CDC HIV guidelines for HIV diagnosis in laboratory settings

HIV-1/2 antigen/antibody combination immunoassay

(+) → HIV-1/2 antibody differentiation immunoassay

(-) → Negative for HIV-1 and HIV-2 antibodies and p24 Ag

HIV-1 (+) → HIV-1 antibodies detected
HIV-2 (-) → HIV-2 (+) → HIV antibodies detected

HIV-1 (-) → HIV-2 (-) → HIV-1 NAT

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

(+) indicates reactive test result
(-) indicates nonreactive test result
NAT: nucleic acid test
Objective

- Compare the performance of two FDA-approved HIV1/2 differentiation assays within the algorithm
  - Bio-Rad Multispot HIV1/ HIV2 Rapid Test
  - Bio-Rad Geenius HIV1/ HIV2 Supplemental Assay
Bio-Rad Multispot HIV1/HIV2 Rapid Test

- 30 μl of plasma/serum

Reactivity to:
- gp41 HIV-1 envelope recombinant protein
- gp41 HIV-1 envelope peptide (IDR)
- gp36 HIV-2 envelope peptide

Operator interpretation

Screening or supplemental

Interpretations as supplemental test:
- HIV-1 or HIV-2 positive
- HIV undifferentiated
- HIV-1 indeterminate
- Negative

Dilution protocol
- 1:10 or 1:100 dilution for undifferentiated

Discontinued in 2016
Bio-Rad Geenius HIV1/HIV-2 Supplemental Assay

Approval Date: October 24, 2014

Indications:

- Single-use immunochromatographic assay for the confirmation and differentiation of individual antibodies to Human Immunodeficiency Virus Types 1 and 2 (HIV-1 and HIV-2) in fingerstick whole blood, venous whole blood, serum, or plasma samples (EDTA, heparin, and sodium citrate).

- Intended for use as an additional, more specific test to confirm the presence of antibodies to HIV-1 and HIV-2 for specimens found to be repeatedly reactive by diagnostic screening procedures. The assay may also be used to confirm the presence of antibodies to HIV-1 and/or HIV-2 in pediatric subjects (i.e., children as young as 2 years of age).
Bio-Rad Geenius HIV1/HIV-2 Supplemental Assay

- 5μl of plasma/serum, 15μl whole blood
- In the USA, interpretation by reader
- Assay interpretation by the Geenius software:
  - HIV Negative
  - HIV-1 Indeterminate
  - HIV-2 Indeterminate
  - HIV Indeterminate
  - HIV-1 Positive
  - HIV-2 Positive
  - HIV-2 Positive with HIV-1 cross-reactivity
  - HIV Positive Untypeable
**Multispot vs. Geenius**

- Protocols take about the same time, but Multispot has many more steps.
Sample sets and analysis

- **Assay performance in early HIV-1 infections**
  - Relative sensitivity of assay’s performance with 50% cumulative frequency analysis
    - 17 commercial seroconverters with HIV-1 WB positive

- **Plasma specimens from STOP study**
  - *Screening Targeted Populations to Interrupt On-going Chains of HIV transmission with Enhanced Partner Notification (STOP) study*
  - Subset of 158 tested with Abbott Architect, Bio-Rad Multispot, and Abbott m2000 viral load (San Francisco) were tested with Geenius
  - Architect-false reactive (32 SF, 37 NY, 23 NC)
    - 87 were tested with GSHIV Ag/Ab Combo and Geenius at CDC
Sample sets and analysis II

- Analysis was performed considering previous testing
  - 85 Early HIV-1 infection: Ag/Ab Combo-pos/MS-neg or –ind/NAT-pos
  - 41 Established HIV-1 infection: Ag/Ab Combo-pos/MS-pos or -undiff
  - 92 False reactive: Architect-pos/NAT-neg

- HIV-2 established infections characterized at CDC
  - 16 plasma specimens from USA
  - 25 plasma specimens from Ivory Coast
Results of the Performance Evaluation of HIV Supplemental Assays
Days before Western blot positive

-25
-20
-15
-10
-5
0

WB POSITIVE

Antigens (26)
Bioflex Combo (21.5)
Architect Combo (18.5)
BioRad Combo (18)
Determine Combo (15.5)
Advia (14)
Abbott CS+O (12)
Aloka/Revel/DPH/IVY (7)
Supra/Combo (5)
Multiplex (Supp) (5)
Onquick (3)

IgG immunoassays
IgG and/or IgM rapid tests
IgG/IgM immunoassays
Antigen/Antibody Combo assays/rapid test
Nuclei Acid test
HIV-1 Infections

Among early infections, Geenius confirmed five more infections than MS, but eight established HIV-1 infections required NAT with Geenius.

Geenius confirmed eight infections that required 1:10 dilution and one undifferentiated with MS.

Three specimens with Geenius HIV-2 indeterminate results would have needed HIV-2 NAT.

McNemar’s analysis showed no significant difference ($p=0.7893$) between MS and Geenius (NAT vs. no NAT required or positive result).

