Optimization and Calibration of Less Sensitive and Avidity Modified Protocols for the Vitros Immunodiagnostic Products Anti-HIV-1+2 Assay for Detection of Early HIV Infections

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Serologic Testing Algorithms for Recent HIV Seroconversion (STARHS) assays, also termed Recent Infection Testing Algorithms (RITAs), are used to distinguish recent from long-standing HIV infections.

- Perform a sensitive HIV Ab detection assay to screen population
- Test confirmed HIV Ab+ samples by a less-sensitive (detuned) avidity modified HIV Ab assay
- If negative on the less/sensitive/avidity assay, the individual is within a recent infection “window period”.

High titer and avidity HIV-1 IgG immune responses to the STARHS/RITA assays are usually complete by one year following HIV infection; dynamic period is from 2-11 months following infection.

New STARHS/RITA assays (less-sensitive/avidity/other) requires well characterized specimens collected in the dynamic period following HIV infection, as well as samples from infected subjects with long-standing infections including categories prone to “false recent” results (AIDS; HAART, EC).
STARHS/RITA and Misclassification

Mean (subtype B) 170 days (95% CI=162, 183)

Cutoff

Courtesy B. Branson, CDC
Vironostika HIV-1 EIA System

- Uses a viral lysate as the capture antigen.
- Low sensitive version uses a difficult, 2-step 1:20,000 dilution.
- Inter assay calibration done by using a calibrator standard supplied by the CDC.
  - Screening: \[
  \frac{\text{Sample OD-Negative OD}}{\text{CDC calibrator OD-Negative OD}}
  \]
  - Confirmatory for samples under a result of 2.0
    \[
    \frac{\text{Median sample OD-median negative OD}}{\text{CDC calibrator OD-Negative OD}}
    \]
- Window period at 1.0 SOD is 170 days (95% CI:163-183 days)
- BioMerieux sold its license to manufacture this kit to Avioq which recently received FDA approval.
VITROS Anti-HIV 1+2 Assay

Product Overview
Principles of Procedure: Two stage reaction

1) HIV antibody present in the sample binds with HIV recombinant antigen coated on the wells. Unbound sample is removed by washing.

2) Horseradish peroxidase (HRP)-labeled recombinant HIV antigens are added in the conjugate reagent. The conjugate binds specifically to any human anti-HIV-1 or anti-HIV-2 (IgG and IgM) captured on the well in the first stage. Unbound conjugate is removed by washing.

The bound HRP conjugate is measured by a luminescent reaction. The amount of HRP conjugate bound is indicative of the level of anti-HIV-1 and anti-HIV-2 present.

4 Antigens Coat Well
4 Ag-conjugated to HRP
• 3 Ag for HIV-1
   (Env 13, Env 10, p24)
• 1 Ag for HIV-2
  (Env AL)
Ortho Vitros Assay

- Currently there are 1450 VITROS ECi customers in the US, 350 using their HIV detection system.
- This can be used in a low sensitive (diluted in plasma) version or an avidity (chaotropic agent incubation step).
- There is potential for doing the dilution step on board the machine. For now, the dilution is being done off board.
LS-VITROS and Avidity Development

- Optimize dilution factor for LS assays by calibration to Vironostika LS-EIA.
- Test samples in avidity assay (Chawla et al.) of the assay.
- Investigate proficiency and precision of assay using CDC proficiency panels.
- Calculate window periods at different cut-offs.
1:400 Dilution

**Left Diagram:**
- LS-Vitros S/C vs. LS-Vironostika SOD
- $R^2 = 0.7368$
- $y = 8.337x + 11.643$
- LS-Ortho (y) = 20

**Right Diagram:**
- LS-Vitros S/C vs. BED SOD
- $R^2 = 0.5451$
- $y = 8.7572x + 14.133$
- LS-Ortho (y) = 21
Vitros Avidity Studies

Previous studies have been done using the Axsym platform to look at HIV-1 avidity.

(Chawla et al. J Clin Micro Feb 2007)
Blood Donors
2007 ARC Interdonation Intervals

<table>
<thead>
<tr>
<th>Inter-donation interval</th>
<th>Number of subjects</th>
<th>Mean EIA (Standard deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>535</td>
<td>3.59 (2.36)</td>
</tr>
<tr>
<td>&lt;120</td>
<td>18</td>
<td>0.04 (0.10)</td>
</tr>
<tr>
<td>120–365</td>
<td>54</td>
<td>1.11 (1.50)</td>
</tr>
<tr>
<td>&gt;365</td>
<td>109</td>
<td>2.49 (2.16)</td>
</tr>
</tbody>
</table>
Vitros Avidity

Avidity Index

Interdonation Interval
Vitros vs BED

\[ y = 0.0289x + 0.2801 \]

\[ R^2 = 0.4673 \]

N=710
Vironostika vs Vitros

\[ y = 0.0756x + 0.2715 \]
\[ R^2 = 0.6471 \]

N = 710
Screening S/C vs. Confirmatory S/C

y = 0.9904x - 0.2358
R² = 0.997

Screening S/C cutoff = 10

Confirmatory S/C cutoff = 10

BBI# 1  BBI# 2  BBI# 4  BBI# 5

Li  (BBI# 1)  Li  (BBI# 1)  Li  (All)

