Evaluation of an In-house Molecular HIV-1 Test to Assess Mother-to-Child HIV-1 Transmission in Angola (the APEHC Cohort)

P-B3

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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 (≈30€ 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

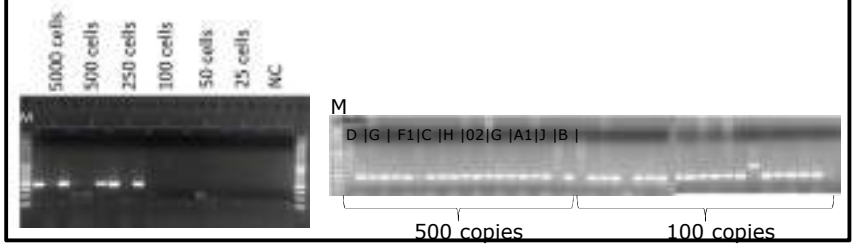
- The assay is a qualitative nested PCR based on new primers targeting the integrase (IN) gene of the most prevalent HIV-1 subtypes and recombinant forms found in Angola.
- One-hundred DBS from HIV-1-infected adults were used as positive controls; fifty DBS from HIV-1 seronegative healthy volunteers 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 MEGA6.
- 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 chelex-100 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

Isolate	Sampling date	Country of Origin (Province)	Genotype (IN)
1-09AOHDP110	2009	Angola (Luanda)	D
2-09AOHDP157	2009	Angola (Luanda)	G
3-09AOHDP237	2009	Angola (Luanda)	F1
4-09AOHDP34	2009	Angola (Luanda)	C
5-93AOHDC251	1993	Angola (Cabinda)	H
6-01PTHDECJN	1998	Portugal (Lisbon)	CRF02_AG
7-00PTHDEEBB	2000	Portugal (Lisbon)	G
8-93AOHDC249	1993	Angola (Cabinda)	A1
9-93AOHDC253	1993	Angola (Cabinda)	J

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



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

	Positive controls (N=100)			Negative controls (N=50)	Total
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	11	9	6	0	36
Negative	66	7	1	50	124
Total	77	16	7	50	160
% detection	14.3	56.3	85.7	0	-

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 of <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**

- **Negative results were obtained with all uninfected children_Specificity 100%**
- **Positive PCR results were obtained with all infected children_ Sensitivity 100%**

	APEHC infants Serological test (n=126)		
EID in-house test*	Positive	Negative	Total
Positive	3	0	3
Negative	0	123	123
Total	3	123	126

- **MTCT rate in the APEHC was 2.4% between January 2012 and October 2014.**
- **This low rate likely reflects the high standard of care of HIV infected children and mothers at HDP in Luanda**

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.**

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