Is It Always Necessary for the ARCHITECT 4th-Generation HIV-1/2 Ag/Ab Combo Assay to be Repeatedly Reactive before Moving Forward in the Centers for Disease Control and Prevention (CDC) HIV Screening Algorithm?

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Introduction

Currently, there are three Food and Drug Administration (FDA) approved 4th-generation assays:

- The Abbott Architect HIV Ag/Ab Combo assay (June, 2010) Chemiluminescent magnetic microparticle-based immunoassay (CMIA)
- The Bio-Rad GS HIV Combo Ag/Ab assay (July, 2011) Enzyme immunoassay (EIA)
- The ADVIA Centaur HIV Ag/Ab Combo (CHIV) assay (June, 2015) CMIA
4th Gen Testing Algorithm for HIV-1 & HIV-2

Sensitive HIV-1/2 immunoassay (eg, fourth-generation Ag/Ab assay)

≥1.0 (+)  
(-) <1.0 ←------- S/CO

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

“Orthogonal”

e.g., Multispot test with consideration for a more rapid turn-around-time; Geenius identifies HIV-1 (gp160, gp41, p31, p24) and HIV-2 (gp140, gp36)

- Branson, JAIDS 2010;55:S102-5
- Clinical & Laboratory Standards Institute 2011, M53-A: Vol.31 No.13
S/CO Distribution by Stage of HIV-1 Infection

Ramos et al. Poster CROI 2015

N = 199

A: ARCHITECT
B: GSCombo

<table>
<thead>
<tr>
<th>Infection Status</th>
<th>Negative</th>
<th>Acute</th>
<th>Recent</th>
<th>Established</th>
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<tr>
<td>N</td>
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<td>35</td>
<td>15</td>
<td>50</td>
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<tr>
<td>Median</td>
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<td>0.27</td>
<td>16.6</td>
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<tr>
<td>IQR</td>
<td>0.09, 0.13</td>
<td>4.7, 74</td>
<td>5.8, 14</td>
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<td></td>
<td>0.25, 0.29</td>
<td>14</td>
<td>218, 449</td>
<td>688, 1008</td>
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</table>
Objectives

Since the ARCHITECT S/CO increases initially with viral replication (HIV-1 p24 antigen) and then following the rise in HIV specific antibodies, we sought to:

- Determine the correlation of the ARCHITECT S/CO values between the first, second and third replicates
- Determine whether the ARCHITECT S/CO value could be used to modify this requirement for replicate testing
- Determine a S/CO threshold for a single test without losing ARCHITECT assay sensitivity or specificity, shortening the turn-around time for moving in the HIV screening algorithm.
Methods

- A retrospective analysis was done using ARCHITECT test results obtained from our primary screening HIV diagnostic algorithm between May 2011 to September 2015

- Specimens were obtained from an academic hospital referral laboratory and research HIV-1 vaccine trials

- The testing algorithm interpretation for S/CO:
  - Initially reactive (S/CO ≥1.0) both replicate S/CO <1.0 was considered non-reactive;
  - One or both of the replicates ≥1.0 was considered reactive and tested with the discriminatory Multispot test, and HIV-1 RNA when indicated.
Results

- From 43,518 specimens tested, 975 were initially S/CO \( \geq 1.0 \); from these a total of 187 (19.2\%) were repeatedly S/CO \(< 1.0\) and defined as ARCHITECT non-reactive.

  Median S/CO [interquartile range (IQR); total range] values:
  - Negative results (N=42,543): 0.13 [0.11-0.16 ; 0.07-0.99]
  - First run (N=187): 1.9 [1.4-3.5 ; 1.0-31]
  - Second run (N=187): 0.15 [0.12-0.19 ; 0.07-0.53]
  - Third run (N=187): 0.15 [0.12-0.18 ; 0.07-0.51]

  Bland-Altman analysis (bias±2SD) between first-second: 3.8±8
  Bland-Altman analysis (bias±2SD) between second-third: 0±0.1
  Mann-Whitney-Wilcoxon between second-third, p=0.44
Results

- Of the 788 repeatedly-reactive specimens, 784 specimens were dually reactive and four specimens (0.5%) were discordant among replicates.

