Update on HIV Diagnostic Testing

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THE ENEMY
Acknowledgements

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Bernard Branson, MD---- CDC
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Outline of Today’s Presentation

• Describe changes in HIV testing technology

• Outline relative sensitivity of immunoassays during early HIV infection

• Experience detecting Acute HIV Infection

• Performance of the recently proposed Alternative HIV Diagnostic Algorithm
HIV Prevalence

Where Do You Live??

www.CDC.GOV
Why Do We Need Better HIV Testing Algorithms?

• **50,000** new people infected with HIV-1 annually in the U.S.

• Routine testing detects HIV infection earlier, allows for earlier treatment, and limits spread of infection.

• HIV antibody tests miss acute infections due to long “serological window period” (antibody results are negative despite presence of infection)
  
  – Acute infection: time of first detection of HIV (RNA, DNA or viral antigen) until first detection of confirmed HIV-1 specific antibodies
  
  – It takes ~22 days after infection for detectable HIV antibodies to first appear → window period.

  – **Patients with acute HIV infections are at a higher risk of transmitting HIV**
The Public Health Service recommends that no positive HIV-1 test results be given to clients/patients until:

1. a screening test has been repeatedly reactive on the same specimen and

2. a supplemental, more specific test such as the Western blot has been used to validate those results.
1989 “State of the Art”
“Generations” of Technological Advancement
1st and 2nd Generation HIV EIA

Antigen
1st - Viral lysate
2nd - Recombinant proteins or synthetic peptides

Plasma/serum

IgG HIV antibody

Enzyme-detection

Color reagent

Detects HIV IgG

Quest Diagnostics

Courtesy B. Branson-CDC
HIV-1 Antibody 1\textsuperscript{st} and 2\textsuperscript{nd} Gen Screening EIA

EIA Plate

EIA Plate Reader
HIV-1 Antibody Confirmatory Testing

Western Blot

IFA

ANATOMY OF HIV-1
3rd Generation “Sandwich” Antibody EIA

Antigen: Recombinant proteins or synthetic peptides

Plasma/serum

HIV antibody

IgG

IgM

enzyme

Enzyme-detection

HIV antigen

Detects HIV IgM or IgG

Color reagent

Courtesy B. Branson-CDC
Current HIV-1/2 “Third Generation”-Based Algorithm

• Screen with HIV-1/2 assay (IVD)
  – Confirm reactives in duplicate

• Reflex to HIV-1 Western blot (IVD) if screen is “repeatedly reactive”

• Reflex to HIV-2 EIA (IVD) if Western blot is negative or indeterminate

• Reflex to HIV-2 Immunoblot (RUO) if HIV-2 EIA is repeatedly reactive

CDC MMWR, July 17, 1992

Centers for Disease Control / Food and Drug Administration testing algorithm for use with combination HIV-1 / HIV-2 enzyme immunoassays (EIAs)

† An immunofluorescence assay (IFA) for HIV-1 antibodies has recently been licensed by the Food and Drug Administration and can be used instead of Western blot. Positive and negative IFA results should be interpreted in the same manner as similar results from Western blot tests. An indeterminate IFA should first be tested by HIV-1 Western blot and then as indicated by the Western blot results.

†† Perform HIV-2 EIA only if there is an identified risk factor for HIV-2 infection.
4th Generation HIV-1/2 Antigen/Antibody "Combo" Assay

Plasma/serum

HIV antibodies

p24 antigen

Enzyme-detection

HIV antigen

p24 antibody

Dectes IgM or IgG antibody or p24 antigen

Color reagent

Color reagent

Quest Diagnostics

Courtesy B. Branson-CDC
Selected HIV Tests FDA-Approved in the Last 10 Years
ADVIA® Centaur™ CP HIV 1/O/2 Enhanced (EHIV)

