Detection of HIV-2 proviral DNA by using single or multiplex real-time PCR

Timothy C. Granade, MS
Microbiologist
HIV Diagnostics Conference
December 12 – 14, 2012
General characteristics of HIV-2 infections

- HIV-2 generally less infectious than HIV-1
- HIV-2 has a longer asymptomatic phase than HIV-1
- HIV-2 proviral loads are lower than those for HIV-1
- HIV-2 plasma viral loads are less than HIV-1 (~30 fold) and associated with CD4 counts
- HIV-2 therapy is significantly different from regimens for HIV-1
- US prevalence is thought to be very low
Identification of HIV-2 infections
Diagnostic methods

- HIV-2 specific EIA for antibodies to HIV-2
- HIV-1/2 EIA for antibodies to both viruses
- HIV-2 WB – research use only
- HIV-1/2 rapid tests
- HIV-1/2 differentiation tests
  - Genie II, Immuno-comb, Multi-spot, Inno-LIA
- HIV-2 DNA PCR
- HIV-2 RT-PCR
- Nested PCR
Nucleic acid amplification testing for HIV-2

Challenges

- Variability within and between subtypes
  - 8 HIV-2 subtypes,
  - A and B have become established epidemics

- Amplification in multiple gene regions may improve isolate coverage

- Target selection
  - Low viral load in HIV-2 infections
  - Proviral DNA may be a better choice for diagnostic

- Lack of specimens for assay development and validation
Developmental approach/assay design

- **Target regions**
  - Integrate, Protease, Long Terminal Repeat (LTR)
- RNaseP amplification as an internal control
- Real-time PCR (TaqMan)
- Panel of HIV-2 plasmids
- Performance assessment using individual and multiplexed primer pairs
Specimens

- **Assay development**
  - Panel of plasmids (12 LTR, 12 Pol) containing HIV-2 gene regions (LTR, polymerase) for assessment of amplification efficiency and sensitivity
    - 9 subtype A, 2 subtype B, 1 subtype AB
  - Whole blood spiked with known copies of HIV-2 plasmids
  - Whole blood known to be HIV 1/2 negative (n=52) (specificity)
  - Whole blood from known HIV-1 positive individuals (n=36) (specificity)
  - HTLV I (n=8) and HTLVII (n=7) infected cell lines (specificity)

- **Initial validation**
  - PBMCs from 10 known HIV-2 infected individuals
Four sets of primer and probe (TaqMan) selected

<table>
<thead>
<tr>
<th>Gene regions</th>
<th>Oligo designation</th>
<th>Amplicon (bp)</th>
<th>MAC239</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2LTR1</td>
<td>cp1354</td>
<td>85</td>
<td>529-549</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>cp1355</td>
<td></td>
<td>529-549</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>cp1356R</td>
<td></td>
<td>565-586</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>cp1367</td>
<td></td>
<td>613-590</td>
<td>R</td>
</tr>
<tr>
<td>H2LTR2</td>
<td>cp1362</td>
<td>87</td>
<td>700-725</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>cp372B</td>
<td></td>
<td>744-725</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>cp1328</td>
<td></td>
<td>786-762</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>cp1330B</td>
<td></td>
<td>786-762</td>
<td>R</td>
</tr>
<tr>
<td>H2PRO</td>
<td>cp1350</td>
<td>87</td>
<td>2521-2545</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>cp1351</td>
<td></td>
<td>2521-2545</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>cp1352</td>
<td></td>
<td>2521-2545</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>cp1353R</td>
<td></td>
<td>2548-2572</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>cp1268R</td>
<td></td>
<td>2548-2572</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>cp1339</td>
<td></td>
<td>2607-2577</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>cp1340</td>
<td></td>
<td>2607-2577</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>cp1341</td>
<td></td>
<td>2607-2577</td>
<td>R</td>
</tr>
<tr>
<td>H2INT</td>
<td>cp1345</td>
<td>111</td>
<td>5058-5088</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>cp1346</td>
<td></td>
<td>5058-5088</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td>cp1300R</td>
<td></td>
<td>5111-5088</td>
<td>P</td>
</tr>
<tr>
<td></td>
<td>cp1301</td>
<td></td>
<td>5168-5138</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>cp1302</td>
<td></td>
<td>5168-5138</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>cp1303</td>
<td></td>
<td>5168-5138</td>
<td>R</td>
</tr>
</tbody>
</table>
Realtime PCR detection of HIV-2 by four individual primer/probe sets

