New Guidelines for HIV Diagnosis

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Disclosure

• None (Dr. Slev)
• Roche Diagnostics (Dr. Hillyard)
Objectives

• Explain the advances in HIV diagnostics, including fourth generation Ag/Ab combination HIV screening assays.
• Describe the new CDC HIV diagnostic algorithm.
• Understand the evidence in support of the new diagnostic algorithm.
• Use screening and follow-up confirmatory tests appropriately.
HIV Globally

33.3 million people [31.4–35.3 million] living with HIV, 2009

Estimated that only 19% of HIV-infected individuals in the US have undetectable HIV viral load.
Awareness of Serostatus Among People with HIV and Estimates of STD Transmission (US)

- ~25% Unaware of Infection
- ~75% Aware of Infection

Account for:

- ~54 - 70% of New Infections
- ~30 - 46% of New Infections

People Living with HIV/AIDS: 1,039,000 - 1,185,000

New Sexual Infections Each Year: ~32,000
Transmission in Males

Adult and Adolescent Males Living with an AIDS Diagnosis, by Transmission Category, 1985–2009—United States and 6 U.S. Dependent Areas

- Male-to-male sexual contact
- Injection drug use (IDU)
- Male-to-male sexual contact and IDU
- Heterosexual contact a
- Other b

No. of persons (in thousands)

Year

Note: All displayed data have been statistically adjusted to account for reporting delays and missing risk-factor information, but not for incomplete reporting.

a Heterosexual contact with a person known to have, or to be at high risk for, HIV infection.
b Includes hemophilia, blood transfusion, perinatal exposure, and risk factor not reported or not identified.
Transmission in Females

Adult and Adolescent Females Living with an AIDS Diagnosis, by Transmission Category, 1985–2009—United States and 6 U.S. Dependent Areas

- Injection drug use
- Heterosexual contact
- Other

Note: All displayed data have been statistically adjusted to account for reporting delays and missing risk-factor information, but not for incomplete reporting.

*Heterosexual contact with a person known to have, or to be at high risk for, HIV infection.

*Includes blood transfusion, perinatal exposure, and risk factor not reported or not identified.
Perinatal Transmission


Diagnoses, No.

Year of diagnosis

Note: All displayed data have been statistically adjusted to account for reporting delays, but not for incomplete reporting.
2006 CDC Guidelines
“Universal Testing”

• Routine HIV
  voluntary, not based on risk

• Opt-Out
  option to decline, general consent for care includes HIV testing

• Population
  13 -64 years old

• Venue
  inpatient services, ED, urgent care, STD clinics, substance abuse and correctional facilities
Human Immunodeficiency Virus

Types

HIV-1

HIV-2

Groups

Major

Non M/Non O

Outlier

Subtypes/Clades

A B C D E F G H I J K

Circulating Recombinant Forms (CRF)
Persons at risk for HIV-2 infection include

- Sex partners of a person from a country where HIV-2 is endemic
- Sex partners of a person known to be infected with HIV-2
- People who received a blood transfusion or a nonsterile injection in a country where HIV-2 is endemic
- People who shared needles with a person from a country where HIV-2 is endemic or with a person known to be infected with HIV-2
- Children of women who have risk factors for HIV-2 infection or are known to be infected with HIV-2

HIV-2 testing also is indicated for

- People with an illness that suggests HIV infection (such as an HIV-associated opportunistic infection) but whose HIV-1 test result is not positive
- People for whom HIV-1 Western blot exhibits the unusual indeterminate test band pattern of gag (p55, p24, or p17) plus pol (p66, p51, or p32) in the absence of env (gp160, gp120, or gp41)
HIV Infection Course

- **Primary infection**
- **Acute HIV syndrome**
- **Clinical latency**
- **Viremia**