- Chemiluminescent immunoassay
- 3rd generation format
  - HIV-1: gp41, p24
  - HIV-2: gp36
  - group O
- Time to result <1 hour
- FDA-approved July 2006
Random Access Multiplatform analyzers for HIV testing

On-board Refrigeration of Multiple Different Assays
Random Access Multiplatform analyzers for HIV testing

STAT sample requests without pausing
Ortho VITROS ECi/ECiQ

- Chemiluminescent immunoassay

- 3rd generation format
  - HIV-1: gp41, gp120, p24
  - HIV-2: gp36
  - group O

- Time to result <1 hour

- Repeat only borderline results

- FDA-approved March 2008
Abbott Architect 4th Generation Ag/Ab Combo Assay

- Chemiluminescent immunoassay
- Detects p24 antigen and HIV antibody
- Time to result: 29 mins
- FDA-approved June 2010
Bio-Rad GS HIV Combo Ag/Ab EIA

• Microwell plate EIA

• 3rd generation Ab format:
  - HIV-1: gp160
  - HIV-2: gp36
  - Group O

• p24 antigen

• FDA-approved July 2011
Alere Determine™ Combo Rapid HIV 1/2 Ag/Ab Test

- CLIA moderate complexity
- Distinguishes Ag from Ab
- Whole blood, serum plasma
- FDA-approved August 2013
- 26 seroconverters were analyzed with 14 tests
- 17 seroconverters with WB positive used for cumulative frequency analysis
Sequence of Test Positivity Relative to WB (plasma)
166 specimens, 17 Seroconverters - 50 % Positive Cumulative Frequency

Days before WB positive

APTIMA RNA (-26)
Architect Ag/Ab Combo (-20)
Bio-Rad Ag/Ab Combo (-19)
Determine Ag/Ab Combo (-15)
Advia (-14)
Vitros (-13)
GS 1/2+O (-12)
INSTI (-9)
Multi-Spot (-7)
Reveal G3, Avioq, DPP (-6)
COMPLETE HIV-1/2 (-5)
HIV-1/2 STAT-PAK (-5)
Unigold (-2)
OraQuick (-1)
Vironostika (+2)
WB positive

Oral Fluid (Avioq)

25 20 15 10 5 0 +40


Quest Diagnostics


Luo et al, J Clin Virol 2013

Courtesy B. Branson-CDC
**Why Does It Matter?**

- Increased sensitivity for detection of HIV infection among frequently-tested MSM in Seattle

- 192 infected with HIV
  - 153 (80%) detected by oral fluid Ab rapid test
  - 169 (88%) detected by serum Ab immunoassay
  - 23 (12%) detected only by RNA
    - (15/16 tested detected by Ag/Ab immunoassay)

  - Stekler et al, Clin Inf Dis 2009
Performance of 4 antibody tests – Clinical Testing, San Francisco

<table>
<thead>
<tr>
<th>Test</th>
<th>Acute HIV</th>
<th>Established HIV</th>
<th>All HIV</th>
<th>Sensitivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraQuick, Oral</td>
<td>0/11</td>
<td>110/116 (94.8%)</td>
<td>110/127</td>
<td>86.6%</td>
</tr>
<tr>
<td>OraQuick, Fingerstick</td>
<td>0/18</td>
<td>226/228 (99.1%)</td>
<td>226/246</td>
<td>91.9%</td>
</tr>
<tr>
<td>Vironostika EIA (1st Gen Ab)</td>
<td>0/22</td>
<td>262/262 (100%)</td>
<td>262/284</td>
<td>92.3%</td>
</tr>
<tr>
<td>GS EIA (3rd Gen Ab)</td>
<td>3/7</td>
<td>97/97 (100%)</td>
<td>100/104</td>
<td>96.2%</td>
</tr>
</tbody>
</table>

*Pilcher et al, PLoS One 2013*
Houston Hospitals, 2011-2013

• 3rd generation Ab EIA laboratory testing

- 238 samples were Repeatedly Reactive but Western blot negative or indeterminate

