Performance of an HIV Diagnostic Algorithm using the Architect HIV AG/AB Combo Assay and Potential Utility of the Sample-to-Cutoff Ratio

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Background

• The effective management of HIV infection requires the ability to distinguish and confirm acute from established infection and to reliably distinguish HIV-1 from HIV-2;

• There is also a requirement to distinguish 4th generation chemiluminescence immunoassay (CMIA) reactivity for HIV-1 p24 antigen from HIV-1/2 antibody.
Proposed testing algorithm for HIV Diagnosis

Sensitive HIV-1/2 Immunoassay
(eg, 4th generation Ag/Ab assay)

(+)

(-)

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

HIV-1/HIV-2 differentiation immunoassay

HIV-1 (+)
HIV-1 antibodies detected
Initiate care (and viral load)

HIV-2 (+)
HIV-2 antibodies detected
Initiate care

HIV-1&2 (-)
RNA

RNA(+)
Acute HIV-1 infection
Initiate care

RNA (-)
Negative for HIV-1

Branson BM. J Acquir Immune Def Syndr 2010; 55:S102-S105
Objective

• To determine the performance of the Abbott Architect Ag/Ab Combo Assay (4th generation assay) in the clinical setting to detect acute infection and explore the utility of the sample/cutoff (S/CO) values to predict the HIV infection status.
Methods

• Retrospective analysis of 15,076 clinical HIV-1/2 test results between May 2011 and July 2012

• The following Architect Algorithm was used:
  – All repeatedly reactive samples were tested with the Multispot HIV-1/2 rapid test (Bio-Rad) (MS) with the current package insert interpretation;
  – Confirmation with HIV-1 Western blot (Genetic Systems) (WB); Abbott m2000rt RealTime HIV-1 RNA; HIV-2 antibody test (Focus Diagnostics); HIV-2 RNA (J Clin Virol 2012;55:128-33) as indicated;
  – “Presumptive” clinical reports were issued based on the MS rapid test outcome and a final report was issued based on the appropriate confirmatory test results.
Results

Figure 1: Schematic diagram of the 4th generation HIV-1/2 testing algorithm.

15,076 Clinical Samples screened using Architect HIV Ag/Ab Combo

Period of time
May 2011 – Jul 2012

First test non-reactive or both duplicate re-tests non-reactive

Non-reactives 14,682

55 had S/CO between 0.70 - 0.99

S/CO (0.13) [0.11 - 0.16]

First test reactive with at least one duplicate re-test reactive

Reactives 394

(Median) [IQR]

Multispot Innuomaoassay (MS) HIV-1 / HIV-2 differentiation

2.6% with S/CO ≥1

337 MS Reactive HIV-1 85.5%

53 MS Non-reactive 13.5%

4 MS Reactive HIV-1 & HIV-2 1.0%

VL (+) VL (-)
“Presumptive positive for HIV-1”

The S/CO values were significantly different between the two WB groups; Mann-Whitney rank sum (P<0.001)

Confirmed with the detection of HIV-1 RNA from a follow-up specimen
Dual reactivity at a 1:100 dilution

### Bands:

- **HIV-1**
  - S/CO, WB, VL
  - 160 (+/-)
  - 160, 120, 31, 24
  - All
- **HIV-2**
  - IB, VL
  - 1.5, Ind, NA
  - 231, Pos, TND
  - 635, Pos, TND
  - 739, Pos, 5,589

### IQR:
- Interquartile range
- Neg: Negative
- Pos: Positive
- Ind: Indeterminate
- TND: Target non-detected
- NA: Non-applicable

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S/CO: Sample / Cutoff ratio
WB: Western Blot
Ind: Indeterminate
VL: Viral load
TND: Target non-detected

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12/12/12 2012 HIV Diagnostic Conference
Median S/CO 55 [IQR 2.1-75]

“HIV-1 negative”

“HIV-1 infection not confirmed”
Performance of the 4\textsuperscript{th} generation HIV testing algorithm for Multispot confirmation only

<table>
<thead>
<tr>
<th>MS Confirmation Scenario(^\dagger) (N=394)</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive Predictive Value</th>
<th>Negative Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WB, IB, NAAT</td>
<td>97.1</td>
<td>91.5</td>
<td>98.8</td>
<td>81.1</td>
</tr>
<tr>
<td>WB or IB only</td>
<td>96.8</td>
<td>86.0</td>
<td>97.9</td>
<td>79.6</td>
</tr>
<tr>
<td>NAAT for MS-negative only; WB, IB, NAAT for MS-dual HIV-1/2 only</td>
<td>97.1</td>
<td>91.5</td>
<td>98.8</td>
<td>81.1</td>
</tr>
</tbody>
</table>

