HIV Testing Update

Speakers

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Updated HIV Testing Terminology and Technology

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APHL Webinar

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. Tradenames are used for informational purposes and does not constitute an endorsement by CDC.
Terminology

- **Immunoassay (IA)** – a biochemical test that detects the presence of a substance in a biological specimen using the binding of an antibody to its antigen
- **Enzyme immunoassay (EIA or ELISA)** – an immunoassay that uses the catalyzing properties of an enzyme for detection of an immunological reaction
- **Chemiluminescent assay (CIA or CMIA)** – an immunoassay in which the signal is generated by a compound that emits light as the result of a chemical reaction
- **Nucleic acid test (NAT) (qualitative)** – molecular assays for detection of the presence of viral nucleic acids (DNA or RNA); NOTE: Sometimes referred to as nucleic acid amplification test (NAAT)
- **Nucleic acid test (NAT) (quantitative)** – molecular assays for quantification of viral nucleic acids (DNA or RNA); NOTE: Sometimes referred to as viral load assays
- **Initial or Screening test** – first test done in the field or in an algorithm
- **Supplemental test** – test used in an algorithm to “confirm” a previous result
Serologic Assays

Lysate indirect IgG

Peptide/recombinant protein indirect IgG

Conjugated Synthetic Peptide IgG/IgM

Antigen/Antibody Combo
Serology Technology

Bio-Rad GS HIV Combo Ag/Ab

~ 3-4 hours

Abbott Architect Ag/Ab Combo Assay**

~30 mins

BioPlex® 2200 HIV Ag-Ab ** #

~ 1 hour

Alere Determine™ HIV-1/2 Ag/Ab #

~25 mins

ADVIA Centaur® HIV Ag/Ab Combo** #

~ 1 hour

- Need HIV algorithm data #
- Automated platforms – multiple pathogens **
Rapid Test Terminology

• Characteristics
  – Individual sample
  – Produces result in 30 min or less
  – Currently 3 general platforms
    • Lateral Flow
      – Sample flows “up” test strip over antigen lines
      – Often CLIA waved
    • Dual Path Platform (DPP)
      – Sample and reagents flow from different directions
    • Flow Thru (Immuno-concentration)
      – Sample flows thru membrane containing antigens
      – Generally moderate complex
CLIA Waived

OraQuick Advance 2002

Uni-Gold Recombigen 2003

Clearview Complete HIV 1/2 2006

Clearview HIV 1/2 Stat Pack 2006

DPP® HIV 1/2 Assay 2012, 2014

Determine 2013, 2014

Insti 2010, 2012
Moderate Complexity

Reveal G3
2003

Geenius™
2014

Multispot HIV-1/HIV-2
2004
NAT Terminology

• PCR – Polymerase chain reaction
  – Cyclic biochemical reaction that requires multiple temperatures
  – RT- PCR
    • Reverse Transcriptase PCR
      – RNA to cDNA (Viral Load)
    • Real-Time PCR
      – Allows real time monitoring of product

• TMA- Transcription mediated amplification
  – Uses gene transcription as the basis for amplification- isothermal
Current NAT Technology

Hologic Aptima HIV-1 Qualitative NAT

Roche COBAS® AmpliPrep/COBAS® TaqMan® HIV-1*

Abbott RealTime m2000*

Roche cobas® 6800*

* Not FDA approved for diagnosis
Laboratory Markers of HIV Infection

Days since detectable RNA

HIV RNA (plasma)

HIV p24 Ag

IgM

IgG

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.

