HIV Diagnostic Testing:  
A New Laboratory Algorithm

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Objectives

• Describe new HIV testing technologies that improve detection of HIV
• Present the updated laboratory testing algorithm and explain
• Illustrate the benefits of the recommended algorithm through laboratory cases
HIV Transmission

- ~52% of new sexually-transmitted infections in the US involve infected persons unaware of their infection
  - including 11% due to persons in the acute phase of HIV infection (Pinkerton, 2011, *AIDS Behav*)
HIV Testing Algorithm: 1989 to 2013

- Screen for HIV antibodies
  - Enzyme Immunoassay (EIA)
  - Rapid test
- If reactive, confirm by HIV-1 Western blot (WB)
- If WB is negative or indeterminate, run HIV-2 EIA (1992)

EIA Tests Have Become More Sensitive Over Time

<table>
<thead>
<tr>
<th>EIA Generation</th>
<th>Year of 1st FDA approval</th>
<th>What it detects</th>
<th>Typical time to detection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st/2nd</td>
<td>1985/1998</td>
<td>HIV-1 antibodies IgG only</td>
<td>42-60 days</td>
</tr>
<tr>
<td>3rd</td>
<td>2003</td>
<td>HIV-1, HIV-2 Ab IgM and IgG</td>
<td>21-24 days</td>
</tr>
<tr>
<td>4th</td>
<td>2010</td>
<td>HIV-1 p24 antigen HIV-1, HIV-2 Ab IgM and IgG</td>
<td>14-15 days</td>
</tr>
</tbody>
</table>
FDA-approved 4th Generation Ag/Ab Combo Tests

2 Lab-based EIA tests
- ARCHITECT HIV Ag/Ab Combo (Abbott, 2010)
- GS HIV Ag/Ab Combo EIA (Bio-Rad, 2011)
- HIV-1 p24 Ag and HIV-1/HIV-2 Ab detected but not distinguished

1 Rapid test
- Intended for POC use when CLIA waived
  - Determine HIV-1/2 Ag/Ab Combo test (Alere, 2013)
- HIV-1 p24 Ag and HIV-1/2 Ab are detected separately

Western blot doesn’t meet today’s needs for HIV detection

- Designed for specificity
  - Specific bands must be present to be positive
- Interpretation is subjective
- False negative and Indeterminate results occur
  - Early infection, late infection, HIV-2, other misc situations
- Turnaround time extended by need to batch
A new algorithm was developed for HIV diagnostic testing

- More sensitive initial test improves allows detection of acute infection
  - Ability to detect established infections is maintained
- A single supplemental assay can confirm HIV-1 and HIV-2 antibodies
- RNA test is used to confirm acute infection
- Indeterminate results and need for follow-up diagnostic testing reduced
CDC/APHL HIV Diagnostic Testing Algorithm

1. **HIV-1/2 Ag/Ab combo immunoassay (4th generation)**
   - (+) Negative for HIV-1 and HIV-2 antibodies and p24 Ag*
   - (–) HIV-1/2 antibody differentiation immunoassay

2. HIV-1/HIV-2 antibody differentiation immunoassay
   - HIV-1 (+) HIV-2 (–) Positive for HIV-1 antibodies
     - Initiate care
   - HIV-1 (–) HIV-2 (+) Positive for HIV-2 antibodies
     - Initiate care
   - HIV-1 (+) HIV-2 (+) Positive for HIV antibodies
     - Initiate care
   - HIV-1 (–) or indeterminate HIV-2 (–) HIV-1 RNA assay
     - RNA (+) Positive for HIV-1
       - Initiate care
     - RNA (–) Negative for HIV-1

3. **HIV-1/HIV-2 Antibody Differentiation Immunoassay**
   - Multispot HIV-1/HIV-2 Rapid Immunoassay (Bio Rad) is only test FDA approved for this step (Mar 2013)
   - Detects and differentiates HIV-1 and HIV-2 antibodies
   - Rapid test, but not CLIA waived; Result in 30 min
   - Can run up to 10 at a time
   - Less expensive than WB

* Peptide HIV-1
* Peptide HIV-2
* Recombinant HIV-1
* Procedural Control
Algorithm relies on results from a combination of tests

- There is no single confirmatory test
  - Multispot is not a direct substitute for WB
- Accuracy of MS was verified in combination with EIA\(\text{3rd} \) \& \(\text{4th} \) gen) but not with POC rapid tests