\(^\dagger\) MS, Multispot; WB, Western blot (HIV-1); IB, Immunoblot (HIV-2); NAAT, Nucleic acid amplification test for HIV-1 or HIV-2
Log-linear relationship between HIV-1 RNA level and Architect assay sample/cutoff chemiluminescence (S/CO) (N=10 acute infections)

\[
Y = 0.9X - 4.31 \\
R^2 = 0.91
\]

\[S/CO = 1: \\
\text{HIV-1 RNA log}_{10} \text{ copies/mL} \\
4.78\ [95\%\ CI,\ 4.68-4.88]^{\dagger}
\]

\[60,300\ [48,000-75,900]\ RNA\ c/mL\]
Twelve acute infection cases (abstract 10 + 2 new†) plus extended dilution of one sample (*) to ≤1 S/CO

<table>
<thead>
<tr>
<th>Copies/mL</th>
<th>Log copies/mL</th>
<th>S/CO</th>
<th>Log S/CO</th>
</tr>
</thead>
<tbody>
<tr>
<td>8,335</td>
<td>3.92</td>
<td>0.54</td>
<td>-0.27</td>
</tr>
<tr>
<td>12,500</td>
<td>4.10</td>
<td>0.76</td>
<td>-0.12</td>
</tr>
<tr>
<td>16,700</td>
<td>4.22</td>
<td>0.98</td>
<td>-0.01</td>
</tr>
<tr>
<td>25,000*</td>
<td>4.40</td>
<td>1.11</td>
<td>0.05</td>
</tr>
<tr>
<td>97,440</td>
<td>4.99</td>
<td>2.1</td>
<td>0.32</td>
</tr>
<tr>
<td>133,300</td>
<td>5.12</td>
<td>1.5</td>
<td>0.18</td>
</tr>
<tr>
<td>150,000†</td>
<td>5.18</td>
<td>15</td>
<td>1.18</td>
</tr>
<tr>
<td>387,300</td>
<td>5.59</td>
<td>2.1</td>
<td>0.32</td>
</tr>
<tr>
<td>397,600</td>
<td>5.60</td>
<td>2</td>
<td>0.30</td>
</tr>
<tr>
<td>3,326,000</td>
<td>6.52</td>
<td>36</td>
<td>1.56</td>
</tr>
<tr>
<td>8,924,000</td>
<td>6.95</td>
<td>76</td>
<td>1.88</td>
</tr>
<tr>
<td>&gt;10,000,000</td>
<td>7.00</td>
<td>74</td>
<td>1.87</td>
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<td>&gt;10,000,000</td>
<td>7.00</td>
<td>151</td>
<td>2.18</td>
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<tr>
<td>&gt;10,000,000</td>
<td>7.00</td>
<td>208</td>
<td>2.32</td>
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<tr>
<td>&gt;10,000,000†</td>
<td>7.00</td>
<td>169</td>
<td>2.23</td>
</tr>
</tbody>
</table>
Correlation between HIV-1 RNA $\log_{10}$ copies/mL versus $\log_{10}$ S/CO for 12 acute infections + dilutions (*)
Receiver Operator Curve for S/CO and Viral load for acute infection (N=55 Multispot non-reactive)†

Area under the curve: 0.74
Bias: 0.002
CI 95% (0.545 – 0.935)

† 53 abstract samples + two new
Summary

• A modified 4th generation HIV screening algorithm used by the University of Washington Clinical Retrovirology Laboratory, detected 14 acute infections and one HIV-2 infection from among 15,076 clinical tests performed over a 13-month period;
  – Ten acute HIV-1 infections were identified from among 53 Multispot non-reactive specimens and a single HIV-2 infection was identified from among four Multispot dually HIV-1/HIV-2 reactive specimens;
  – There were an additional four acute/early HIV-1 infections and three possibly “false-positives” [WB indeterminate] from among the 337 MS HIV-1 positive tests, and one false-positive from among the four MS dual HIV-1/2 positive tests that would have been missed without complementary NAAT testing.
• There was a significant separation for the Architect S/CO median [IQR] values for HIV-1 infection from among the MS-negative specimens:
  – False-positive (N=43): 2.3 [1.4-5.0]
  – Acute (N=10): 19 [2.7-76]
  – Established (N=330): 793 [444-1,040]

• There was a strong linear relationship ($R^2=0.91$) between the Architect S/CO value and the HIV-1 RNA level in acute infection that requires further study (i.e., a larger sample size) to explore whether there is an acceptable S/CO value <1.0 that should trigger HIV NAAT to enhance the detection of acute infection.
Conclusions

• For this study, the Abbott 4th generation assay with Mulitspot HIV-1/2 confirmation had a suitable test performance for detecting acute and established HIV infection without the current mandate to use Western blot for confirmation; however, this is predicated on using NAAT alone for MS-negative specimens with reserved use of Western blot (HIV-1), Immunoblot (HIV-2) and HIV-1/2 NAAT for Mulitspot dual HIV-1/2 infection.

• The broad dynamic range of the Abbott S/CO ratio may provide some guidance for NAAT testing at a S/CO < 1 but this requires further study.

• These conclusions may not apply to HIV-1 vaccine recipients because of vaccine induced seropositivity (VISP) for which Western blot still has a role.
Acknowledgements

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