New Approach for Sequence of Test Reactivity
NATs on the Horizon

Hologic Panther

Cepheid GeneXpert® System

Diagnostics for the Real World
SAMBA

Liat™ Analyzer Roche
HIV DIAGNOSTICS INFORMATIONAL UPDATES

APHL HIV and Viral Hepatitis Subcommittee

Monica Parker, Ph.D.
Wadsworth Center, NYSDOH
Chair, APHL HIV/Viral Hepatitis Subcommittee
Faculty Disclosure

The Association of Public Health Laboratories adheres to established standards regarding industry support of continuing education for healthcare professionals. The following disclosures of personal financial relationships with commercial interests within the last 12 months as relative to this presentation have been made by the speaker(s):

Monica Parker, PhD
“Nothing to disclose”.
New HIV Diagnostic Tests

• Information was gathered on three new HIV diagnostic tests
  – ADVIA Centaur® HIV Ag/Ab Combo Assay
  – BioPlex® 2200 HIV Ag-Ab Assay
  – Geenius™ HIV 1/2 Supplemental Assay

• The update is available at:
  http://www.aphl.org/aphlprograms/infectious/hiv/Documents/2015_Info...12_16_FINAL.pdf
ADVIA Centaur® HIV Ag/Ab Combo

Siemens Healthcare Diagnostics

• FDA approved June 2015
• Chemiluminescent microparticle immunoassay (CMIA)
• Detects HIV-1 p24 Ag, HIV-1 and HIV-2 antibodies
  – Does not distinguish analytes
  – Reactive: repeat in duplicate
  – Time to result ~ 1 hour

Centaur, Centaur XP
• Automated
• Random Access
• Immunoassays for other pathogens
BioPlex® 2200 HIV Ag-Ab Assay

Bio-Rad Laboratories

- FDA approved July 2015
  - Approved for plasma and serum
- Multiplex flow immunoassay
- Detects and differentiates HIV-1 p24 Ag, HIV-1 Ab and HIV-2 Ab
  - Separately coated beads to detect each analyte independently
  - Reactive: repeat in duplicate
  - Index ≥ 1.0 = Reactive
  - Time to result ~ 1 hour
- Not for supplemental use

BioPlex 2200 system
- Automated
- Random Access
- Immunoassays for other pathogens
# Lab-based 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</td>
<td>Fully automated, random access (i2000SR)</td>
<td>Serum, Plasma</td>
<td>Nonreactive Reactive</td>
</tr>
<tr>
<td>(Abbott Diagnostics)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GS HIV Combo Ag/Ab EIA</td>
<td>Manual or semi-automated instrument (Evolis)</td>
<td>Serum, Plasma</td>
<td>Nonreactive Reactive</td>
</tr>
<tr>
<td>(Bio-Rad Laboratories)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADVIA Centaur HIV Ag/Ab Combo</td>
<td>Fully automated, random access (Centaur/Centaur XP)</td>
<td>Serum</td>
<td>Nonreactive Reactive</td>
</tr>
<tr>
<td>(Siemens Healthcare Diag.)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BioPlex 2200 HIV Ag-Ab</td>
<td>Fully automated, random access (BioPlex 2200)</td>
<td>Serum, Plasma</td>
<td>Nonreactive Reactive for HIV Ag-Ab with Reactive for HIV-1 Ag and/or Reactive for HIV-1 Ab and/or Reactive for HIV-2 Ab or Reactive, Undifferentiated</td>
</tr>
<tr>
<td>(Bio-Rad Laboratories)</td>
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</tbody>
</table>
Geenius™ HIV 1/2 Supplemental Assay

Bio-Rad Laboratories

• FDA approved in Oct 2014 for supplemental use only
• Replacement for Multispot – Discontinued by Jul 2016
• Differentiates HIV-1 and HIV-2 antibodies
• DPP™ design, Protein A colloidal gold (IgG)
• Result within 30 min

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™ and Multispot: Features

<table>
<thead>
<tr>
<th><strong>Multispot</strong></th>
<th><strong>Geenius</strong></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>

- HIV-2 gp36 peptide
- HIV-1 gp41 recomb
- HIV-1 gp41 peptide
- Control
<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>
Test results unique to Geenius - I

