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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
Evolution of HIV Tests: Four Generations
Evolution of HIV Tests

- 1st generation: whole viral lysate, detects IgG antibody
EIA

IFA

Western blot

1st Generation
Testing Recommendation since 1989

- Repeatedly reactive screening test

- A supplemental, more specific test such as the **Western blot** has been used to validate those results
1989: State of the Art
Evolution of HIV Tests

- 1\textsuperscript{st} generation: whole viral lysate, detects IgG antibody
- 2\textsuperscript{nd} generation: synthetic peptides, detects IgG antibody
OraQuick Advance

Multispot HIV-1/HIV-2

Clearview Complete

MedMira Reveal G3

INSTI

Chembio Stat Pak

2nd Generation
Evolution of HIV Tests

- 1\textsuperscript{st} generation: whole viral lysate, detects IgG antibody
- 2\textsuperscript{nd} generation: synthetic peptides, detects IgG antibody
- 3\textsuperscript{rd} generation: detect IgM and IgG antibodies
Bio-Rad GS HIV-1/2 PLUS O
2003

Siemens Advia Centaur
2006

Ortho Vitros
2008

3rd Generation

Unigold Recombigen
2003
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 antibodies
- 4th generation: detects IgM, IgG antibodies, p24 antigen
Abbott Architect Ag/Ab Combo  
2010

Bio-Rad Ag/Ab Combo  
2011

Control  Antigen  Antibody

Determine Combo Ag/Ab Rapid Test  
2013

4th Generation
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 antibodies
- **4th generation**: detects IgM, IgG antibodies, p24 antigen
  - “Combi” tests: detect both HIV-1 and HIV-2 antibodies
  - “Combo” tests: detect both antigen and antibody

- Nucleic acid tests (NAT): detect HIV RNA
APTIMA Qualitative HIV-1 RNA Assay

2006

- Plasma or serum
- FDA-approved for HIV diagnosis
HIV Infection and Laboratory Markers

Relative Seroconversion Sensitivity (Plasma)

- 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

Why Does It Matter?

- Sensitivity among frequently-tested MSM in Seattle

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

- Stekler et al, Clin Inf Dis 2009
It’s not only about 4th generation...

- 3rd generation laboratory testing, 2 screening programs in Houston hospital EDs

  - 238 repeatedly reactive EIA, Western blot negative or indeterminate
  - Sent for NAT testing

  - 26 (10.9%) positive = acute HIV infection: IgM antibody detected, IgG tests (WB) negative
Major change with new algorithm: Continue beyond IgG serology.
Acute Infection: Increased Risk of Sexual Transmission of HIV

1/30 - 1/200

Virus 75-750 times more infectious

1/1000 - 1/10,000

1/500 - 1/2000

1/100 - 1/1000

Acute Infection:  Increased Risk of Sexual Transmission of HIV

Ma, J Virol 2009

Cohen & Pilcher, J Infect Dis. 2005
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 seen at least 3 times before diagnosis

-  
  Kahn et al, NEJM 199

-  
  Weintrob et al, Arch Int Med 2003
## Clinical Manifestations

**101 seroconverters, HIVNET cohort 1995-98**

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Percent</th>
<th>Median Duration Days (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any symptom</td>
<td>85%</td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>56%</td>
<td>9 (5-29)</td>
</tr>
<tr>
<td>Fever</td>
<td>55%</td>
<td>5 (4-10)</td>
</tr>
<tr>
<td>Pharyngitis</td>
<td>43%</td>
<td>7 (5-10)</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>36%</td>
<td>7 (4-14)</td>
</tr>
<tr>
<td>Rash</td>
<td>16%</td>
<td>8 (6-14)</td>
</tr>
</tbody>
</table>

- Celum et al, JID 2001
Acute HIV: 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
Limitations of Current 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 that can detect most antibody-negative persons during highly infections acute infection stage.
HIV-2 Infection

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

- **Demographic/behavioral information usually not available to laboratory**

- **HIV-2 Remains uncommon in U.S., but**
  - Does not respond to NNRTIs, some PIs (first line therapy)
  - Undetectable by HIV-1 viral load tests

