Evaluation of the Xpert® HIV-1 Qual Assay and Xpert® HIV-1 Viral Load Assay

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Xpert® HIV-1 Viral Load Evaluation:

**Unique features of this assay:**
- Single target – 3’ end of the 5’ LTR
- Accepts fresh and frozen plasma
- Uses both a low & high IQC, traceable to WHO international standard

**Demographics:**
- 724 eligible subjects
  - 205 (28.3%) females & 519 (71.7%) males
  - Average age 44.5 ± 11.3 years (range 18-83 yr)

**Inclusion Criteria:**
- Clinician ordered HIV-1 Viral Load (VL)
- >18 years of age
- Known ART status
- Plasma same freeze/thaw cycle

**Exclusion Criteria:**
- Previously enrolled in study
- Specimen not properly collected

**Results:**

**Assay Performance:**
Overall rate of assay success was 96.9%
Linear range: 40-10^7 copies/mL (cp/mL)
Validated across Group M subtypes, Groups N & O

**Specificity in HIV-1 sero-negative blood band donors:**
- 100% specificity;
- 109/109 were HIV-1 negative,
- 95% CI: 96.7-100.0

**Regression Analysis**
- Deming: \( y = -0.1771 + 1.0589x \)
- SLR: \( y = -0.1772 + 1.0417x \)
- \( r = 0.9847, R^2 = 0.9696 \)

**Specimens with HIV-1 VL Results for Either Assay Not Quantified by the Other:**

<table>
<thead>
<tr>
<th>No. Specimens</th>
<th>Xpert (cp/mL)</th>
<th>Abbott (cp/mL)</th>
<th>Values (cp/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Not Detected</td>
<td>&gt;40</td>
<td>Abbott: 43, 44, 44, 81</td>
</tr>
<tr>
<td>2</td>
<td>&gt;40</td>
<td>Detected &lt;40</td>
<td>Xpert: 40, 43</td>
</tr>
<tr>
<td>1</td>
<td>&gt;40</td>
<td>Not Detected</td>
<td>Xpert: 51</td>
</tr>
<tr>
<td>24</td>
<td>Detected &lt;40</td>
<td>&gt;40</td>
<td>Abbott: 40-167 (median 64)</td>
</tr>
</tbody>
</table>

**Results by Result Classification**

<table>
<thead>
<tr>
<th>Xpert HIV-1 VL</th>
<th>Abbott RealTime-HIV-1 Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV Detected &gt;40 cp/mL</td>
<td>HIV Detected &lt;40 cp/mL</td>
</tr>
<tr>
<td>390</td>
<td>2</td>
</tr>
<tr>
<td>HIV Detected &lt;40 cp/mL</td>
<td></td>
</tr>
<tr>
<td>87</td>
<td>38</td>
</tr>
<tr>
<td>HIV Not Detected</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>494</td>
</tr>
</tbody>
</table>

Concordance = 74.9%

**Consecutive Specimens Collected Without Bias by Result Classification**

<table>
<thead>
<tr>
<th>Xpert HIV-1 VL</th>
<th>Abbott RealTime-HIV-1 Assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV Detected &gt;40 cp/mL</td>
<td>HIV Detected &lt;40 cp/mL</td>
</tr>
<tr>
<td>97</td>
<td>2</td>
</tr>
<tr>
<td>HIV Detected &lt;40 cp/mL</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>34</td>
</tr>
<tr>
<td>HIV Not Detected</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>18</td>
</tr>
<tr>
<td>Total</td>
<td>125</td>
</tr>
</tbody>
</table>

Concordance = 70.9%

**SUMMARY:**
- Good overall agreement between Xpert® HIV-1 VL and Abbott RealTime HIV-1 Assays
  - Slightly lower quantification with Xpert® at low end
  - Slightly higher overall detection rate with Xpert®
  - Xpert® allows flexibility for testing both frozen & fresh plasma specimens
  - Xpert® allows for HIV-1 VL testing nearer the patient reducing TAT and loss to follow up

*CE-IVD mark for in vitro diagnostic use. *For Investigational Use Only in the U.S.

**Acknowledgement:** Cepheid Inc. supported HIV-1 VL Study
**Xpert® HIV-1 Qualitative Evaluation:**

### Age Distribution:

<table>
<thead>
<tr>
<th>Category</th>
<th>N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>~6 weeks - 18 months</td>
<td>258</td>
<td>62.2</td>
</tr>
<tr>
<td>&gt;18 months - 7 years</td>
<td>29</td>
<td>7.0</td>
</tr>
<tr>
<td>&gt;7 years</td>
<td>128</td>
<td>30.8</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>415</td>
<td>100</td>
</tr>
</tbody>
</table>

### Demographics:

415 eligible subjects;
223 (53.6%) females, 186 (44.8%) males
505 specimens tested: 106 WB + 399 DBS

### Results:

#### Whole Blood Workflow

1. Collect ≥100 µl whole blood and transfer to EDTA microtainer tube or lavender tube
2. Use the 1mL pipette to transfer 0.75 mL sample reagent into the cartridge
3. Use the transfer pipette to transfer 100 µl whole blood into the cartridge
4. Scan cartridge barcode
5. Load into GX and close door

#### DBS Workflow

1. Collect DBS with 60-70 µl whole blood per spot
2. Transfer one DBS into the sample reagent bottle and mix. Incubate in Thermomixer at 56°C, 500 rpm for 15 min
3. Transfer all liquid into chamber 3 with the 1 mL transfer pipette
4. Scan cartridge barcode
5. Load into GX and close door

### WB Concordance:

104/106 (98.1%)
PPA: 98.2% (95% CI: 90.3-100)
NPA: 98.0% (95% CI: 89.6-100)

### DBS Concordance:

387/399 (97.0%)
PPA: 95.6% (95% CI: 91.8-98)
NPA: 98.5% (95% CI: 95.6-99.7)

#### Specificity in HIV-1 sero-negative adult blood donors:

100% specificity; 1014/1014 were HIV-1 negative, 95% CI: 99.6-100.0

- 512 WB and 502 DBS specimens were analyzed

### SUMMARY:

Excellent agreement between the Xpert® and Roche HIV-1 Qual assays

Xpert® HIV-1 Qual is applicable for near patient testing with results available in under 2 hours, with the potential for immediate confirmatory testing for:
- Early infant diagnosis
- Screening of high-risk sero-negative adults

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**Acknowledgement:** Cepheid Inc. supported the HIV-1 Qual Study
• **Potential Applications**
  – Reference Laboratories
  – Hospitals
  – Urgent Clinics
  – Physician Offices
  – Antenatal Clinics
  – Non-for-Profit Community Care Centers
  – Mobile Clinics/ Vans
  – Outreach Programs
  – In-Home Testing

• **Improve Patient Care:** *Same day results support better clinical decisions and may reduce loss to follow up*

• **Increase Efficiency:** *Rapid results enable immediate intervention to save lives at birth and earlier adjustments to appropriate therapy*

• **Strengthen Communities:** *Quick decisions can help reduce morbidity or mortality in HIV-infected infants and reduce drug resistance*