  Median S/CO [IQR ; total range] values:
  - First run (N=788): 651 [285-880 ; 1.0-1441]
  - Second run (N=788): 653 [265-889 ; 0.9-1447]
  - Third run (N=788): 656 [264-885 ; 0.9-1454]

  Bland-Altman analysis (bias±2SD) between first-second: -4.0±31
  Bland-Altman analysis (bias±2SD) between second-third: 0.5±30
  Kruskal Wallis, p=0.97
ARCHITECT S/CO Distribution Between Replicates

Initially Reactive 19.2%

<table>
<thead>
<tr>
<th>Sample Repetition</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
<th>Median</th>
<th>IQR</th>
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<tr>
<td>1st</td>
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<td>1.9</td>
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<td>3rd</td>
<td>885</td>
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<td>0.15</td>
<td>0.12</td>
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Receiver Operator Curve for ARCHITECT S/CO Initially Reactive Specimens

S/CO of 10
Sensitivity: 89.5%
Specificity: 98.7%
Correctly Classified 94.3%

S/CO of 1.0
Sensitivity: 100%
Specificity: 78.5%
Correctly Classified 88.8%

S/CO of 32
Sensitivity: 84.8%
Specificity: 100%
Correctly Classified 92.7%

Area under the curve: 0.99
CI 95% (0.987 - 0.993)
**ARCHITECT S/CO Initially Reactive at S/CO ≥10**

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<tr>
<th>Architect Status</th>
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<th>Non-Reactive</th>
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<td>788</td>
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<td>20</td>
<td>0.20</td>
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<td>880</td>
<td>889</td>
<td>885</td>
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ARCHITECT S/CO Replicates Reactive with Negative Viral Load (False Positive)

Φ: Negative Viral load
ΦΦ: Positive Viral load or Reactive Multispot

<table>
<thead>
<tr>
<th>Architect S/CO in Log10 Scale</th>
<th>False Positive 13.2%</th>
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<tr>
<td>S/CO≥10 3 (3.3%)</td>
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<td>S/CO&lt;10 32 (4.6%)</td>
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<tr>
<th>Median</th>
<th>S/CO&lt;10</th>
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<tr>
<td>1.8</td>
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<td>1.4</td>
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<td>2.8</td>
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<td>696</td>
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</table>

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Receiver Operator Curve for ARCHITECT S/CO Replicates Reactive and HIV Confirmed

S/CO of 1.0
Sensitivity: 100%
Specificity: 71.1%
Correctly Classified: 83.7%

S/CO of 32
Sensitivity: 91%
Specificity: 100%
Correctly Classified: 96.1%

S/CO of 10
Sensitivity: 95.7%
Specificity: 98.5%
Correctly Classified: 97.3%

Area under the curve: 0.99
CI 95% (0.993 - 0.997)
Results

- Turnaround time of the ARCHITECT from 942 repeatedly-reactive specimens was:
  - Median time [IQR ; total range] values:
    - First/Second run (N=942): **74 min [57-98 ; 34-198]**
    - Second/Third run (N=942): **1 min [1-1 ; 1-33]**
Conclusions

- For samples with an initial ARCHITECT S/CO ≥10, the false-reactive duplicate rate was 1.3% with a sensitivity of 85.5% while the false HIV infection rate was 1.5% with a sensitivity of 95.7%.

- Most of the samples initially ARCHITECT reactive as well as ARCHITECT false positive (negative viral load) were S/CO <10.

- To decrease turnaround time and total screening costs for research testing, all initially reactive research specimens with a S/CO ≥10 could reflex directly to Multispot discriminatory testing and HIV-1 RNA as indicated, while initially reactive specimens with a S/CO between 1 and 10 could be rerun only in singleton after centrifugation.
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