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Short communication

Using a simplified human immunodeficiency virus type 1 p24 antigen assay to diagnose pediatric HIV-infection in Malawi

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ABSTRACT

Background: There is a worldwide need for a pediatric HIV-1 diagnostic test that has a high diagnostic accuracy, is technically simple and cost efficient. The Up24 HIV-1 assay, which requires both the HIV-1 p24 ELISA and the ELAST signal amplification kit, has previously been shown to be a robust tool to diagnose pediatric HIV-1 from dried whole blood spots (DBS) (Cachafeiro et al., JCM 2009;47:459–62[13]). In order to make the assay more accessible to a resource-limited clinical setting, we eliminated the ELAST system, which simplified the Up24 assay, reduced its cost, and tested the accuracy of the modified assay in a rural Malawian hospital.
Objectives: In this proof of concept study, we tested the ability of a simplified Up24 antigen assay, without ELAST, to detect HIV-1 on DBS obtained via heel prick from 6-week-old Malawian infants.
Study design: A case–control study of DBS collected from 113 HIV-infected and 109 HIV-negative infants, using the HIV-1 DNA PCR assay as the reference standard.
Results: The simplified HIV-1 Up24 assay had a sensitivity and specificity of 84% and 98%, respectively. When HIV-1 prevalence is 15%, the positive- and negative-predictive values are 89% and 97%, respectively.
Conclusion: The simplified Up24 assay has a good positive- and a robust negative-predictive values, is easier to perform and has a reduced cost compared to both HIV DNA PCR and Up24 assays. With additional testing, the simplified Up24 assay has the potential to increase global access to pediatric HIV-1 diagnostics.

1. Background

Despite the evidence that timely antiretroviral treatment (ART) in HIV-infected infants reduced death by approximately 75%,[1] only 35% of the HIV-infected children in sub-Saharan Africa (SSA) were receiving ART.[2] The lack of affordable, accessible and accurate HIV-1 infant diagnostic tests is one of the major bottlenecks limiting timely access to ART among children in SSA. In Malawi, it is estimated that 85,488 HIV-infected mothers deliver annually,[3] but in 2009, only three public hospitals located in urban districts in Malawi had capacity to perform HIV DNA PCR. However, in an attempt to increase coverage of infant HIV testing, the Early Infant Diagnosis (EID) Programme collects dry blood samples for centralized PCR testing from 41 of the 425 health facilities delivering prevention of HIV mother-to-child transmission (PMTCT) services.[3,4] One of the EID testing facilities is based at Queen Elizabeth Central Hospital (QECH) located in the city of Blantyre (population ~660,000[5]); QECH is the only public health facility in the southern region of Malawi (population ~5.9 million[3]) with the capacity to conduct HIV DNA PCR. In 2009, QECH performed 8642 tests of which 2297 were from Blantyre city (Hannania Moyo, EID Senior Laboratory Technician, QECH, *personal communication*). Thus, it is estimated that that only ~11% of the Malawian children

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have ready access to HIV DNA PCR. This system poses logistical problems and ultimately limits coverage of ART in children.

Several published studies have demonstrated the ability of HIV-1 p24 antigen detection assays to diagnose pediatric HIV-infection in plasma[6–12] and dried blood spots from venous blood.[13–16] Importantly, the Up24 assay has been validated against HIV-1 subtype C, which comprises >95% of all HIV-infections in Malawi[7,11,12,15–19] and it can be easily adapted to a resource-limited setting.[20] Up24 assays contain two modules, a p24 ELISA and ELAST (ELISA signal amplification kit), and two reagents comprise the ELAST kit: the Biotynil Tyramide and the ELAST HRP, and collectively, the ELAST reagents increase the sensitivity of the p24 ELISA. Before using the ELAST kit, the Biotynil Tyramide needs a single step dilution, and the ELAST HRP needs a two-step serial dilution, which must be experimentally determined for each lot. As a result, in addition to increasing the cost per test of the Up24 assay, the ELAST module adds technical complexity, labor time, and it increases assay variability.

2. Objective

In this proof of concept study, we tested the ability of a simplified Up24 antigen assay, without ELAST, to detect HIV-1 on DBS obtained via heel prick from 6-week-old Malawian infants.