Adapted from Roche and Siemens slides
HIV Serological Response

Typical response following infection

Weeks following infection

Years

HIV p24 Antigen

env anti-HIV

gag anti-HIV

pol anti-HIV
Current HIV Diagnostic Algorithm

1  Screen
    immunoassay (EIA/CIA)
    rapid tests

2  Confirmation
    Western blot (98%)
    IFA
    APTIMA*

*Note: APTIMA, Genprobe (TMA format) qualitative assay only FDA approved nucleic acid amplification test (NAAT) for diagnosis and confirmation
## Screening Assays - Rapid Antibody Tests

(2nd gen - IgG)

<table>
<thead>
<tr>
<th>Assay</th>
<th>Sample Type</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraQuick ADVANCE Rapid HIV-1/2 Antibody Test</td>
<td>Oral fluid</td>
<td>99.3%</td>
<td>99.8%</td>
</tr>
<tr>
<td></td>
<td>Whole Blood</td>
<td>99.6%</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>99.6%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Clearview COMPLETE HIV 1/2</td>
<td>Whole Blood</td>
<td>99.7%</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td>Serum &amp; Plasma</td>
<td>99.7%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Clearview HIV ½ STAT-PAK</td>
<td>Whole Blood</td>
<td>99.7%</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td>Serum &amp; Plasma</td>
<td>99.7%</td>
<td>99.9%</td>
</tr>
<tr>
<td>Reveal G-3 Rapid HIV-1 Antibody Test</td>
<td>Serum</td>
<td>99.8%</td>
<td>99.1%</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>99.8%</td>
<td>98.6%</td>
</tr>
<tr>
<td>Uni-Gold Recombigen HIV</td>
<td>Whole Blood</td>
<td>100%</td>
<td>99.7%</td>
</tr>
<tr>
<td></td>
<td>Serum &amp; Plasma</td>
<td>100%</td>
<td>99.8%</td>
</tr>
<tr>
<td>Multisport HIV-1/HIV-2 Rapid Test</td>
<td>Serum</td>
<td>100%</td>
<td>99.9%</td>
</tr>
<tr>
<td></td>
<td>Plasma</td>
<td>100%</td>
<td>99.9%</td>
</tr>
<tr>
<td>INSTI HIV-1 Antibody Test*</td>
<td>Plasma</td>
<td>99.9%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Whole blood (venipuncture)</td>
<td>99.9%</td>
<td>100.0%</td>
</tr>
<tr>
<td></td>
<td>Whole blood (fingerstick)</td>
<td>99.8%</td>
<td>99.5%</td>
</tr>
</tbody>
</table>
OraQuick® Advance

- Synthetic gp-41 (HIV-1)
- Synthetic gp-36 (HIV-2)
- Goat anti-human IgG

Photograph from CDC: www.cdc.gov/hiv/rapid_testing
Multispot HIV-1/HIV-2

Detections and differentiates between HIV-1 and HIV-2
HIV Ab Screening Assays
(3rd gen – IgM and IgG)

- Third generation assays (IgG/IgM)
- Detect HIV infection on day 22
- Detect HIV-1/HIV-2 and HIV-1 group O depending on the assay
- Several automated platforms
HIV Antigen/Antibody Combination Assays (4th gen – p24 Ag/IgM/IgG)

- Detects both HIV-1 (group O) and HIV-2 antibodies and p24 antigen
- Does not distinguish between Ab+ or Ag+
- Only two FDA – cleared assays

Earlier Detection of HIV Infection: (4^{th} generation)

Detects infection at 2.5 - 3.0 weeks, 5 days earlier than 3^{rd} gen

Relative concentration

- RNA (viral load)
- p24
- HIV IgM
- HIV IgG

HIV-1 infection

weeks

months

years
False Positive Immunoassay Results

- Vaccinations
  - flu, rabies
- HIV vaccine trials
- Autoimmune disease
- Heterophile Antibodies
- Other viral infections
Confirmation by Western Blot

CDC Interpretation Criteria
- positive - presence of 2 of 3 cardinal bands
- negative - absence of all bands
- indeterminate – does not meet + or - criteria
Western Blot “Indeterminate”

