Clinical Performance of the Bio-Rad Multispot HIV-1/HIV-2 Rapid Test to Identify HIV-2 Infection and Cross Reactivity with HIV-1 Western Blot

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Objectives

Evaluate the performance of the Bio-Rad Multispot rapid test in 3\textsuperscript{rd} and 4\textsuperscript{th} generation HIV-1/2 diagnostic assay algorithms to:

- Correctly classify HIV-2 from HIV-1 infections.
- Differentiate between acute/early from chronic HIV-1 infection.
Background

- HIV-2 occurs mainly in West Africa; however, an increasing number of cases have been recognized world-wide.

- Differentiation of HIV-1 from HIV-2 is important as therapeutic monitoring and treatment regimens differ for the two infections.

- HIV-2 infection is often diagnosed only after immunologic deterioration occurs in patients with undetectable HIV-1 viral loads.
Bio-Rad Multispot HIV-1/HIV-2 Rapid Test

- Single-use qualitative ImmunoConcentration™ assay to detect and differentiate circulating HIV antibodies.

Procedural Control
- Goat antihuman IgG
- HIV-1 gp41 recombinant
- HIV-2 gp36 peptides
- HIV-1 gp41 peptides

Occasional samples are “HIV Undifferentiated” ➔ may reflect dual infection or cross-reactivity.
Currently available enzyme immunoassay (EIA) or chemiluminescence microparticle immunoassay (CMIA) HIV-1/2 diagnostic tests **do not distinguish** between HIV-1 and HIV-2 antibodies.

The only FDA-approved assay which distinguishes HIV-1 from HIV-2 antibodies.

Suggested as a supplemental test in the proposed 4th generation HIV diagnostic algorithm to also distinguish between acute/early infection and chronic HIV-1 infection.
Sequence of Test Positivity Relative to WB

* Bio-Rad
** Abbott

Modified after, Masciotra et al 2011;52(S1):S17-22

Days Before Positive WB

- WB Indeterminate
- Bio-Rad Multisport Rapid Test
- WB Positive

Architect HIV Ag/Ab Combo (4th Gen)** Genetic Systems HIV-1/HIV-2

20 15 10 0
HIV Serology Algorithms

HIV-1/2 plus O EIA (3rd gen)
- gp160 and p24 recombinant gp36 peptide

Multisport

HIV-1 - HIV-2 -
- HIV-1 WB
- HIV-1 WB
- HIV-1 WB
- HIV-2 IB
- HIV-1 WB
- HIV-2 IB

WB: Bio-Rad Genetic Systems
HIV-1 Western Blot (WB)

HIV-1/2  Ag/Ab Combo (4th gen)

Multisport

HIV-1 WB
- HIV-1 WB
- HIV-1 WB
- HIV-2 IB
- HIV-1 WB
- HIV-2 IB

WB Neg/Ind
- Recombin/1/2 EIA (Abbott)

HIV-1 - HIV-2 -
- HIV-1 WB
- HIV-1 WB
- HIV-1 WB
- HIV-2 IB
- HIV-1 WB
- HIV-2 IB

HIV-1/2 -
- HIV-2 -
- HIV-1 +
- HIV-2 +
- HIV-1 +
- HIV-2 +

IB: Focus Diagnostic
HIV-2 Immunoblot (IB)
Method: Review HIV-1/HIV-2 Clinical Testing at UW Retrovirus Laboratory

A total of 46,061 clinical samples from Harborview Medical Center and UW Medical Center were screened for HIV between Aug 2008 and Jul 2012.

Additional research samples were also tested but are not included in this presentation.
# Clinical HIV Testing

<table>
<thead>
<tr>
<th>Screening Test</th>
<th>Interval</th>
<th>Number Tested</th>
<th>Reactives</th>
<th>Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genetic Systems HIV-1/HIV-2 Plus O EIA (3rd Gen)*</td>
<td>Aug 2008 - Apr 2011</td>
<td>30,885</td>
<td>599</td>
<td>1.94%</td>
</tr>
<tr>
<td>Architect HIV Ag/Ab Combo (4th Gen)**</td>
<td>May 2011 - Jul 2012</td>
<td>15,076</td>
<td>394</td>
<td>2.61%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>46,061</td>
<td>993</td>
<td>2.42%</td>
</tr>
</tbody>
</table>

* Bio-Rad  
** Abbott
# Multispot Results for EIA (3rd Gen) and CMIA (4th Gen) Reactive Samples

