may be due to co-infection with both HIV-1 and HIV-2 (rare).*</td>
</tr>
</tbody>
</table>

*Note: Differentiation features managed by proprietary algorithm.
Point of Care Rapid HIV Testing
Approved Tests for the Point of Care Algorithms

Uni-Gold Recombigen

Multispot HIV-1/HIV-2

OraQuick ADVANCE

Reveal G3

Clearview Complete HIV 1/2

Clearview HIV 1/2 Stat Pak

Slide presented by Dr. Branson at the 2007 HIV Diagnostics Conference
<table>
<thead>
<tr>
<th>Product</th>
<th>Specimen type</th>
<th>CLIA Category</th>
<th>Exp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unigold Recombigen HIV</td>
<td>Whole Blood, Serum, Plasma</td>
<td>Waived, Moderate Complexity</td>
<td>1 yr</td>
</tr>
<tr>
<td>OraQuick ADVANCE HIV 1/2</td>
<td>Oral Fluid, Whole Bld Plasma</td>
<td>Waived, Moderate Complexity</td>
<td>6 mos.</td>
</tr>
<tr>
<td>Reveal G3 Rapid HIV-1</td>
<td>Serum, Plasma</td>
<td>Moderate Complexity</td>
<td>1 yr</td>
</tr>
<tr>
<td>Clearview STAT-PAK HIV-1/2</td>
<td>Whole Blood Serum, Plasma</td>
<td>Waived, Moderate Complexity</td>
<td>2 yrs</td>
</tr>
<tr>
<td>Clearview Complete HIV-1/2</td>
<td>Whole Blood Serum, Plasma</td>
<td>Waived, Moderate Complexity</td>
<td>2 yrs</td>
</tr>
<tr>
<td>Alere Determine</td>
<td>Whole Blood</td>
<td>Waived, Moderate Complexity</td>
<td>15 mo</td>
</tr>
</tbody>
</table>
## FDA Approved HIV Rapid Tests

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufacturer</th>
<th>Analyte</th>
<th>Specimen Type</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>FDA Approval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unigold Recombigen HIV</td>
<td>Trinity Biotech</td>
<td>HIV-1</td>
<td>Whole blood, Serum, Plasma</td>
<td>100%</td>
<td>99.7%</td>
<td>Dec. 2003</td>
</tr>
<tr>
<td>OraQuick ADVANCE HIV 1/2</td>
<td>Orasure Technologies</td>
<td>HIV-1</td>
<td>Whole blood, Oral fluid, Plasma</td>
<td>99.6% BL</td>
<td>100% BL</td>
<td>June 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-2</td>
<td></td>
<td>99.3% OF</td>
<td>99.8% OF</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>99.9% plasma</td>
<td>99.9% plasma</td>
<td></td>
</tr>
<tr>
<td>Reveal G3 Rapid HIV-1</td>
<td>MedMira</td>
<td>HIV-1</td>
<td>Serum, Plasma</td>
<td>99.8%</td>
<td>99.1% serum</td>
<td>Oct. 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>98.6% plasma</td>
<td></td>
</tr>
<tr>
<td>Clearview STAT-PAK HIV-1/2 &amp; Complete HIV-1/2</td>
<td>Inverness Med.</td>
<td>HIV-1</td>
<td>Whole blood, Serum, Plasma</td>
<td>99.7%</td>
<td>99.9%</td>
<td>May 2006</td>
</tr>
<tr>
<td></td>
<td><a href="http://www.invernessmedicalpd.com">www.invernessmedicalpd.com</a></td>
<td>HIV-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Determine HIV-1/2 Ag/Ab</td>
<td>Alere</td>
<td>HIV-1 Ab</td>
<td>Whole blood, serum, plasma</td>
<td>99.9</td>
<td>99.7% BL</td>
<td>2013,2014</td>
</tr>
<tr>
<td></td>
<td><a href="http://www.alerehiv.com">www.alerehiv.com</a></td>
<td>HIV-2 Ab</td>
<td></td>
<td></td>
<td>99.6% serum</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HiV-1 Ag</td>
<td></td>
<td></td>
<td></td>
<td>99.7% plasma</td>
<td></td>
</tr>
</tbody>
</table>
Non-Clinical Site Algorithms

- Single rapid test with immediate linkage to clinical provider if reactive
- Single rapid test followed by lab-based follow up testing if reactive
  - WDPH algorithm for CTS
- Single rapid test immediately followed up a 2nd rapid test on-site if reactive
  - If both tests reactive, link to HIV care
  - If second test nonreactive, refer to laboratory or clinical provider for follow-up testing
Testing Strategy for Point of Care HIV Rapid Testing Facilities

**Two Rapid Tests** (A1/A2) Performed in Sequence on Blood (A1 and A2 must be different rapid tests)

- **A1**
  - [HIV-1 or HIV-1/2 rapid test (Blood)]
  - **A1+**
  - **A1-**
    - Negative for HIV-1 and HIV-2 antibodies*

- **A2**
  - [HIV-1 or HIV 1/2 rapid test from a different manufacturer (blood)]
  - **A1+ A2+**
    - Presumptive positive for HIV-1 or HIV-2 antibodies; requires medical follow-up for further evaluation and testing
  - **A1+ A2-**
    - Inconclusive rapid test result; requires additional testing

*If using an HIV-1 only rapid test, Negative for HIV-1 antibodies only
POC Two Test Algorithm

• Requires that technicians be proficient with 2 tests
  – Limited training, expertise required
• Little chance for false-positive or false-negative algorithm results
• Likely to work well in high prevalence settings
• In low prevalence areas majority of specimens will require lab resolution
POC Testing using Multi-Rapid Test Algorithms

- Addresses low return rate for confirmatory test results experienced in some settings
- Can optimize care and treatment for HIV-infected persons
  - HC settings can aid in definitive diagnosis and therefore inform clinical decisions quickly
  - All settings can facilitate referral to HIV care, especially with mobile and transient populations
- Can optimize prevention
  - Messages about HIV test results can be clearer and more persuasive; optimizes prevention and care engagement
  - Facilitates provision of partner services
Oral Fluid Testing

- Not part of CDC’s recommended algorithm
- Requires a testing algorithm that includes the Western blot
- Does not detect infection as early as blood tests
More Information


• Link to 2016 HIV Diagnostic Conference: http://hivtestingconference.org/
Questions are guaranteed in life; Answers aren't.