Average Ct values of 12 plasmid panels
Comparison of singleplex and multiplex PCR reactions
LTR and Pol plasmid dilution panel (A2267)
Assay sensitivity: biPLEX reactions

LTR and Pol Plasmid Panels (GB87)

GB87 LTR

GB87 Pol
Realtime PCR of HIV-2 plasmids diluted in whole blood (biplex reaction)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Input</th>
<th>WB Extract</th>
<th>Plasmid</th>
<th>WB Extract</th>
<th>Plasmid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LTR 1 + LTR 2</td>
<td>Pro + Int</td>
<td></td>
<td></td>
</tr>
<tr>
<td>310319</td>
<td>20000</td>
<td>28.44</td>
<td>29.44</td>
<td>30.2</td>
<td>30.58</td>
</tr>
<tr>
<td>310319</td>
<td>2000</td>
<td>33.03</td>
<td>32.58</td>
<td>33.91</td>
<td>34.13</td>
</tr>
<tr>
<td>310319</td>
<td>200</td>
<td>38.34</td>
<td>36.86</td>
<td>37.75</td>
<td>44.46</td>
</tr>
<tr>
<td>310319</td>
<td>20</td>
<td>No ct</td>
<td>42.07</td>
<td>40.89</td>
<td>42.62</td>
</tr>
<tr>
<td>310319</td>
<td>2</td>
<td>No ct</td>
<td>No ct</td>
<td>40.2</td>
<td>No ct</td>
</tr>
<tr>
<td>60415 K</td>
<td>20000</td>
<td>29.83</td>
<td>29.08</td>
<td>30.06</td>
<td>29.77</td>
</tr>
<tr>
<td>60415 K</td>
<td>2000</td>
<td>32.59</td>
<td>32.43</td>
<td>34.17</td>
<td>33.64</td>
</tr>
<tr>
<td>60415 K</td>
<td>200</td>
<td>36.32</td>
<td>34.75</td>
<td>37.46</td>
<td>36.85</td>
</tr>
<tr>
<td>60415 K</td>
<td>20</td>
<td>No ct</td>
<td>No ct</td>
<td>39.91</td>
<td>No ct</td>
</tr>
<tr>
<td>60415 K</td>
<td>2</td>
<td>No ct</td>
<td>No ct</td>
<td>No ct</td>
<td>No ct</td>
</tr>
</tbody>
</table>
Realtime PCR of HIV-2 DNA from patient PBMCs

**Biplex vs Single Reaction**

- **LTR1+LTR2**
- **LTR1**
- **LTR2**

Sample: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10

**Pro+Int**, **Pro**, **Int**

Sample: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10
Specificity

• 100% specificity
  – HIV-1/2 negative whole blood extracts (n=52)
  – Whole blood extracts of known HIV-1 reactive specimens (n=36)
  – HTLV I/II infected cell lines (n= 15)
Summary
HIV-2 Real-time PCR

- Limit of detection for four primer sets individually was ~ 40 input copies (24 plasmids)
- Multiplexing with three primer sets (LTR1, LTR2 and Pro) performed equally well as the individual primer sets
- Multiplexing with three primer sets (LTR2, Pro, Int) caused significant interference with the integrase amplification
- Two biplex reactions (LTR1 + LTR2) / (Pro + Int) may perform better than each primer set individually
- Amplification was observed in 10 patient PBMCS known to be infected with HIV-2
- 100% specificity
Conclusions

• Singleplex and Multiplex real-time PCR assays were developed that detect proviral DNA of HIV-2 subtypes A and B

• Additional validation of patient specimens is needed before implementing for clinical use
Acknowledgements

Chou-Pong Pau
Susan K. Wells
Ae Youngpairoj
Kelly A. Curtis
Silvina Masciotra
Donna Rudolph
Michele Owen