<table>
<thead>
<tr>
<th>MS Result</th>
<th>Number of Samples</th>
<th>Confirmatory Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non Reactive</td>
<td>50 (3rd Gen)</td>
<td>HIV-1 Viral Load</td>
</tr>
<tr>
<td></td>
<td>53 (4th Gen)</td>
<td></td>
</tr>
<tr>
<td>Reactive, HIV-1</td>
<td>882</td>
<td>WB* Pos 871</td>
</tr>
<tr>
<td></td>
<td></td>
<td>WB* Ind 11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.3%</td>
</tr>
<tr>
<td>Reactive, HIV-2</td>
<td>3</td>
<td>IB** Pos 3</td>
</tr>
<tr>
<td>Reactive, HIV-1&amp;2 (Post-dilution)</td>
<td>5</td>
<td>IB** Pos 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IB** Neg 4</td>
</tr>
</tbody>
</table>

* WB: Bio-Rad Genetic Systems HIV-1 Western Blot (WB)
** IB: Focus Diagnostic HIV-2 Immunoblot (IB)
Profiles for 3rd Generation EIA – Reactive, Multispot HIV-2-Reactive Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>HIV-1 Spot</th>
<th>HIV-2 Spot</th>
<th>HIV-2 IB</th>
<th>HIV-1 WB</th>
<th>HIV-1 WB Bands</th>
<th>Reported as</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>+</td>
<td>Pos</td>
<td>Ind</td>
<td>55, 40, 31, 24</td>
<td>HIV-2</td>
</tr>
<tr>
<td>2</td>
<td>-</td>
<td>+</td>
<td>Pos</td>
<td>Pos</td>
<td>160, 55, 31, 24</td>
<td>HIV-2</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>Pos</td>
<td>Pos</td>
<td>160, 31, 24</td>
<td>HIV-2</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>+/-</td>
<td>Neg</td>
<td>Pos</td>
<td>All</td>
<td>HIV-1</td>
</tr>
</tbody>
</table>
Profiles for 4\textsuperscript{th} Generation CMIA Reactive, Multispot HIV-2 Reactive Patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>HIV-1 Spot</th>
<th>HIV-2 Spot</th>
<th>HIV-2 IB</th>
<th>HIV-1 WB</th>
<th>HIV-1WB Bands</th>
<th>HIV-1 RNA</th>
<th>HIV-2 RNA\textdagger</th>
<th>Reported as</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>+</td>
<td>+</td>
<td>Pos</td>
<td>Pos</td>
<td>160, 120</td>
<td>TND</td>
<td>17</td>
<td>HIV-2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>31, 24</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>+/-</td>
<td>Neg</td>
<td>Pos</td>
<td>All</td>
<td>TND</td>
<td>ND</td>
<td>HIV-1</td>
</tr>
<tr>
<td>7</td>
<td>+</td>
<td>+/-</td>
<td>Neg</td>
<td>Pos</td>
<td>All</td>
<td>5,589</td>
<td>ND</td>
<td>HIV-1</td>
</tr>
<tr>
<td>8</td>
<td>+/-</td>
<td>+/-</td>
<td>Neg</td>
<td>Ind</td>
<td>160 (+/-)</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

ND: Not done
TND: Target not detected
\textdagger\text{HIV-2 RNA: UW developed and validated, CLIA-compliant assay (Chang, M., Gottlieb, G., Dragavon, J., et al, J Clin Virol 2012:55;128-33)}
HIV-2 Cross Reactivity on HIV-1 WB

- The most commonly observed HIV-1 WB bands from HIV-2 infected patients were gag p24 and pol p31 (100%), followed by env gp160 (75%), gag p55 (50%) and env p120 and gag p40 (25%).

- Patients 2, 3, 5 (from previous slides) would have been incorrectly identified as confirmed HIV-1
Conclusions

- Weakly reactive HIV-2 MS, in the presence of HIV-1 MS, likely reflect cross reactivity with HIV-1 antibody.

- The MS performed well as the confirmatory test for screening algorithms that used either 3rd or 4th generation HIV-1/2 assays.
Conclusions, cont’d

Eight of 993 (0.8%) EIA/CMIA repeatedly-reactive specimens were HIV-2 reactive by MS testing but only the 4 samples with strong reactions in the HIV-2 MS test confirmed by HIV-2 IB.

3 samples (0.03%) would have been otherwise misclassified as HIV-1 positive if only the HIV-1 WB was used to confirm the initially reactive screening test result.
Acknowledgments

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