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

\(^1\)Torian et al, Clinical Infectious Disease 2010 \(^2\)MMWR, July 2011
HIV Testing Recommendations from CDC/APHL
HIV-1/2 Combination Ag/Ab immunoassay

- (+) → Neg for HIV-1 and HIV-2 antibodies and p24 Ag
- (-) → HIV-2 antibodies detected

HIV-1/HIV-2 antibody differentiation immunoassay

- HIV-1 (+) → HIV-1 antibodies detected
- HIV-2 (-) → HIV-2 antibodies detected
- HIV-1 (-) → HIV-2 (+) → HIV antibodies detected → NAT
  - NAT (+) → Acute HIV-1 infection
  - NAT (-) → Neg for HIV-1
- HIV-1 (+) → HIV-2 (+) → HIV antibodies detected → NAT
  - NAT (+) → Acute HIV-1 infection
  - NAT (-) → Neg for HIV-1
- HIV-1 (-) or indeterminate

Available at: http://stacks.cdc.gov/view/cdc/23447
4th Generation Ag/Ab Combination Assays

- Detect HIV-1 p24 antigen and IgM and IgG antibodies against either HIV-1 or HIV-2

- Reactive test results do not distinguish antigen from antibody

- Insufficient data for rapid Ag/Ab test to recommend it as first test in algorithm
4th Generation Ag/Ab Assay vs. RNA

- RNA+/ 3rd gen-negative specimens detected by 4th generation EIA:
  - 38 of 46 (83%) – Australia*
  - 10 of 14 (71%) – CDC AHI study**
  - 51 of 61 (84%) – CDC panel***

- 4 days after RNA – 9 seroconversion panels***

* Cunningham P, HIV Diagnostics Conf 2007
** Patel P, CROI 2009
*** Owen M, CROI 2009
FDA-approved HIV-1/HIV-2 Antibody Differentiation Assay
Why not go directly to NAT?

- Cost: most specimens will be resolved with antibody tests
- Turnaround time: positive antibody results now feasible in <A 90 minutes
- Fails to diagnose acute infection (Stage 0), HIV-2
- NAT is undetectable in 3% to 5% of Western blot-positive specimens
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

- APHL and CDC contracted 2 referral laboratories (NY State and FL) as reference laboratories for APTIMA from other public health labs
Where’s my Western blot?
Phoenix ED 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 (32.4%) had Acute HIV Infection (antibody negative)

- Median viral load:
  - Patients with acute infections: 3.6 million
  - Patients with established infections: 27,000

-MMWR June 21, 2013
Implementation: Massachusetts
First 12 months

- 7,984 specimens tested
  - 258 (3.2%) positive for HIV-1
  - 1 (0.01%) positive for HIV-2
  - 8 (0.10%) acute HIV infections
    - 6 = 3rd gen EIA negative, WB negative
    - 2 = 3rd gen EIA reactive, WB indeterminate
Suggested Reporting Language for the HIV Laboratory Diagnostic Testing Algorithm

November 2013

http://stacks.cdc.gov/view/cdc/23447
Implications

- Faster turnaround time for antibody test results

- Ability to detect acute HIV infection
  - New case definition: HIV Stage 0
  - Focus for partner services and intervention efforts

- Ability to detect HIV-2 infections
  - False-Negative results with viral load tests
  - Do not respond to many ARVs, e.g. NNRTI’s and several protease inhibitors
Summary

- New assays detect HIV infection sooner
- Updated recommendations allow earlier, more accurate diagnosis of HIV infection
  - Acute HIV infection plays a major role in sustaining onward HIV transmission
  - New testing algorithm will routinely detect acute HIV infection and HIV-2
Additional Information

- 2011 Journal of Clinical Virology Supplement
  - Open access:
  - www.journalofclinicalvirology.com

- 2013 Journal of Clinical Virology Supplement
  - Open access:
  - www.journalofclinicalvirology.com

- CDC HIV testing web site
  - http://www.cdc.gov/hiv/testing/index.html