<table>
<thead>
<tr>
<th>EIA type</th>
<th>Kit name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>4\text{th} Gen</td>
<td>Abbott Architect HIV Ag/Ab Combo Assay</td>
<td>Abbott Laboratories</td>
</tr>
<tr>
<td></td>
<td>GS HIV Ag/Ab Combo EIA</td>
<td>Bio-Rad Laboratories</td>
</tr>
<tr>
<td>3\text{rd} Gen</td>
<td>Abbott PRISM HIV O Plus Assay</td>
<td>Abbott Laboratories</td>
</tr>
<tr>
<td></td>
<td>ADVIA Centaur HIV 1/O/2 Enhanced Assay</td>
<td>Siemens Healthcare Diagnostics</td>
</tr>
<tr>
<td></td>
<td>GS HIV-1/HIV-2 + O EIA</td>
<td>Bio-Rad Laboratories</td>
</tr>
<tr>
<td></td>
<td>Ortho Vitros Anti HIV 1+2</td>
<td>Ortho-Clinical Diagnostics</td>
</tr>
</tbody>
</table>

Where do rapid tests fit in?

- Limited sensitivity for CLIA-waived rapid tests
- Rapid tests may still be used for screening, but not considered part of the algorithm
  - When confirming, lab must begin with EIA (4\text{th} gen) and if reactive, continue through steps of algorithm
Alere Determine is a 4th generation rapid test

- Ag/Ag Combo Rapid Test
- Ag & Ab detected separately
- Not CLIA waived yet – lab use only, for now
- Acceptable uses:
  - Initial screening: Yes
  - Step 1 of algorithm: No
- Validation data for its use in the algorithm are still needed

4th gen EIAs > Determine > 3rd gen EIAs > Other rapid tests

Accuracy for Established Infections

<table>
<thead>
<tr>
<th>Author</th>
<th>Study design</th>
<th>Reactive on 3rd gen EIA</th>
<th>Positive on WB</th>
<th>Positive by Algorithm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Styer et al, 2011 JCV</td>
<td>Retrospective analysis of clinical test data</td>
<td>1578 HIV-1 pos</td>
<td>1546 (98%)</td>
<td>1578 (100%)</td>
</tr>
<tr>
<td>Wesolowski et al, 2011</td>
<td>Retrospective testing of archived specimens</td>
<td>2,202 WB pos</td>
<td>2202 (100%)</td>
<td>2201 (99.95)</td>
</tr>
<tr>
<td>Masciotra et al, 2011</td>
<td>Retrospective testing of archived specimens</td>
<td>416 WB-pos</td>
<td>416 (100%)</td>
<td>415 (99.8%)</td>
</tr>
</tbody>
</table>

These and other studies conclude that the new algorithm is as good or better than the original algorithm at identifying established HIV infections.
Accuracy for Acute Infections

<table>
<thead>
<tr>
<th>Author</th>
<th>Study design</th>
<th>Initial test/## reactive</th>
<th>Positive by Algorithm (MS + RNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masciotra et al. 2011</td>
<td>Seroconversion panel samples, n=228</td>
<td>3\textsuperscript{rd} gen (Vitros)/110 4\textsuperscript{th} gen (Abbott)/135</td>
<td>15 (6.6%) 41 (17.9%)</td>
</tr>
<tr>
<td>Nasrullah et al. 2013</td>
<td>Seroconversion panels samples n=230</td>
<td>3\textsuperscript{rd} gen (BioRad) 4\textsuperscript{th} gen (BioRad)</td>
<td>12 (5.2%) 36 (15.7%)</td>
</tr>
<tr>
<td>Linley et al. 2013</td>
<td>WB neg and indet PHL samples, n= 570</td>
<td>3\textsuperscript{rd} gen EIA reactive 570 WB Ind/Neg 220 WB Ind 350 WB Neg</td>
<td>55 (9.6%) 47 (21.4%) 8 (2.3%)</td>
</tr>
</tbody>
</table>

New algorithm is better at detecting acute and early infections because of initial and supplemental tests improved sensitivity

Accuracy for HIV-2 Infections

- Torian et al. 2010 Clin Inf Dis; 51:1334-42
- 40/62 (64.5%) persons infected with HIV-2 were initially diagnose with HIV-1
  - 85% of HIV-2 cases had a positive HIV-1 WB
  - 40/42 (95%) HIV-2 infected persons correctly identified with MS
- Styer et al. 2011: 5/5 correctly identified as HIV-2 by MS
Where do things stand in NYS?

- Draft recommendations are posted on CDC website: http://www.cdc.gov/hiv/pdf/policies_Draft_HIV_Testing_Alg_Rec_508.2.pdf
  - Official recommendations from CDC still under review
- NYSDOH Interim guidelines for laboratories sent on May 16, 2013
- Update planned following release of CDC recommendations

What is Wadsworth’s role?