<table>
<thead>
<tr>
<th>HIV-1 infections</th>
<th>Total</th>
<th>HIV negative</th>
<th>HIV-1 indeter</th>
<th>HIV-1 positive</th>
<th>HIV-2 indeter</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARC-positive/MS-negative</td>
<td>74</td>
<td>65</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>ARC-positive/MS-indeterminate</td>
<td>11</td>
<td>5</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>ARC-positive/MS-HIV-1 positive</td>
<td>40</td>
<td>3</td>
<td>5</td>
<td>32*</td>
<td></td>
</tr>
<tr>
<td>ARC-positive/MS-undifferentiate</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HIV infections were confirmed with nucleic acid testing; indeter: indeterminate

* eight required 1:10 dilution with MS
HIV-2 infections

<table>
<thead>
<tr>
<th>HIV-2 infections</th>
<th>Total</th>
<th>HIV negative</th>
<th>HIV-2 indeter</th>
<th>HIV-2 positive</th>
<th>HIV-2 pos (HIV-1 XR)</th>
<th>HIV untyp</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRC-positive/MS-HIV-2 positive</td>
<td>40</td>
<td>1</td>
<td>2**</td>
<td>15</td>
<td>15</td>
<td>7***</td>
</tr>
<tr>
<td>BRC-positive/MS-undifferentiate</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

indeter: indeterminate; HIV-1 XR: cross-reactivity with HIV-1; untyp: untypable

HIV infections were confirmed with nucleic acid testing

** one required 1:10 dilution with MS
*** four required 1:10 dilution and two 1:100 dilution with MS

- Ten HIV-2 infections required NAT for confirmation with Geenius while only one with MS
- Seven of those ten required the dilution protocol with MS
- McNemar’s analysis showed significant differences between MS and Geenius ($p=0.0159$)
False reactive specimens

- **Of ARC-false reactive specimens (HIV NAT negative)**
  - 95.6% were MS-negative
  - 93.5% were Geenius-negative
- **HIV-2 reactivity contributed to decrease specificity**
- **Of 87 available ARC-false reactive**
  - 6.9% (6) were Bio-Rad Combo-repeatedly reactive
    - 1 MS-indeterminate/Geenius HIV-1 indeterminate
    - 1 MS-indeterminate/Geenius HIV-1 positive

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<table>
<thead>
<tr>
<th>HIV-negative (false reactive)</th>
<th>Total</th>
<th>HIV negative</th>
<th>HIV-1 indeter</th>
<th>HIV-1 positive</th>
<th>HIV-2 indeter</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARC-positive/MS-negative</td>
<td>88</td>
<td>84</td>
<td>1</td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>ARC-positive/MS-indeterminate</td>
<td>2</td>
<td></td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ARC-positive/MS-HIV-2 positive</td>
<td>2</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>HIV infections were confirmed with nucleic acid testing; indeter: indeterminate</strong></td>
<td></td>
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</tr>
</tbody>
</table>

Geenius results
Geenius HIV-2 indeterminate results

- Among HIV-1 and ARC-false reactive samples, gp140 only reactivity was observed.

- HIV-2 indeterminate result showed different reactivity among HIV-2 infections.

<table>
<thead>
<tr>
<th>Boca Bioliastics Data</th>
<th>Geenius conclusion</th>
<th>Detected Bands Geenius</th>
<th>MP diagnostics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multispot HIV-2</td>
<td>HIV-2 INDETERMINATE</td>
<td>gp36 CTRL</td>
<td>HIV-2 WB - 125, 80, 68, 56, 53, 36, 26</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>HIV-2 INDETERMINATE</td>
<td>gp36 CTRL</td>
<td>80, 68, 56, trace, 53, 36, 27, 26</td>
</tr>
<tr>
<td>POSITIVE</td>
<td>HIV-2 INDETERMINATE</td>
<td>gp36 CTRL</td>
<td>80, 68, 56, 53, 36</td>
</tr>
</tbody>
</table>
Summary

- Overall, MS confirmed 40 HIV-1 and 40 HIV-2 infections, while Geenius confirmed 38 HIV-1 and 31 HIV-2 infections.

- Due to the higher HIV-2 indeterminates, more NAT testing is required with Geenius compared to MS.

- High number of HIV-2 indeterminate results with Geenius among HIV-1 and HIV-false reactive specimens (gp140 reactivity); while true HIV-2 positive specimens were due to gp36.

- MS showed slightly better specificity than Geenius.
Conclusions

- MS and Geenius had comparable performance in identifying HIV-1 infections using the recommended laboratory diagnostic algorithm

- Increased the number of HIV-2 indeterminate results with Geenius, which may require more nucleic acid testing
Acknowledgements

HIV Diagnostics and Incidence Team
- Krystin A. Price
- William Fowler
- Vickie Sullivan
- Sarah Adams
- Michele Owen
- Silvina Masciotra

STOP Team
- Cindy Gay
- Emily Westheimer
- Stephanie Cohen
- Lisa Hightow-Weidman
- Philip Peters