Li  (BBI# 1)  Li  (BBI# 1)  Li  (All)
Vitros Detuned and Avidity Assays
Window period calculations from Seroconversion panels

- 357 (LS-Vitros) and 350 (Vitros Avidity) longitudinal observations from 70 subjects were used in the window period estimation.
- Days since seroconversion was assumed to be the midpoint between last negative and first positive test dates when the interval was ≤120 days.
- Slope & intercept from a random effects regression, and LS S/C or avidity index were the explanatory variables in the multiple imputation regression model.
- The date that the subject reaches cut-off value was linearly interpolated from the last known date below the first known date above the avidity index cut-off value. If the subject does not reach the cut-off value, the observation is censored at the last date above the cut-off.

Statistical Analysis done by Debra Hanson.
<table>
<thead>
<tr>
<th>Assay, Cutoff Value</th>
<th>Mean Window Period</th>
<th>Standard Deviation</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>S/C Ratio, 5.0</td>
<td>61.0</td>
<td>8.7</td>
<td>43.9, 78.1</td>
</tr>
<tr>
<td>S/C Ratio, 10.0</td>
<td>115.0</td>
<td>18.1</td>
<td>79.6, 150.4</td>
</tr>
<tr>
<td>S/C Ratio, 15.0</td>
<td>178.2</td>
<td>29.1</td>
<td>121.1, 235.4</td>
</tr>
<tr>
<td>S/C Ratio, 20.0</td>
<td>238.8</td>
<td>38.6</td>
<td>163.1, 314.5</td>
</tr>
<tr>
<td>S/C Ratio, 30.0</td>
<td>387.5</td>
<td>65.2</td>
<td>259.8, 515.2</td>
</tr>
</tbody>
</table>

Seroconversion panels 357 samples from 70 subjects
Ortho Vitros

Mean Window Period Estimates

<table>
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<tr>
<th>Assay, Cutoff Value</th>
<th>Mean Window Period</th>
<th>Standard Deviation</th>
<th>95% Confidence Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avidity, 0.50</td>
<td>145.3</td>
<td>25.0</td>
<td>96.4, 194.3</td>
</tr>
<tr>
<td>Avidity, 0.60</td>
<td>179.8</td>
<td>26.8</td>
<td>127.3, 232.3</td>
</tr>
<tr>
<td>Avidity, 0.70</td>
<td>243.0</td>
<td>29.7</td>
<td>184.8, 301.2</td>
</tr>
<tr>
<td>Avidity, 0.80</td>
<td>368.2</td>
<td>42.9</td>
<td>284.2, 452.2</td>
</tr>
</tbody>
</table>

Seroconversion panels 350 samples from 70 subjects
Caveat: False Incident Cases

- False-positive EIAs not confirmed with an HIV-1 Western blot or IFA (i.e., diagnostic algorithms with poor specificity)
- Poor specimen handling during processing or shipping
- Chronic infection, inflammation (too much antibody)
- HIV subtype heterogeneity
- Persons with advanced HIV disease (AIDS)
- Persons who have taken antiretroviral (ARV) agents 6 months before test
Validation Panels

- Sensitivity and window period calculations.
  - Clade B Seroconversion panels from HIVNet (collaboration with the CDC)
  - Clade C Seroconversion panels from Caprisa Studies in South Africa (collaboration with Salim Kalim)
  - Multi-clade Seroconversion panels from Nigerian cohort (collaboration with Kevin Delaney at CDC)

- Specificity
  - Long term non progressors/Elite controllers
  - JHU Specificity Panels
    - JHU ER study: predominantly chronically infected individuals
    - AIDS patients with low CD4 counts
    - HAART treated patients with low viral load
## Validation Panels

Cutoff and false Incidence comparison of specificity panels (% Incident):

<table>
<thead>
<tr>
<th>False Recency Rates</th>
<th>Vitros LS-Eci S/C</th>
<th>Vitros Avidity Index</th>
<th>BED SOD</th>
<th>Vironostika LS-EIA SOD</th>
<th>BioRad Avidity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13</td>
<td>16</td>
<td>18</td>
<td>20</td>
<td>60</td>
</tr>
<tr>
<td>JHU ER Study (n=297; Vitros avidity n=247)</td>
<td>12%</td>
<td>15%</td>
<td>16%</td>
<td>18%</td>
<td>21%</td>
</tr>
<tr>
<td>CD4&lt;50 (n=140)</td>
<td>6%</td>
<td>7%</td>
<td>11%</td>
<td>14%</td>
<td>6%</td>
</tr>
<tr>
<td>HAART CD4&gt;400 VL&lt;50 (n=134)</td>
<td>17%</td>
<td>23%</td>
<td>25%</td>
<td>29%</td>
<td>42%</td>
</tr>
</tbody>
</table>
Conclusions

- Calibrated the LS-Vitros assay to the Vironostika.
- Calculated the window period for LS- and avidity Vitros.
- Investigated false recency rates of challenge panels.
Work to do

- Window periods for combined assays.
- Investigate other chaotropic agents for avidity assay.
- Test with other clades to look at clade variation.
Thank you!

BSRI
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