**Introduction**

Mother-to-child-transmission (MTCT) rate has decreased sharply in recent years in most of the sub-Saharan Africa, however, 220,000 children acquired HIV-1 in 2014. PCR detection of proviral DNA is the most sensitive method for early infant diagnosis (EID) of HIV-1 infection. Commercial kits are available but they have poor sensitivity with divergent non-B subtypes and high costs (≥30C per test) which limit their use in resource-limited settings. The HIV-1 epidemic in Angola is driven by highly divergent strains of all group M subtypes, except B, as well as multiple recombinant forms (CRFs and URFs) making EID a challenge in this setting. The aim of this study was to develop and validate a qualitative, inexpensive and sensitive "in-house" HIV-1 EID assay on heel prick dried blood spots (DBS) from infants of the Hospital da Divina Providência (HDP) in Luanda, Angola and determine the current HIV-1 MTCT rate in the Angolan Perinatal HIV Cohort (APEHC).

**Materials and Methods**

- The assay is a qualitative nested PCR based on new primers targeting the integrase (IN) gene of the most prevalent HIV-1 subtypes and recombinant forms found in Angola.
- One-hundred DBS from HIV-1-infected adults were used as positive controls; fifty DBS from HIV-1 seronegative healthy volunteers were used as negative controls.
- The analytical sensitivity was assessed with: 1) ACH-2 cells containing a single, integrated HIV-1 subtype B DNA copy per cell; 2) Recombinant plasmids containing HIV-1 IN (927pb) of subtypes A-J and CRF02_AG from Angolan and Portuguese clinical samples; the sequences were subtyped by Maximum Likelihood (ML) phylogenetic analysis with MEGAS.
- Plasmids and ACH-2 cells were diluted in HIV seronegative blood by 5 log_{10} serial dilutions and spotted in Whatman® Human ID blood stain cards.
- DNA was extracted from DBS using exon-10 resin and a fragment of IN (194 pb) was amplified by nested PCR. CCR5 gene was also amplified as an internal control.
- The clinical sensitivity was assessed using DBS from 126 HIV-1-exposed infants enrolled in the APEHC from HDP. The median age was 1 month and 50% were girls. Definitive diagnosis of HIV-1 infection was based on serology at month 12.

**Results**

Recombinant reference plasmids represent the complexity of the HIV-1 strains circulating in Luanda

- **Performance of the assay in DBS samples collected from HIV-1 infected adult patients**

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Sampling date</th>
<th>Country of Origin</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-0A90HDP116</td>
<td>2009</td>
<td>Angola (Luanda)</td>
<td>A1</td>
</tr>
<tr>
<td>2-0A90HDP157</td>
<td>2009</td>
<td>Angola (Luanda)</td>
<td>G</td>
</tr>
<tr>
<td>3-0A90HDP237</td>
<td>2009</td>
<td>Angola (Luanda)</td>
<td>PI</td>
</tr>
<tr>
<td>4-0A90HDP34</td>
<td>2009</td>
<td>Angola (Luanda)</td>
<td>C</td>
</tr>
<tr>
<td>5-0A90HDC251</td>
<td>1999</td>
<td>Angola (Luanda)</td>
<td>A1</td>
</tr>
<tr>
<td>6-G1P196</td>
<td>1998</td>
<td>Portugal (Lisbon)</td>
<td>CRF02_AG</td>
</tr>
<tr>
<td>7-07HDC290</td>
<td>1997</td>
<td>Angola (Luanda)</td>
<td>B</td>
</tr>
<tr>
<td>8-0A90HDC249</td>
<td>1997</td>
<td>Angola (Luanda)</td>
<td>A1</td>
</tr>
<tr>
<td>9-0A90HDC255</td>
<td>1999</td>
<td>Angola (Luanda)</td>
<td>G</td>
</tr>
</tbody>
</table>

Limit of detection of the assay is 4 HIV-1 DNA copies (in ACH-2 cells) and 1-10 copies based on the recombinants plasmids

EID in-house test

- **Undetectable viral load (<20 cop/mL)**
- **Viral load of 20-1,000 cop/mL**
- **Viral load of >1,000 cop/mL**

Positive controls (N=100) | Negative controls (N=50) | Total
---|---|---
Positive | 11 | 6 | 0 | 36
Negative | 66 | 7 | 1 | 124
Total | 77 | 16 | 7 | 160

% detection | 14.3 | 56.3 | 85.7 | 85

The probability of detecting HIV-1 proviral DNA with our EID test in the 100 seropositive and 50 seronegative samples tested was:

- 0% when the patients were not infected with HIV-1
- 14.3% when the patients had a plasma viral load <20 copies/mL
- 56.3% when the patients had a plasma viral load of 20-1,000 copies/mL
- 85.7% when the patients had a plasma viral load >1,000 copies/mL

**Discussion**

- **Negative results were obtained with all uninfected children** Specificity 100%
- **Positive PCR results were obtained with all infected children** Sensitivity 100%

**Conclusion**

- The DBS in-house EID assay has a remarkably low limit of detection with highly divergent viruses of all subtypes.
- The high clinical sensitivity and specificity makes its use suitable for complex HIV-1 epidemics for EID of HIV-1 infection.
- The low (2.4%) HIV-1 MTCT rate within the APEHC shows the importance of establishing PMTCT programs and also the high standards of health care provided at HDP.
- The exceedingly simplicity and low cost per test suggests that implementation of this assay in Angola and in other less-resourced countries will be possible allowing the early treatment of HIV-1-infected infants.

**Acknowledgements**

To the patients and the collaborating centers for their participation. This work was supported by Fundação para a Ciência e Tecnologia (FCT, Portugal) (projects: PTDC/SAU-EPI/115290/2009, PTDC/SAU-EPI/112240/2010, and VD/H/SAU/001/2011), part of the EDCTP programme supported by the European Union. Francisco Martin is supported by a FCT PhD fellowship (SFRH/BD/87488/2012)

**References**

- Martin F, Palladino C, Mateus R, Clemente S, Gomes D, Taveira N
- Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Portugal
- Hospital da Divina Providência (HDP), Luanda, Angola
- Centro Hospitalar de Lisboa Ocidental, E.P.E., Portugal
- Centro de Investigação Interdisciplinar Egesa Moniz, (CiiEM) Instituto Superior de Ciências da Saúde Egesa Moniz, Caparica, Portugal