• Indeterminate results may be due to
  infected but in the “window”
  advanced disease, AIDS
  HIV vaccinated
  infected with HIV-2
  uninfected, cross reactivity
  ➢ viral or non-viral bands, recent flu and rabies vaccinations, multiple pregnancies, recipients of multiple transfusions, autoimmune disease
  ➢ study followed 99 blood donors – 91 stable indeterminate Western blot patterns over 30 months

• Indeterminate results require follow-up
  repeat Western blot
  nucleic acid amplification test (NAAT)
HIV-1 vs HIV-2 and Western Blot

Percentage of specimens with each HIV-1 Western blot band in 114 specimens collected from persons infected with HIV-2 and 1761 specimens positive for HIV-1 by Western blot and Multispot HIV-1/HIV2 assay.

<table>
<thead>
<tr>
<th></th>
<th>p17</th>
<th>p24</th>
<th>p31</th>
<th>p40</th>
<th>gp41</th>
<th>p51</th>
<th>p55</th>
<th>p66</th>
<th>gp120</th>
<th>gp160</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HIV-2 (n=114)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>18.4</td>
<td>93.9</td>
<td>83.3</td>
<td>88.6</td>
<td>1.8</td>
<td>74.6</td>
<td>73.7</td>
<td>29.8</td>
<td>10.5</td>
<td>48.3</td>
</tr>
<tr>
<td>Present but weak</td>
<td>14.9</td>
<td>4.4</td>
<td>7.0</td>
<td>9.7</td>
<td>0.9</td>
<td>17.5</td>
<td>17.5</td>
<td>10.5</td>
<td>79.0</td>
<td>22.8</td>
</tr>
<tr>
<td>Absent</td>
<td>66.7</td>
<td>1.8</td>
<td>9.7</td>
<td>1.8</td>
<td>97.4</td>
<td>7.9</td>
<td>8.8</td>
<td>59.7</td>
<td>79.0</td>
<td>29.0</td>
</tr>
<tr>
<td><strong>HIV-1 (n=1761)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>78.8</td>
<td>91.4</td>
<td>95.2</td>
<td>-</td>
<td>97.4</td>
<td>97.2</td>
<td>93.3</td>
<td>95.0</td>
<td>98.6</td>
<td>99.9</td>
</tr>
<tr>
<td>Present but weak</td>
<td>6.3</td>
<td>7.3</td>
<td>2.0</td>
<td>-</td>
<td>1.7</td>
<td>1.4</td>
<td>1.3</td>
<td>2.8</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>Absent</td>
<td>14.9</td>
<td>1.4</td>
<td>2.8</td>
<td>-</td>
<td>0.9</td>
<td>1.4</td>
<td>5.4</td>
<td>2.2</td>
<td>0.8</td>
<td>0.0</td>
</tr>
</tbody>
</table>

HIV-2 Infection Classification by Western Blot

Comparison of two HIV-1 Western blot interpretive criteria applied to specimens collected from 114 persons known to be infected with HIV-2,

<table>
<thead>
<tr>
<th>Current CDC HIV-1 WB criteria</th>
<th>Alternative HIV-1 WB criteria</th>
<th>Negative</th>
<th>Indeterminate</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>1 (0.9)</td>
<td>0 (0.00)</td>
<td>0 (0.0)</td>
<td>1 (0.9)</td>
<td></td>
</tr>
<tr>
<td>Indeterminate</td>
<td>0 (0.0)</td>
<td>60 (52.6)</td>
<td>0 (0.0)</td>
<td>60 (52.6)</td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0 (0.0)</td>
<td>40 (35.1)</td>
<td>13 (11.4)</td>
<td>53 (46.5)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1 (0.9)</td>
<td>100 (87.7)</td>
<td>13 (11.4)</td>
<td>114 (100.0)</td>
<td></td>
</tr>
</tbody>
</table>