➢ All sent for HIV NAT testing

  26 (10.9%) positive = acute HIV infection
Increased Risk of Sexual Transmission of HIV

Virus 75-750 times more infectious

Cohen & Pilcher, J Infect Dis. 2005

Courtesy B. Branson-CDC
Acute HIV Infection: Partner Notification

• Persons with acute HIV infection named
  – 2.5 times as many sex partners
  – 1.9 times as many partners newly diagnosed with HIV

  …as did persons with new diagnosis of established HIV infection

Moore et al, JAIDS 2009
Clinical Syndrome of Acute HIV

- 40-90% develop symptoms of Acute HIV
- 50%-90% with symptoms seek medical care
- Of those diagnosed with Acute HIV, 50% of patients were seen by the health care system at least 3 times before diagnosis

- Kahn et al, NEJM 1999
- Weintrob et al, Arch Int Med 2003
Phoenix Emergency Department Screening --
July 2011 through February 2013

• 4th gen screening of patients who had blood
drawn
  – 15% of patients declined testing
  – 13,014 patients tested
  – 37 (0.3%) new HIV infections
    • 12 of these 37 (32.4%) had Acute HIV Infection (antibody negative)

• Median viral load:
  – Patients with acute infections: 3.6 million cp/mL
  – Patients with established infections: 25,000 cp/mL

-MMWR June 21, 2013
New HIV Algorithms Made Possible By

• Availability of:
  • 4th generation HIV Ag/Ab combo assay
  • HIV-1/HIV-2 differentiation immunoassay: moderately complex, rapid test (Multispot – U.S. FDA cleared)
  • HIV nucleic acid tests (NAT) for sensitive detection of HIV RNA
• New available tests shorten the “window period”
• Window period: 4th gen Ag/Ab combo test 16 days
  3rd gen HIV1/2 test 22 days
  HIV Western blot 40 days
Limitations of the Traditional Algorithm

• 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 that can detect most antibody-negative persons during highly infectious acute infection stage
CDC/APHL Proposed New HIV Testing Algorithm

4th generation HIV-1/2 immunoassay

(+)  
(-)  
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 (+)  
NAT (-)  
Acute HIV-1 infection  
Negative for HIV-1
Goals of New HIV Testing Algorithm

- Identify more patients with Acute HIV infection
- Allow for more rapid confirmation of infection
- Allow for definitive detection of HIV-2 infection
- Eliminate “Indeterminate” WB results that are inherent in traditional HIV Diagnostic testing algorithm
Multispot HIV-1/HIV-2 Rapid Test

- CLIA moderate complexity with serum, plasma
- Perform test in 15 minutes
- Shelf life: 1 year refrigerated, 3 months room temperature
- FDA-approved for use in multistep HIV diagnostic algorithm March 2013
Several timed reagent & wash steps
FDA-approved HIV-1/HIV-2 Antibody Differentiation Assay

- Reactive Control
- Recombinant HIV-1
  - Peptide HIV-2
  - Peptide HIV-1
Nucleic Acid Test (NAT) for Diagnosis

- APTIMA HIV-1 qualitative RNA assay is the **only** NAT FDA-approved for diagnosis

- Clinicians can order HIV-1 **viral load** tests as “confirmation” of a reactive screening EIA, but labs cannot use them as a reflex part of the algorithm without first performing a CLIA analytical validation
Validation Studies of the New Algorithm

Validation Results – NY State

38,257 specimens – 1,659 GS HIV-1/2 Plus O (3rd gen) EIA repeatedly reactive

Current Algorithm

- 1,546 HIV-1 positive
  - 32 discrepant specimens:
    - 28 indeterminate
    - 4 negative

- 48 indeterminate

New Algorithm

- 1,579 HIV-1 positive (MS)
  - 32 discrepant specimens:
    - 29 RNA+
    - 3 follow-up specimens +