HIV-2 positive with HIV-1 cross-reactivity

- Antibody to HIV-2 is confirmed
  - Criteria for HIV-1 positivity are met, but only one HIV-1 envelope band (gp160 or gp41) is present
- From PI: Of 200 known HIV-2 Ab positive samples, Geenius result was interpreted as:
  - HIV-2 Positive for 38.50% (77/200)
  - HIV-2 with HIV-1 cross-reactivity for 54.00% (108/200)
  - HIV Untypable (undifferentiated) for 6.00% (12/200)
  - HIV-2 indeterminate for 1.50% (3/200)
- Next step: Refer to care for HIV-2 infection
Test results unique to Geenius - II

HIV-2 Indeterminate

- PI: Repeat to confirm before reporting
  - If repeat is non-reactive, record as non-reactive and proceed to HIV-1 RNA
  - If result recurs on repeat, result is confirmed; report as HIV-2 indeterminate

- Next step for HIV-2 indeterminate will be addressed later in presentation
Test results unique to Geenius - III

HIV Indeterminate

• Bands present for both HIV-1 and HIV-2 but criteria to be called positive not met for either

• Since HIV-1 indeterminate is a component of this result, HIV-1 NAT is needed
  – Additional guidance will follow
2016 HIV DIAGNOSTICS CONFERENCE

The New Landscape of HIV Testing in Laboratories, Public Health Programs and Clinical Practice
Overview of the 2016 Conference

• Abstracts presented: 36 Oral presentations, 4 Roundtables, 30 Posters

• Performance data:
  – New diagnostic tests
  – Recommended lab algorithm, and alternatives

• Impact of testing strategies on turn-around and linkage to care

• New challenges for surveillance programs

• New approaches to clinical staging, incidence estimation
Access to Conference Materials

• Website: [http://hivtestingconference.org](http://hivtestingconference.org)
  – Program book containing all abstracts
  – Slides from oral presentations and posters

• Conference will be featured in an issue of Journal of Clinical Virology
  – Conference summary
  – 3-4 invited papers from the conference
New Technology and its Impact on the CDC/APHL Laboratory Testing Algorithm

Laura Wesolowski, Ph.D.

May 6, 2016

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Recommended Laboratory Algorithm
http://www.cdc.gov/hiv/testing/lab/guidelines/

HIV-1/2 Ag/Ab combination immunoassay

(+)

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

(-) indicates negative test results
NAT: nucleic acid test
Geenius HIV 1/2 Supplemental Test

- Rapid test with automated reader; differentiates HIV-1 from HIV-2.
  - Additional results
    - HIV-2 indeterminate
    - HIV indeterminate
    - HIV-2 positive, HIV-1 cross reactivity
  - Established HIV-1, 100% concordance Multispot; 1.6% HIV positive untypable (Bennett, Diagnostics conference)
  - No false-positive algorithm results Ag/Ab and Geenius (Delaney, Dx conference)
Geenius HIV 1/2 Supplemental Test

- HIV-2 indeterminates?
  - Delaney: 3 HIV-2 indeterminates
    - 2 from 165 false positive Ag/Ab or IgM Ab (gp140 only)
    - 1 acute HIV-1 (gp140 only)
  - Bennett: 2 HIV-2 indeterminates
    - from 60 false positive Ag/Ab (gp140 only)
  - Luo: 8 HIV-2 indeterminates
    - 2 HIV-2s (gp36 only)
    - 3 acute HIV-1 (gp140 only)
    - 3 false pos Ag/Ab (gp140 only)
Geenius HIV 1/2 Supplemental Test

- Data needs
  - Cost data for Geenius
  - HIV indeterminates
  - Performance on whole blood
  - If Geenius HIV-2 IND or HIV IND, conduct HIV-1 NAT. If HIV-1 NAT negative, test with HIV-2 test.
Determine HIV-1/2 Ag/Ab Combo

- CLIA-waived rapid test- fingerstick whole blood
- CLIA-moderately complex-serum, plasma, or whole blood
- Distinguishes Ag from Ab, but does not differentiate HIV-1 Ab from HIV-2 Ab
- Interpreted subjectively (no reader)
Determine HIV-1/2 Ag/Ab Combo