3. Study design

IRB approval was obtained from the Malawi College of Medicine Research Ethics Committee and the Ohio State University. Additional permission was obtained from the Malawi Ministry of Health to use archived, de-identified DBS samples from infants who attended the Thyolo District Hospital. *A priori* sample size calculations indicated that 136 HIV-infected and 118 HIV-negative samples would give an 80% power at the 0.05 significance level to detect differences from the HIV-1 DNA PCR test when the sensitivity or specificity of the HIV-1 Up24 test is below 90%. Infant DBS samples from the Thyolo District Hospital are currently tested for HIV-1 at Queen Elizabeth Central Hospital (referral hospital in Southern Malawi) with the Roche Amplicor HIV-1 DNA PCR assay when the infant is 6 and 10 weeks old, and again 6 weeks after breastfeeding has stopped. The DBS cards were stored in ziploc bags containing dessicant and a humidity detector card, and the samples were excluded if the humidity detector card showed abnormalities. DBS samples from HIV-exposed 6-week-old infants who had an unequivocal negative or positive DNA PCR result were selected. Three 6 mm punches from a single DBS were collected and transported to Thyolo District Hospital for HIV-1 Up24 antigen testing by a Malawian laboratory technician who was masked to the HIV-1 DNA PCR results. Alliance® HIV-I p24 ELISA kits (Perkin Elmer) were purchased from Separation Scientific SA (Honeydew, South Africa) and Up24 assays were performed as reported by Cachafeiro et al.,[13] with the modifications described in Table 1. When the simplified Up24 ELISA and HIV DNA PCR were discordant at any of the cutoff values, a third DBS was sent to the UNC Core Retrovirology lab for an additional HIV-1 DNA PCR assay (Roche HIV-1 DNA PCR v1.5). The tiebreaker results from UNC lab were considered the true HIV-1 status. Sensitivity, specificity, and likelihood ratios[22] were determined from contingency tables and positive- and negative-predictive values (PPV and NPV, respectively) for each proposed cutoff were calculated according to the following equations:

$$\mathrm{PPV} = \frac{\mathrm{sen} \times p}{(\mathrm{sen} \times p) + ([1 - \mathrm{spec}] \times [1 - p])},$$

$$\mathrm{NPV} = \frac{\mathrm{spec} \times (1 - p)}{[\mathrm{spec} \times (1 - p)] + [(1 - \mathrm{sen}) \times p]}$$

Table 1
Differences in assay conditions between this publication and Cachafeiro et al., JCM (2009). DBS: dried blood spot; SDS: sodium dodecyl sulfate; HRP: horse radish peroxidase; ELAST: ELISA amplification system.

Characteristic	Cachafeiro et al., JCM (2009)[13]	This publication
Sample	Two disks punched from DBS	Three disks punched from DBS
Specimen preparation buffer	No SDS	0.3% SDS
Volume of elution buffer	250 μl	275 μl
Input volume of sample into wells	250 μl	270 μl
Detector antibody incubation	1 h	2 h
HRP incubation	15 min	90 min
ELAST (signal amplification)	Present	Absent

where "sen" equals assay sensitivity, "spec" equals assay specificity, and "*p*" equals the theoretical prevalence of the HIV-1 in the pediatric population.

4. Results

A total of 222 DBS samples from 113 HIV-infected and 109 HIV-negative infants were assayed with the simplified HIV-1 p24 ELISA. The modifications listed in Table 1 were implemented to compensate for the decreased sensitivity of the Up24 assay in the absence of ELAST amplification step. In order to optimize the sensitivity and specificity of the simplified Up24 assay, several assay cutoffs were evaluated, including the following: the average of the negative controls (NC) multiplied by two (2NC); NC plus 0.05 OD units (NC + 0.05); or NC + 2 standard deviations (SD), NC + 3SD, or NC + 5SD. The data in Table 2A indicate that NC + 3SD had the highest sensitivity and lowest specificity, while NC + 5SD had the highest specificity, an intermediate sensitivity, and the highest positive likelihood ratio (Table 2B).

In order to explore the ability of the simplified Up24 assay to correctly diagnose pediatric HIV-1 infection, we calculated the positive- and negative-predictive values for the five p24 assay cutoff-definitions over a plausible range of HIV-1 prevalence values (1–25%). Fig. 1 (top) shows that NC + 5SD had the most robust PPV over the entire prevalence range, with a PPV of 89% at 15% HIV-prevalence. Fig. 1 (bottom) shows that all cutoff values had robust NPVs, with NC + 5SD falling just slightly below the best cutoff values, with a NPV of 97% at 15% HIV-prevalence.

5. Discussion

This study was done in a setting where overall rate of HIV-1 mother-to-child transmission (MTCT) without any preventive intervention was previously estimated at approximately 28%.[23,24] With the roll-out of prevention of MTCT programs, the MTCT prevalence is likely to have decreased to approximately 15%.[25,26] Using the NC + 5SD cutoff, the PPV was between 84% and 89% at a theoretical HIV-1 prevalence of 10–15%; these data, in combination with the high positive likelihood ratio (45.8), demonstrate that the simplified Up24 antigen detection assay may be acceptable in resource-limited settings. However, a confirmatory test may still be required before starting HIV-infected infants on ART, especially if the estimated HIV-1 prevalence falls below 10%. The high NPV (>96%) at a pediatric HIV-1 prevalence of less than 15% and the low negative likelihood ratio (0.16) demonstrates that a negative result is very good at excluding infant HIV-infection. This means