Validation and Clinical Use of a HIV-2 Viral Load Assay

Linda M. Styer, Thomas Miller, Monica M. Parker
Wadsworth Center, New York State Department of Health

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Clinical guidelines recommend HIV viral load testing to monitor treatment response.

There are no FDA-approved HIV-2 viral load tests.

Our goal was to develop a HIV-2 viral load assay and validate it for clinical use.

1. Assay Design

2. Validation

3. Clinical Use
The duplex real time RT-PCR assay detects HIV-2 RNA and internal control virus (MHV).

**HIV-2**

**Mouse Hepatitis Virus (MHV)**

**Coronavirus**

- Spike (S) protein
- Membrane (M) protein
- Envelope (E) protein
- Nucleocapsid (N) protein + genomic RNA

**LTR**

**MGB**

**FAM**

**Q**

**HEX**

**Q**
The duplex real time RT-PCR assay detects HIV-2 RNA and internal control virus (MHV).

20 ul internal control virus

200 ul or 900 ul plasma

Total nucleic acid
Nuclisens EasyMAG

HIV-2 high +

HIV-2 neg

MHV

HIV-2 low +
Each run includes calibrators and controls that need to fall within defined ranges.
The assay detects several strains of HIV-2 and doesn’t detect other human viruses.

<table>
<thead>
<tr>
<th>HIV-2 Strains</th>
<th>Result</th>
<th>Other Viruses</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>7924A (A)</td>
<td>Pos</td>
<td>HIV-1 (B, C)</td>
<td>Neg</td>
</tr>
<tr>
<td>60451K (A)</td>
<td>Pos</td>
<td>HTLV-1</td>
<td>Neg</td>
</tr>
<tr>
<td>CDC77618 (A)</td>
<td>Pos</td>
<td>HBV (B)</td>
<td>Neg</td>
</tr>
<tr>
<td>MVP-15132 (A)</td>
<td>Pos</td>
<td>HCV (1a,2a,3a)</td>
<td>Neg</td>
</tr>
<tr>
<td>CDC310072 (A)</td>
<td>Pos</td>
<td>SIV Strains</td>
<td></td>
</tr>
<tr>
<td>ROD (A)</td>
<td>Pos</td>
<td>SIV mac251</td>
<td>Pos</td>
</tr>
<tr>
<td>7312A (A/B)</td>
<td>Pos</td>
<td>SIV hu</td>
<td>Pos</td>
</tr>
<tr>
<td>CDC310319 (B)</td>
<td>Pos</td>
<td>SIV smE660</td>
<td>Pos</td>
</tr>
</tbody>
</table>
The assay accurately quantifies specimens over a wide dynamic range and can detect as few as 7 IU/ml.

<table>
<thead>
<tr>
<th>Vol (μl)</th>
<th>LOD (IU/ml)</th>
<th>LLOQ (IU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>32</td>
<td>130</td>
</tr>
<tr>
<td>900</td>
<td>7</td>
<td>29</td>
</tr>
</tbody>
</table>
The assay delivers accurate results based on samples exchanged with another lab.
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19/30 (63%) within 0.5 log
28/30 (93%) within 1.0 log
The HIV-2 viral load assay was approved for clinical use in April 2012.

- **HIV-2 Viral Load Assay Validation Study**
- **Validation Review**
- **Annual Competency Assessment**
- **Semiannual Proficiency Testing**
- **Lot-to-lot Testing of Reagents**
- **Instrument Calibration Multisystems Agreement**
- **Electronic reporting to NYSDOH**
We have tested 25 specimens from 13 individuals with the HIV-2 viral load assay.

At least one HIV-2 RNA + specimen from 11/13 (84.6%)

HIV-2 RNA negative, HIV-2 antibody positive

12/13 patients from NYC
Quantifiable viral load values range from 2.3 – 4.9 \log_{10} \text{ IU/ml} (200-80,000 \text{ IU/ml}).
Quantifiable viral load values range from 2.3 – 4.9 \( \log_{10} \) IU/ml.
We developed a sensitive and accurate clinical assay to quantify HIV-2 RNA.

Assay is well controlled,...

...sensitive, accurate...

...and includes QA/QC components required for clinical use.