- Early infection
  - Plasma
    - Ag not detected, most acutes (Laperche, Rosenberg, Kilembe, Duong, Conway, Faraoni)
    - Ag detected at about 3 million copies/mL (Pandori, Dx conference)
    - Detects infection earlier than IgM-sensitive assays, not as early as lab-based Ag/Ab assays (Masciotra, JCV)
    - Detected 40-76% early infections (Delaney, Masciotra, Patel JCV)
  - Whole blood
    - 0% Ag sensitivity, acutes (Lewis, AIDS)
    - Detects infection 2 days after plasma (Masciotra, CROI)
Determine HIV-1/2 Ag/Ab Combo

- Sensitivity
  - Early/established infection
    - Whole blood: 84.6% (Stekler, JCV)
    - Serum: 88.9% (Gillis, Dx conference)
  - Established infection
    - Plasma: 99.6%-100% (Delaney, Masciotra, Dx conference)
- Specificity
  - Plasma: 98.9% (Delaney); No false positive algorithm results
  - Serum: 98.9% (Wester, NHPC)
  - Whole blood: 98.3%-99.9% (6 sources)
Determine HIV-1/2 Ag/Ab Combo

- Data needs
  - Sensitivity using whole blood
  - Performance data using Determine with serum or plasma as the initial test in the algorithm
    - Specificity of Ag to determine whether to go directly to NAT
BioPlex 2200 HIV Ag-Ab assay

- Lab-based screening assay for detection and differentiation of HIV-1 Ag, HIV-1 Ab, and HIV-2 Ab in serum or plasma.
  - Early infection detection similar to lab Ag/Ab tests (Masciotra, Delaney)
  - 100% sensitivity, established HIV-1 (Salmona, JCM and Delaney)
  - 100% sensitivity, HIV-2 (package insert)
  - 99.5% specificity (Salmona)
  - No false positive algorithm results (Delaney)
BioPlex 2200 HIV Ag-Ab assay

- Data needs
  - Specificity of Ag reactivity
  - Data on performance of Bioplex with Geenius and NAT
  - If HIV-2 Ab reactive, reflex to an antibody differentiation supplemental test, and if that is HIV-2 negative, an HIV-1 NAT. If HIV-1 NAT negative, conduct another HIV-2 assay.
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 (+) untypable
HIV antibodies detected

HIV-1 (-) HIV-2 (-) or indeterminate

NAT

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

Bio-Plex Determine

Geenius

*Test for HIV-2 if Geenius HIV or HIV-2 IND OR Bioplex HIV-2 + and Geenius HIV-2 NEG
Laura Wesolowski
Email: lig7@cdc.gov

For more information, contact CDC
1-800-CDC-INFO (232-4636)

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Polling Question 1

If the Alere Determine antigen/antibody rapid test (performed on serum or plasma) was recommended as an alternative first test in the algorithm would your laboratory use it?

A. Yes
B. No
C. I don’t know
Polling Question 2

Is your laboratory using the BioPlex 2200 HIV Ag-Ab Assay?

A. Yes, currently using it
B. Not currently, planning to validate and use in the next 6-12 months
C. No plans to use the assay
Polling Question 3

What is your laboratory’s plan for supplemental antibody testing for serum/plasma specimens after the discontinuation of Multispot in July 2016?

A. Verify and Use Bio-Rad Geenius
B. Send out for supplemental antibody testing-Commercial
C. Send out for supplemental antibody testing-Other public health lab
D. Validate and Use HIV Antibody rapid test
E. Validate and Use HIV Antibody immunoassay
F. Undecided
G. None of the above
Polling Question 4

If there was an HIV-1 NAT test that was FDA approved for monitoring HIV-1 Viral load (quantitative) and for diagnosing HIV-1 infection (qualitative RNA similar to the APTIMA) would your laboratory be likely to bring it on?

A. Yes
B. No
C. I don’t know
Polling Question 5

Which of the following are barriers to HIV NAT testing? (Select all that apply)

A. Cost of platform/reagents
B. Current diagnostic option is too labor intensive
C. Lack of a viable test option for your lab needs
D. Do not receive appropriate samples for NAT
E. None of the above
Questions???