Diagnosis of HIV-2 infection by an HIV-2 total nucleic acid qualitative assay using the Abbott m2000 platform

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The CDC algorithm for HIV diagnosis requires HIV-2 NAT.
Issues for the diagnosis of HIV-2

- Undetectable plasma RNA viral loads in ~30% of ART-naïve patients
- Unaddressed issues in the CDC 4th Generation HIV testing algorithm
  - HIV-2 Ab indeterminate by differentiation immunoassays
  - Undifferentiated HIV infection (dual HIV-1/2 infection vs. cross-reactive serology)
  - HIV-2 acute infection
Objective

- HIV-2 sero-indeterminate, undifferentiated HIV-1/HIV-2 or “false positive Combo” cases
- Infants born to HIV-2 infected mothers

HIV-2 RNA plasma quantitative assay
  ↓
  Detected
  ↓
  HIV-2 positive

HIV-2 total nucleic acid (TNA) assay
Assay overview

- Adapted from the current UW clinical HIV-2 plasma RNA quantitative assay since Dec. 2011 (Chang et al. JCV 2012)
- HIV-2 specific primers/probe, without cross-reactivity with HIV-1
- Sample: ca. 1-million peripheral white blood cells (WBC) prepared from EDTA whole blood
Assay overview

- Abbott m2000 platform: a DNA & RNA extraction protocol and a laboratory-defined qualitative program
- Endogenous (human Ribonuclease P gene) and exogenous (in-house RNA transcript) controls were used to assess sample quality and assay inhibition
Abbott laboratory-defined qualitative program

Cut-off (CO) cycle number: determined by evaluating cycle numbers of low-positive controls

Delta Cycle (DC): calculated for each sample as CO minus the target cycle number.
Assay precision

- Positive Control: ca. 180 copies of linear HIV-2 pROD plasmid spiked in 1 million human WBC
- Negative control: 1 million WBC
- 24 replicas of Positive and Negative controls were carried out in two days
  - An additional 4 replicas of controls were tested each day for 8 days
Assay precision

- ca. 180 copies of linear pROD plasmid spiked in 1 million WBC

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<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td>5.76 DC</td>
</tr>
<tr>
<td><strong>Total Standard Deviation</strong></td>
<td>0.40 DC</td>
</tr>
<tr>
<td><strong>Total CV</strong></td>
<td>6.94%</td>
</tr>
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- All negative controls were tested negative for the assay
## Sensitivity (limit of detection)

The LOD is estimated to 25 copies/million cells (95% CI, 13-37 copies/million cells).

<table>
<thead>
<tr>
<th>Copies of HIV-2&lt;sub&gt;pROD9&lt;/sub&gt; plasmid/million cells</th>
<th>Number tested</th>
<th>Number Detected</th>
<th>Percent detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>46</td>
<td>8</td>
<td>8</td>
<td>100</td>
</tr>
<tr>
<td>23</td>
<td>8</td>
<td>7</td>
<td>88</td>
</tr>
<tr>
<td>12</td>
<td>8</td>
<td>5</td>
<td>63</td>
</tr>
<tr>
<td>6</td>
<td>8</td>
<td>5</td>
<td>63</td>
</tr>
<tr>
<td>3</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Assay sensitivity for HIV-2 infected patient samples

- 30 HIV-2 seropositive patients
  - 23 with non-detectable (<8 copies/mL) plasma HIV-2 RNA
  - 7 with detectable plasma HIV-2 RNA up to 2.06 \( \log_{10} \) copies/mL plasma
  - All 30 WBC samples tested positive by HIV-2 TNA assay (DC median = 6.34; DC range, 1.07-9.39)
Diagnostic performance with CDC 4th generation algorithm

- Comparing patients’ WBC tested by HIV-2 TNA assay to the corresponding plasma tested by Abbott HIV Ag/Ab Combo assay and Bio-Rad Multispot HIV-1/-2 Rapid Test
- Tested samples from 25 HIV-negative donors, 30 HIV-2-infected and 25 HIV-1-infected patients
Diagnostic performance

<table>
<thead>
<tr>
<th></th>
<th>Abbott HIV Ag/Ab Combo assay and Bio-Rad Multispot HIV-1/-2 Rapid Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV-2 TNA assay</td>
<td>HIV-2 Ab reactive</td>
</tr>
<tr>
<td>Detected</td>
<td>29</td>
</tr>
<tr>
<td>Not-detected</td>
<td>0</td>
</tr>
</tbody>
</table>

*Sample contained the lowest signal-to-cutoff ratio, 5.89, and was non-reactive by Multispot

Agreement between two methods: 98.75%
### Multispot-undifferentiated HIV-1/HIV-2 cases

<table>
<thead>
<tr>
<th>ID</th>
<th>Plasma HIV-1 RNA c/mL</th>
<th>Plasma HIV-2 RNA c/mL</th>
<th>WBC HIV-1 TNA</th>
<th>WBC HIV-2 TNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>HD01</td>
<td>TND#</td>
<td>TND</td>
<td>Detected</td>
<td>1.38 DC</td>
</tr>
<tr>
<td>HD02</td>
<td>85120</td>
<td>2592</td>
<td>Detected</td>
<td>11.60 DC</td>
</tr>
<tr>
<td>HD06</td>
<td>510596</td>
<td>311</td>
<td>Detected</td>
<td>5.62 DC</td>
</tr>
<tr>
<td>HD09</td>
<td>TND</td>
<td>TND</td>
<td>Detected</td>
<td>8.08 DC</td>
</tr>
<tr>
<td>HD12</td>
<td>TND</td>
<td>TND</td>
<td>Detected</td>
<td>Detected*</td>
</tr>
<tr>
<td>H2A036</td>
<td>TND</td>
<td>136</td>
<td>Detected</td>
<td>7.96 DC</td>
</tr>
<tr>
<td>HD13</td>
<td>&lt;40</td>
<td>28726</td>
<td>TND</td>
<td>9.50 DC</td>
</tr>
<tr>
<td>HD10</td>
<td>13730</td>
<td>TND</td>
<td>Detected</td>
<td>TND</td>
</tr>
</tbody>
</table>

#Target Not detected

*Detected by a second HIV-2 probe*
Limitations

- When serology and TNA detection are discordant, need to consider Poisson distribution and compartmentalization of HIV-infected cells.

- As with any diagnostic test, results from the UW HIV-2 TNA qualitative assay should be interpreted in conjunction with other clinical and laboratory findings.
Conclusions

- Diagnostic testing application for 4th generation HIV algorithm
  - For cases such as HIV sero-indeterminate or undifferentiated orthogonal results by HIV-1/HIV-2 differentiation immunoassays

- Future plans
  - Test white blood cells from infants born to HIV-2 infected mothers and provide the assay for the clinical service

- Assay feasibility
  - Reliable Abbott m2000 platform, requiring minimal 0.5mL EDTA whole blood, completing one run in 8 hours
Acknowledgements

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