- Referral HIV testing services
  - Rapid test confirmation, including DBS
  - Fill gaps in test availability (e.g. HIV-2)
  - Special services (e.g. Pediatric HIV testing)
- RNA referral testing
  - Wadsworth and AIDS Institute are partnering to assist NYS hospital labs with implementing algorithm
  - Wadsworth/APHL NAT demonstration project
- Lab standards and guidance in development
  - Including guidelines for validation for VL tests
Wadsworth has used Multispot and Aptima HIV-1 RNA for years

How does the HIV Diagnostic Algorithm for Laboratories compare to the former Western blot strategy in practice?

Case Studies from Wadsworth
Cases 1 & 2: When a negative is not a negative

- Two cases in August 2012 submitted for confirmation of reactive rapid test
  - Case 1: EIA reactive (High S/C), Multispot negative, Western blot negative, HIV-1 RNA detected
  - Case 2: EIA reactive (High S/C), Multispot HIV-1 indeterminate, Western blot negative, HIV-1 RNA detected
- Both cases were picked up by rapid test, but WB was completely negative

There are many examples of acute and early HIV infections that are missed by Western blot.

Case 3: Positive HIV-1 WB
End of story?

- Blood from NYC resident submitted to WC for rapid test confirmation
- HIV-1 and HIV-2 Ab reactivity on MS persisted at 1:10 and 1:100 dilutions
- 2\textsuperscript{nd} specimen suitable for RNA testing requested

<table>
<thead>
<tr>
<th>Test</th>
<th>Spec 1</th>
<th>Spec 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV 1/2+O EIA</td>
<td>Repeat reactive</td>
<td></td>
</tr>
<tr>
<td>HIV-1 Western blot</td>
<td>Positive</td>
<td>All bands</td>
</tr>
</tbody>
</table>

HIV-1/HIV-2 dual infection confirmed
Case 3 continued

• Initial test requisition from Case 3 had no information suggesting possible HIV-2 exposure
  – No further testing indicated by the old strategy
• Contacted submitter after initial results
  – 2002, HIV neg prior to immigrating to U.S.
  – 2006, Sex with person from Gambia
  – 2011, Symptoms of fatigue, tested for HIV

Labs typically don’t get risk factor info with test requests

Case 4: A positive HIV-1 Western blot means HIV-1 positive, right?

• Dec 2013 - rec’d specimens from HIV-exposed infant (1d and 14d)
  – Multispot HIV-2 antibody positive, HIV-2 RNA not detected
• Jan 2014 – submitter calls “there is a mistake, mom is HIV-1 infected”
  – Mom had tested HIV-1/2 EIA +, HIV-1 WB +
  – Undetectable HIV-1 viral load, low CD4 count
• Jan 2014 – received specimen from mom
  – Reactive HIV-1/2 EIA, HIV-1 WB + (bands: 24, 31, 40, 51/55, 160), Multispot HIV-2 antibody positive, HIV-2 RNA not detected

HIV-2 infected people often have positive HIV-1 Western blot (with unusual banding pattern). Use of the Multispot will lead to fewer misdiagnosed cases of HIV-2.
Case 5: Weak EIA, probably a false positive?

- Reported negative tests in Puerto Rico in May 2012 and March 2013
- Specimen 1 tested in upstate NY hospital lab
- Pos RNA → Suspected acute infection
- Retested at different hospital lab 3 wks later
- Note negative Western blot – no bands

<table>
<thead>
<tr>
<th>Test Performed</th>
<th>Spec 1 Feb ’14</th>
<th>Spec 2 Mar ’14</th>
</tr>
</thead>
<tbody>
<tr>
<td>4th gen EIA</td>
<td>Pos (Bio-Rad)</td>
<td>Low S/CO</td>
</tr>
<tr>
<td>Oraquick rapid test</td>
<td>n/a</td>
<td></td>
</tr>
<tr>
<td>Multispot</td>
<td>HIV-1 Indet</td>
<td></td>
</tr>
</tbody>
</table>

Case 5: Further Evaluation

- Atypical seroconversion pattern
  - Antibodies detected, but remained weak
  - No bands on either Western blot
  - Note negative Oraquick on Specimen 2
- Follow-up (at Spec 2, and 1 wk after)
  - VL = 5.0 and 5.1 log copies/mL
  - CD4 = 88 and 52
- Clinical staff suspect infection present for many months, but blunted Ab response

A very weak or absent antibody response is rare, but the new algorithm will detect these cases.
Summary

• 4th generation HIV Ag/Ab Combo tests provide the best option for detecting HIV infection
• Specific test combinations have been validated for use in the HIV diagnostic algorithm
• In practice, we are seeing the benefits of the algorithm in our laboratory

Questions, Comments?

Questions regarding the HIV Diagnostic Testing Algorithm may be directed to the NYSDOH by email at: hivtesting@health.state.ny.us

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