Comparative Performance of the Geenius™ HIV-1/HIV-2 Supplemental Test in Florida’s Public Health Testing Population

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HIV-1/2 “Combo” immunoassay

(+)

HIV-1(+)
HIV-2(-)

HIV-1 antibodies detected

HIV-2 (+)

HIV-2 antibodies detected

(-)

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

RNA

RNA(+)

Acute HIV-1 infection

RNA(-)

Negative for HIV-1

HIV-1/HIV-2 antibody differentiation immunoassay

*Additional testing required to rule out dual infection
HIV-1/HIV-2 Differentiation Immunoassays (as of March 1, 2016)

FDA approved, Oct. 2014

Serum Control
Recombinant HIV-1 gp41

July 29, 2016 Product Withdrawal

Peptide HIV-2 gp36  Peptide HIV-1 gp41

Multispot HIV-1/HIV-2

Geenius™ HIV-1/2 Supplemental Assay

1 gp36
2 gp140
3 p31
4 gp160
5 p24
6 gp41
Control
# Geenius HIV-1/HIV-2 Interpretation Criteria

## Assay Interpretation by the Geenius™ Software

The Geenius™ Software detects the presence or absence of Bands 1-6 above (and the Control band), determines the presence or absence of antibodies to HIV-1 and/or HIV-2, and generates an “HIV-1 Result” that is Positive, Indeterminate, or Negative, and an “HIV-2 Result” that is Positive, Indeterminate, or Negative. The following table indicates the criteria employed by the Geenius™ Software to interpret the HIV-1 Result and HIV-2 Result and provide an “Assay Interpretation.”

<table>
<thead>
<tr>
<th>HIV-1 result</th>
<th>HIV-2 result</th>
<th>Assay Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>HIV NEGATIVE</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Negative</td>
<td>HIV-1 INDETERMINATE&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Negative</td>
<td>Indeterminate</td>
<td>HIV-2 INDETERMINATE&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Indeterminate</td>
<td>HIV INDETERMINATE&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Positive</td>
<td>Indeterminate</td>
<td>HIV-1 POSITIVE</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
<td>HIV-2 POSITIVE</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>Positive</td>
<td>HIV-2 POSITIVE</td>
</tr>
</tbody>
</table>
| Positive     | Positive     | **HIV-2 POSITIVE with HIV-1 cross-reactivity:** Antibody to HIV-2 confirmed in the sample. HIV-1 positivity (with only one HIV-1 envelope band, gp160 or gp41), is due to cross-reactivity and precludes confirmation of HIV-1.*
*Note: Differentiation features managed by proprietary algorithm.

**Untypable (undifferentiated):** Antibodies to HIV-1 and HIV-2 confirmed in the sample. This may occur in an HIV-2 positive sample with significant cross-reactivity to HIV-1, or may be due to coinfection with both HIV-1 and HIV-2 (rare).*
*Note: Differentiation features managed by proprietary algorithm.

<sup>a</sup> *HV-1 band(s) detected but did not meet the criteria for HIV-1 Positive*

<sup>b</sup> *HV-2 band(s) detected but did not meet the criteria for HIV-2 Positive*

<sup>c</sup> *HIV band(s) detected but did not meet the criteria for HIV-1 Positive or HIV-2 Positive*
February 2016

HIV Diagnostic Informational Updates

This issue contains information about FDA Approved HIV Diagnostic assays including: ADVIA Centaur® HIV Ag/Ab Combo Assay, BioPlex® 2200 HIV Ag-Ab Assay and Geenius™ HIV 1/2 Supplemental Assay.

Both available at: www.aphl.org/aphlprograms/infectious/hiv/pages/default.aspx
Retrospective Study Design
n = 350 (Serum/Plasma)

1) Testing of 250 established HIV-1 positive antibody specimens
   Combo IA r/r, Multispot HIV-1 reactive

2) Testing of 60 false positive HIV Combo specimens
   Combo IA r/r, Multispot HIV-1 nonreactive or indeterminate,
   HIV-1 NAT nonreactive

3) Testing of 20 Algorithm-defined acute stage HIV-1 specimens
   Combo IA r/r, Multispot HIV-1/2 nonreactive, HIV-1 NAT reactive

4) Testing of 10 Algorithm-defined early stage HIV-1 specimens
   Combo IA r/r, Multispot HIV-1 indeterminate, HIV-1 NAT reactive

5) Testing of 10 known HIV-2 antibody positive specimens
# HIV-1 Established Infections

**Combo IA r/r, Multispot HIV-1 reactive**  
\[ n = 250 \]

<table>
<thead>
<tr>
<th>Multispot HIV-1/HIV-2</th>
<th>( n )</th>
<th>Geenius™ HIV-1/HIV-2</th>
</tr>
</thead>
</table>
| HIV-1 reactive, HIV-2 nonreactive (neat) | 243 | HIV-1 Positive, HIV-2 Negative (225)  
HIV-1 Positive, HIV-2 Indeterminate (16)  
(8/16 resolved as HIV-2 Negative on repeat)  
HIV Positive – Untypable (2)  
(1/2 resolved as HIV-1 Positive, HIV-2 Negative on repeat) |
| HIV-1 reactive, HIV-2 nonreactive (1:10) | 7 | HIV-1 Positive, HIV-2 Negative (2)  
HIV-1 Positive, HIV-2 Indeterminate (3)  
(1/3 resolved as HIV-2 Negative on repeat)  
HIV Positive – Untypable (2)  
(1/2 resolved as HIV-1 Positive, HIV-2 Indeterminate on repeat) |