Sensitivity of Current Assays

Rapid Tests (mostly 2nd gen)
- Oraquick
- Unigold
- Statpak/Complete
- Reveal G2 & G3
- Multispot

3rd gen
- GS + O
- Vitros
- Advia

4th gen
- Architect
- Aptima
- NAAT

Days before Western blot positive

Adapted from Masciotra et al. Journal of Clinical Virology 2011.
Western Blot Disadvantages

• Diagnostic Considerations
  insensitive compared to current screening assays
  indeterminate/inconclusive results - follow-up
  HIV-2 misdiagnosis

• Practical Considerations
  access
  expense
  turn around time
*Could be an IgM sensitive Ab immunoassay if Ag/Ab combination assay is unavailable

AACC. Clinical Laboratory News. 2010
## GS Combo Ag/Ab and Long Standing HIV Infection Sensitivity (4th generation)

<table>
<thead>
<tr>
<th>Population</th>
<th>N</th>
<th>GS HIV Combo Ag/Ab</th>
<th>Licensed HIV-1/HIV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Known HIV-1 Ab positive US</td>
<td>100</td>
<td>1000 (100%)</td>
<td>1000 (100%)</td>
</tr>
<tr>
<td>Known HIV Ab positive, Non-US</td>
<td>200</td>
<td>200 (100%)</td>
<td>200 (100%)</td>
</tr>
<tr>
<td>AIDS</td>
<td>100</td>
<td>100 (100%)</td>
<td>100 (100%)</td>
</tr>
<tr>
<td>Known HIV-1 Ab positive, pediatric</td>
<td>40</td>
<td>40 (100%)</td>
<td>40 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>1340</td>
<td>1340</td>
<td>1340 (100%)</td>
</tr>
</tbody>
</table>

Adapted from Bentsen et al. Journal of Clinical Virology 2011.
## GS HIV Combo Ag/Ab Specificity (4th generation)

<table>
<thead>
<tr>
<th>Low Risk Population</th>
<th>Number tested</th>
<th>GS HIV Ag/Ab Combo</th>
<th>Repeatedly reactive Samples</th>
<th>Specificity (#negative/total)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Repeatedly Reactive (% Reactive)</td>
<td>WB positive (%positive)</td>
<td>HIV-2 positive (%positive)</td>
</tr>
<tr>
<td>Health insurance applicants</td>
<td>2000</td>
<td>6 (0.30%)</td>
<td>2</td>
<td>0 (0.00%)</td>
</tr>
<tr>
<td>Normal blood donors</td>
<td>2000</td>
<td>0 (0.0%)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Pregnant women</td>
<td>1000</td>
<td>2 (0.20%)</td>
<td>1</td>
<td>0 (0.00%)</td>
</tr>
<tr>
<td>Military recruits</td>
<td>1000</td>
<td>3 (0.30%)</td>
<td>1</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Healthy pediatric subjects</td>
<td>100</td>
<td>0 (0.0%)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Total</td>
<td>6100</td>
<td>11 (0.18%)</td>
<td>4</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

Adapted from Bentsen et al. Journal of Clinical Virology 2011.
## Architect Ag/Ab Combo Performance Data (4\textsuperscript{th} generation)

<table>
<thead>
<tr>
<th>Result</th>
<th>No of samples</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 infected (n-3386)</td>
<td>HIV-1 Uninfected (N=7551)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Initial screening</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3384</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>7459</td>
<td></td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td>99.94%</td>
<td>98.78%</td>
</tr>
<tr>
<td><strong>Retest screening</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>3384</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>2</td>
<td>7513</td>
<td></td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td>99.94%</td>
<td>99.50%</td>
</tr>
<tr>
<td><strong>Result Acute Infection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Performance</td>
<td></td>
<td></td>
<td>82.76%</td>
</tr>
</tbody>
</table>

Adapted from Chavez et al. Journal of Clinical Virology 2011
## Multispot vs. Western Blot