- 75 required RNA
  - 3 RNA detected
  - 63 RNA-negative

- 9 indeterminate (RNA unsuitable)

-Styer et al, J Clin Virol, 2011
Validation Studies of the New Algorithm

Validation Results – NY State
38,257 specimens – 1,659 GS HIV-1/2 Plus O (3rd gen) EIA repeatedly reactive

Current Algorithm
- 5 HIV-2
  - 112 HIV-2 EIA on WB-neg or indeterminate specimens
  - 5 Multispot supplemental
- 36,649 (99.95%) correctly reported negative

New Algorithm
- 5 HIV-2 (Multispot)
- 36,661 (99.98%) correctly reported negative

-Styer et al, J Clin Virol, 2011
Performance of a fourth-generation HIV screening assay and an alternative HIV diagnostic testing algorithm

Muazzam Nasrullah\textsuperscript{a}, Laura G. Wesolowski\textsuperscript{a}, William A. Meyer III\textsuperscript{b}, S. Michele Owen\textsuperscript{a}, Silvina Masciotra\textsuperscript{a}, Craig Vorwald\textsuperscript{c}, William J. Becker\textsuperscript{d} and Bernard M. Branson\textsuperscript{a}

\textit{AIDS} 2013, 27:731–737
New Algorithm Performance in Low Risk and Confirmed Infections

• Quest Diagnostics obtained three sets of de-identified residual serum/plasma specimens:
  – 10,014 specimens from life insurance applicants, a population that typically has low HIV prevalence (<0.1%);
  – 493 previously tested 3rd Generation GS HIV-1 western blot-positive specimens from life insurance applicants;
  – 20 previously tested 3rd Generation GS HIV-1 western blot-indeterminate specimens submitted for diagnostic testing.

• In addition, CDC obtained 230 serial plasma specimens from 26 US donors early in the process of HIV seroconversion.

  - Nasrullah et al, AIDS 2013
• 13 of 10,014 Life Insurance specimens were repeatedly reactive by 3rd or 4th Gen screen

• 2 of 13 were WB and Multispot Positive

• Two 3rd Gen and nine 4th Gen screen results from these 13 specimens were considered biologic false positive screens

• Two HIV-2 infections picked up by 4th Gen Multispot from ≈ 500 HIV-1 WB Positives

- Nasrullah et al, AIDS 2013
**New (and Old) Algorithm Results in HIV-1 Seroconverters**

Table 3. Number of specimens detected by the current and alternative laboratory HIV testing algorithm among 26 HIV-1 seroconverters (230 specimens).

<table>
<thead>
<tr>
<th>Screening immunoassay</th>
<th>MS/NAT</th>
<th>WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Third-generation</td>
<td>102***</td>
<td>56**</td>
</tr>
<tr>
<td>Fourth-generation</td>
<td>130***</td>
<td>56**</td>
</tr>
</tbody>
</table>

MS, Multispot; NAT, nucleic acid test; WB, western blot.

*P < 0.001 (102 vs. 130).

**P < 0.001 (102 vs. 56 and 130 vs. 56).

- Nasrullah et al, AIDS 2013
So What Happens to HIV Western Blots ??

Support Groups
Now Forming
in Your Lab

Courtesy B. Branson-CDC
How are results reported with the new algorithm?

Suggested Reporting Language for the HIV Laboratory Diagnostic Testing Algorithm


(Search: APHL HIV Reporting Language)
Summary

• New assays detect HIV infection sooner

• Acute HIV infection plays a major role in sustaining onward HIV transmission

• New testing algorithm will routinely detect acute HIV infection and provide better discrimination of HIV-1 from HIV-2


3. **HIV Laboratory Diagnosis: New Tests and a New Algorithm**
   Journal of Clinical Virology – open access

4. **Criteria for Laboratory Testing and Diagnosis of Human Immunodeficiency Virus Infection; Approved Guideline.**
   *Clinical and Laboratory Standards Institute*  CLSI document M53-A