- 100% concordance for HIV positivity (includes Untypable results)
- 7 (2.8%) initial Multispot results required dilution testing per package insert
- 23 (9.2%) initial Geenius results required repeat testing per package insert
- Geenius repeat testing resolved 10/23 (43%) as HIV-1 Pos/HIV-2 Neg
**HIV-1/2 Combo False Positives**

Combo IA r/r, **Multispot HIV-1 nonreactive or indeterminate, HIV-1 RNA nonreactive**

\( n = 60 \)

<table>
<thead>
<tr>
<th>Multispot HIV-1/HIV-2</th>
<th>n</th>
<th>Geenius™ HIV-1/HIV-2</th>
</tr>
</thead>
</table>
| HIV-1 nonreactive, HIV-2 nonreactive (neat) | 57 | HIV-1 Negative, HIV-2 Negative (54)  
HIV-2 Indeterminate, HIV-1 Negative (2)  
(2/2 remained HIV-2 Indeterminate on repeat, both HIV-2 RNA Not Detected)  
HIV-1 Indeterminate, HIV-2 Negative (1) |
| HIV-1 indeterminate, HIV-2 nonreactive (neat) | 3 | HIV-1 Negative, HIV-2 Negative (1)  
HIV-2 Indeterminate, HIV-1 Negative (2)  
(2/2 resolved as HIV-2 Negative on repeat) |

- 59 HIV-1 negative Geenius results vs. 57 HIV-1 nonreactive Multispot results
- All Geenius HIV-2 Indeterminate results were resolved as HIV-2 Negative by repeat testing or HIV-2 RNA.
- All Geenius and Multispot results indicate the need for reflex HIV-1 RNA testing.
HIV-1 Acute Infections
Combo IA r/r, Multispot HIV-1/2 nonreactive, HIV-1 RNA reactive
  n = 20

<table>
<thead>
<tr>
<th>Multispot HIV-1/HIV-2</th>
<th>n</th>
<th>Geenius™ HIV-1/HIV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 nonreactive, HIV-2 nonreactive (neat)</td>
<td>20</td>
<td>HIV-1 Negative, HIV-2 Negative (16)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-1 Indeterminate, HIV-2 Negative (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-2 indeterminate, HIV-1 Negative (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(1/1 remained HIV-2 indeterminate on repeat)</td>
</tr>
</tbody>
</table>

• Neither Geenius nor Multispot was HIV-1 Positive with any of the 20 algorithm-defined acute infection specimens.

• 3/20 (15%) were HIV-1 indeterminate by Geenius and therefore reclassified as HIV-1 early infections per study design.
**HIV-1 Early Infections**

Combo IA r/r, Multispot HIV-1 indeterminate, HIV-1 RNA reactive

\[ n = 10 \]

<table>
<thead>
<tr>
<th>Multispot HIV-1/HIV-2</th>
<th>n</th>
<th>Geenius™ HIV-1/HIV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-1 indeterminate, HIV-2 nonreactive (neat)</td>
<td>10</td>
<td>HIV-1 Negative, HIV-2 Negative (1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-1 Indeterminate, HIV-2 Negative (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-1 Positive, HIV-2 Indeterminate (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2/3 resolved as HIV-2 Negative on repeat)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-1 Positive, HIV-2 Negative (3)</td>
</tr>
</tbody>
</table>

- 6/10 (60%) were HIV-1 Positive by Geenius and therefore reclassified as HIV-1 established infections per study design.
Known HIV-2 Antibody Positive
Supplied by BioRad Laboratories
n = 10

<table>
<thead>
<tr>
<th>Multispot HIV-1/HIV-2</th>
<th>n</th>
<th>Geenius™ HIV-1/HIV-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-2 reactive, HIV-1 Nonreactive (neat)</td>
<td>5</td>
<td>HIV-2 Positive with HIV-1 cross-reactivity, (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-2 Positive, HIV-1 Indeterminate (1)</td>
</tr>
<tr>
<td>HIV-2 reactive, HIV-1 Nonreactive (1:10)</td>
<td>5</td>
<td>HIV-2 Positive with HIV-1 cross-reactivity, (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HIV-2 Positive, HIV-1 Indeterminate (1)</td>
</tr>
</tbody>
</table>
Repeat testing of Geenius HIV-2 indeterminate results, as recommended in the package insert, resolved 43% - 50% as HIV-2 negative.

Geenius HIV-1/HIV-2 sensitivity compared to Multispot HIV-1/HIV-2 reclassified - 15% (3/20) algorithm-defined acute infections as early infections - 60% (6/10) algorithm-defined early infections as established infections.

Geenius HIV-1/HIV-2 has the potential to further reduce the number of HIV-1 RNA tests needed to resolve discordant algorithm results.

Further studies are needed to determine the impact on differentiation testing for antiretroviral therapy (ART) experienced individuals with viral suppression (i.e. Proof of Positivity testing).

This study further supports the need for simplified and routine HIV-2 RNA testing.
Questions & Thank You

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