<table>
<thead>
<tr>
<th></th>
<th>Multispot Positive</th>
<th>Multispot Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Row %</td>
<td>N</td>
</tr>
<tr>
<td>WB positive</td>
<td>8670</td>
<td>99.9%</td>
<td>8</td>
</tr>
<tr>
<td>WB negative</td>
<td>3</td>
<td>15.8%</td>
<td>16</td>
</tr>
<tr>
<td>WB indeterminate</td>
<td>23</td>
<td>36.5%</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>8696</td>
<td>99.3%</td>
<td>64</td>
</tr>
</tbody>
</table>

Adapted from Torian et al. Journal of Clinical Virology 2011.
NAAT for HIV Diagnosis

- APTIMA® HIV-1 Qualitative Assay (FDA approved 2006)
  – TMA, Hybrid capture for RNA purification
- Screening of high-risk populations
- Known exposure such as needle-stick
- Testing patients with acute HIV-1 symptoms and known exposure
- Screening of newborn babies born to infected mothers
- HIV vaccine studies
- Resolution arm for new screening algorithms
APTIMA® HIV-1 Qualitative Assay

• Poses dilemma for diagnostic algorithm:
  – Only approved test with diagnostic claim few installations, limited test availability
  – Manual test format
  – 1\textsuperscript{st} generation chemistry design, ability to see new strains?

Hybrid Capture Purification  Transcription Mediated Amplification (TMA)  Hybridization Protection Dual Kinetic Detection
## Aptima vs Real-time PCR Tests

<table>
<thead>
<tr>
<th></th>
<th>Aptima Gen-Probe</th>
<th>Real Time Abbott</th>
<th>TaqMan 1.0 Roche</th>
<th>TaqMan 2.0 Roche</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>30 copies/ml</td>
<td>40 copies/ml</td>
<td>43 copies/ml</td>
<td>20 copies/ml</td>
</tr>
<tr>
<td><strong>Genotypes</strong></td>
<td>A-O</td>
<td>A-O</td>
<td>A-G</td>
<td>A-G</td>
</tr>
<tr>
<td><strong>Amplicon control</strong></td>
<td>Strand Capture</td>
<td>closed</td>
<td>UTP/UNG, closed</td>
<td>UTP/UNG closed</td>
</tr>
<tr>
<td><strong>Automation</strong></td>
<td>No (U.S.)</td>
<td>yes</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td><strong>FDA approval</strong></td>
<td>Diagnosis</td>
<td>Monitor</td>
<td>Monitor</td>
<td>Monitor</td>
</tr>
</tbody>
</table>

Note: bDNA (signal amplification assay) has ~1% low positivity rate. In negative samples, not suitable for resolution testing.
Comparative Assay Sensitivities (probit modeling of PI data)

Aptima

Roche 2.0

Abbott

Andrew Wilson
Targets, Chemistry, Issues

Cobas 1.0, 1.5 end-point PCR Roche

CapTaq 2.0 CapTaq 1.0 real-time PCR Roche

Aptima

Real Time Abbott

Pyne, M. J Clin Microbiol, 48(8), 2852-2858.
HIV-1 Proviral DNA Testing

• Whole blood assay, detects RNA and DNA
• Uses
  – Infants born to HIV-1 infected mothers
  – 2 serially positive RNA or DNA tests necessary for diagnosis in infants < 2 years of age
  – Early diagnosis of infants on prophylactic therapy (RNA suppressed)
• Whole blood assay (Roche)
  – 1.0 chemistry
  – CAPTAQ robot
Expanding HIV Diversity in U.S.

Pyne et al. 2012
Expanding HIV Diversity in U.S.

Pyne et al 2012
Molecular Take-Home Points

• Only APTIMA® is approved for HIV diagnosis
  – Automation may eventually occur on Panther platform
• Viral Load tests have equivalent “analytic performance” compared to APTIMA®
  – Guidelines stirred interest in claims for diagnosis
  – Process will be slow
• Precedent for off-label use molecular tests for confirmation/resolution (HCV)
• Proviral HIV-1 DNA testing available, not approved
• Very few LDT HIV-2 RNA assays available
# Comparing Algorithms

<table>
<thead>
<tr>
<th>Algorithm</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td></td>
<td>%</td>
<td>95% CI</td>
</tr>
<tr>
<td><strong>Two-test current algorithm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Architect/WB</td>
<td>99.76</td>
<td>98.65 - 99.06</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>99.08 - 100.00</td>
</tr>
<tr>
<td>GS+O /WB</td>
<td>100.00</td>
<td>99.09 – 100.00</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>99.08 – 100.00</td>
</tr>
<tr>
<td><strong>Three-test proposed algorithm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Architect or GS+O/Multispot/NAAT</td>
<td>99.76</td>
<td>98.65 – 99.96</td>
</tr>
<tr>
<td></td>
<td>100.00</td>
<td>99.08 – 100.00</td>
</tr>
</tbody>
</table>

Adapted from Masciotra et al. Journal of Clinical Virology 2011.
ARUP

Offers both third and fourth generation screening assays

Acute HIV Case
Validating Multispot

<table>
<thead>
<tr>
<th></th>
<th>Multispot negative</th>
<th>Multispot HIV-1 positive</th>
<th>Multispot HIV-2 positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB negative</td>
<td>12</td>
<td></td>
<td></td>
<td>12</td>
</tr>
<tr>
<td>WB positive</td>
<td></td>
<td>9</td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>WB indeterminate</td>
<td>2</td>
<td>5</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>HIV-2 confirmed positive</td>
<td></td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
New Algorithm Benefits

• Improves detection of acute HIV infection
  Ag/Ab Combo Assay
  NAAT confirmation

• Increased detection of HIV-2 infection
  replacing Western blot with Multispot

• Eliminate inconclusive/indeterminate results
  eliminating the Western Blot

• Decrease turn around time & linkage to care
  replacing Western blot with Multispot
New Algorithm Challenges

• Only two platforms currently available for Ag/Ab Combo assays
• Multispot is a rapid test, not approved for confirmation of HIV infection
• There is only one qualitative molecular assay approved for HIV diagnosis (Aptima) that is not automated and therefore not routinely available
• High-throughput quantitative or viral load HIV assays are widely utilized but none is approved for diagnosis
Clinical Considerations

• Ag/Ab combination or 4\textsuperscript{th} assays are the most sensitive screening assays and should be used if acute HIV infection is suspected

• Multispot discriminates between HIV-1 and HIV-2 infection

• Both the Multispot and Western blot can detect established HIV infection, majority of HIV diagnoses

• If a result is positive by 4\textsuperscript{th} gen screening assay but negative by either the Western blot or Multispot (Ab detection only), further testing by molecular assays (NAAT) is necessary

• NAAT testing cannot be used as the second step because it can be negative in:
  
  HIV-2 infection
  
  HIV infected individuals that are elite suppressors/controllers - 0.5%
Thanks

• Orly Ardon
• Jennifer Blackley
• Scott Griffiths
• Michael Pyne
• Melanie Mallory
• Malissa Jones
• Jason Metz
Universal Screening?

- Laws
- Reimbursement

Adapted from JAMA 2011
Transmission in Adults

Adults and Adolescents Living with an AIDS Diagnosis, by Transmission Category, 1985–2009—United States and 6 U.S. Dependent Areas

- Male-to-male sexual contact
- Injection drug use (IDU)
- Male-to-male sexual contact and IDU
- Heterosexual contact
- Other

No. of persons (in thousands)

Year

Note. All displayed data have been statistically adjusted to account for reporting delays and missing risk factor information, but not for incomplete reporting.

* Heterosexual contact with a person known to have, or to be at high risk for, HIV infection.

* Includes hemophilia, blood transfusion, perinatal exposure, and risk factor not reported or not identified.
Global HIV Epidemiology

Detection of HIV by Diagnostic Tests

Symptoms
p24 Antigen
HIV RNA
HIV EIA*
Western blot

Weeks Since Infection
0 1 2 3 4 5 6 7 8 9 10

*3rd generation, IgM-sensitive EIA
*2nd generation EIA
*viral lysate EIA

After Fiebig et al, AIDS 2003; 17(13) 1871-9

Confirmation for HIV-1 Infection

• All repeatedly reactive EIA/CIA screening assay results must be confirmed

• POC results are considered “preliminary positive” results and must also be confirmed

• Confirmation for HIV-1 Infection
  Indirect Immunofluorescence (IFA)
  Western Blot
NA-ACCORD: Increasing Life Expectancy in HIV+ Adults Receiving ART

### GS Combo Ag/Ab & Acute HIV Infection (4th generation)

<table>
<thead>
<tr>
<th>Acute HIV patient</th>
<th>Days from 1st bleed</th>
<th>HIV-1 RNA copies (mL)</th>
<th>GS HIV Combo Ag/Ab</th>
<th>Historical results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>HIV-1/HIV-2 EIA</td>
<td>HIV-1 EIA</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>&gt;500,000</td>
<td>R</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>56</td>
<td></td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>183,850</td>
<td>R</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>10,479</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td></td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>&gt;500,000</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>141</td>
<td></td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>&gt;500,000</td>
<td>R</td>
<td>NR</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td></td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>&gt;500,000</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td></td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>RR</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

Adapted from Bentsen et al. Journal of Clinical Virology 2011.
## Detection of Rare HIV Genotypes

<table>
<thead>
<tr>
<th>Assay Type</th>
<th>HIV-1 group M, non-B</th>
<th>HIV-1 group O</th>
<th>HIV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1/HIV-2/O Ab (3rd gen)</td>
<td>detected</td>
<td>detected</td>
<td>detected</td>
</tr>
<tr>
<td>HIV-1/HIV-2/O Ag/Ab Combination (4th gen)</td>
<td>Ab detected</td>
<td>Ab detected</td>
<td>Ab detected</td>
</tr>
<tr>
<td></td>
<td>Ag sensitivity is assay dependent</td>
<td>Ag sensitivity is assay dependent</td>
<td>Ag sensitivity is assay dependent and HIV-2 detection is dependent on cross-reactivity</td>
</tr>
<tr>
<td>HIV-1 WB</td>
<td>detected</td>
<td>negative indeterminate</td>
<td>negative indeterminate</td>
</tr>
<tr>
<td>HIV-1 RNA NAAT (qualitative)</td>
<td>detected</td>
<td>detected</td>
<td>not detected</td>
</tr>
<tr>
<td>HIV-1 RNA NAAT (quantitative)</td>
<td>detected but quantification is assay dependent</td>
<td>detection and quantification assay dependent</td>
<td>not detected</td>
</tr>
<tr>
<td>HIV-1 RNA NAAT (genotyping)</td>
<td>detected</td>
<td>not detected</td>
<td>not detected</td>
</tr>
</tbody>
</table>
### Acute HIV Infection

(3rd gen, 4th gen, Western blot and NAAT)

---

**Analysis of the current two-test algorithm in acute HIV-1 infections (seroconversion panels).**

<table>
<thead>
<tr>
<th>Screening test</th>
<th>GS+O</th>
<th>Vitros</th>
<th>Advia</th>
<th>Architect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of first positive results</td>
<td>108</td>
<td>110</td>
<td>111</td>
<td>135</td>
</tr>
<tr>
<td>WB positive (n)</td>
<td>56</td>
<td>56</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>WB indeterminate (n)</td>
<td>38</td>
<td>39</td>
<td>39</td>
<td>43</td>
</tr>
<tr>
<td>+NAAT positive (n)</td>
<td>36</td>
<td>37</td>
<td>37</td>
<td>41</td>
</tr>
<tr>
<td>+NAAT negative (n)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>+NAAT not available (n)</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Adapted from Masciotra et al. Journal of Clinical Virology 2011.