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High Sensitivity and Specificity of the Cepheid Xpert® HIV-1 Qualitative Point-of-Care Test among Newborns in Botswana

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Abstract

Background—HIV point-of-care (POC) testing allows for early infant HIV diagnosis and treatment, but POC accuracy at birth and in the setting of antiretroviral prophylaxis for the prevention of mother-to-child HIV transmission (MTCT) is unknown.

Methods—We evaluated the Cepheid Xpert® HIV-1 Qual POC test against the Roche Taqman HIV-1 PCR platform using dried blood spots from 15 HIV-infected and 75 HIV-exposed uninfected newborns. These infants were screened for HIV at <96 hours of life at 5 hospital maternity wards in Botswana; all infants received post-exposure antiretroviral prophylaxis (PEP) and most mothers received 3-drug antiretroviral therapy in pregnancy and at delivery.

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Results—Fourteen of 15 PCR positive samples tested positive by Cepheid POC, yielding a sensitivity of 93.3% (95%CI: 68.1–99.8%). Baseline viral load among positive infants ranged from <40 to >10,000,000 copies/ml, with a median of 2,403 copies/mL. The HIV RNA for the infant with false negative POC testing was 1,661 copies/mL. Of note, two infants with low HIV RNA (< 40 copies/mL and 272 copies/ml) were correctly identified as HIV positive by Cepheid POC. All of the 75 PCR-negative samples tested negative by Cepheid POC, yielding a specificity of 100% (95% CI: 96.1 –100%).

Discussion—Our study demonstrates high sensitivity and specificity for the Cepheid POC assay in the first week of life despite early infection and antiretroviral prophylaxis. This platform may be a useful approach for adding early infant HIV diagnosis to current testing programs.

Keywords

HIV early infant diagnosis; point-of-care testing; Cepheid Xpert®; HIV-1 Qual; Botswana

BACKGROUND

Early infant HIV treatment may reduce viral reservoir and improve long-term treatment outcomes, but diagnosing infants within the first few days of life remains a logistic challenge.^{1,2} Currently, early infant diagnosis requires laboratory equipment and infrastructure as well as skilled personnel.^{3,4} The turnaround time of laboratory tests is often slow, and especially in low-resource settings, patients are frequently lost to follow-up.⁵ To address these issues, the World Health Organization has encouraged the study of platforms for point-of-care (POC) HIV testing at birth.⁶

The Cepheid Xpert® HIV-1 Qualitative (Qual) POC test (WHO-PQ and CE-IVD marked, but not FDA approved) is a qualitative *in vitro* diagnostic test designed to detect HIV-1 total nucleic acids on GeneXpert® Systems using human whole blood and dried blood spots (DBS) in a simple automated manner.⁷ Previous studies have shown good sensitivity and specificity for this platform among adults and older children^{8–12}; however, the utility of this test has not been evaluated during the first week of life, in the setting of extensive antiretroviral prophylaxis for the prevention of mother-to-child HIV transmission (PMTCT).

We evaluated the sensitivity and specificity of the Cepheid Xpert® HIV-1 Qual POC test as compared with the “gold standard” Roche Taqman HIV PCR platform to diagnose infant HIV infection in the first 96 hours of life in Botswana.

METHODS

Study Setting

The study was nested within the ongoing Botswana-Harvard Partnership (BHP) Early Infant Treatment (EIT) Study, conducted at 5 public hospital maternity wards and surrounding maternity clinics in the Gaborone and Francistown regions of Botswana. Laboratory testing was performed at the Botswana-Harvard HIV Reference Laboratory (BHHRL) in Gaborone.

Study Population

The EIT Study consents HIV-infected women for early HIV PCR screening of their newborn infants as soon as possible after birth, and infants found to be HIV-infected are offered enrollment and immediate antiretroviral treatment (ART). Eligibility for the EIT Study included maternal Botswana citizenship and age ≥ 18 years, gestational age at birth ≥ 35 weeks, birth weight ≥ 2000 grams, and infant < 96 hours of age. One Munktel TFN dried blood spot (DBS) card was collected from screened infants via heel stick at each of the health facilities, with collection of 5 spots per infant.

Sample Storage

DBS samples were initially stored at room temperature prior to Roche testing, then transferred to -80°C freezers until additional spots were tested using the Cepheid assay.

Sampling Strategy

All samples that tested positive by Roche were tested by the Cepheid platform. A subset of 75 samples that tested negative by Roche were also systematically selected for testing by Cepheid. To identify a representative group of PCR-negative samples, we retrospectively identified a similar proportion of negative samples from each collection site and from each month of the study, and we then selected samples at random within these groupings.

Laboratory Assays

Roche Cobas Ampliprep/Taqman (CAP/CTM) HIV-1 Testing—Upon arrival at BHHRL, one DBS circle per card was tested for HIV by Roche. The Roche system (Roche Diagnostics, Mannheim, Germany) is based on three major processes: 1) DNA extraction, 2) reverse transcription of target RNA to generate complementary DNA, and 3) PCR amplification of target DNA and cDNA and detection. For DBS specimens, a manual pre-extraction step is required, which involves incubating the DBS sample with 1100 µl of sample pre-extraction buffer (SPEX) at 56°C for 10 minutes in a thermo-mixer vortex at 1000 RPM. Testing was conducted as per the manufacturer's instructions.¹³ All positive tests were confirmed by repeat DBS sampling of the infant and a repeat HIV PCR. HIV RNA testing for HIV-positive mothers and infants was performed on separate plasma specimens using the automated Abbot HIV-1 RNA assay (Abbot Molecular Inc., Des Plaines, IL, USA).¹⁴

Cepheid Xpert HIV-1 Qual Test—For the Cepheid assay (Cepheid, Sunnyvale, CA, United States), one 12 mm diameter DBS was excised from the filter paper and placed in a sample reagent bottle with 1.0 ml of lysis buffer. This vial was incubated in a ThermoMixer for 15 minutes at 56°C while rotating at 500 RPM. All the liquid from the vial was then placed into the Xpert[®] cartridge sample chamber, loaded into the GeneXpert[®] instrument, and tested. Testing was conducted as per the manufacturer's instructions.⁷

Statistical Analysis

We evaluated the sensitivity and specificity of the Cepheid assay as compared with the Roche platform. The 95% confidence intervals were calculated utilizing the exact binomial

distribution. For our positive sample size of 15 samples, the lower bound of the 95% confidence interval was 82% for exact concordance between tests.

RESULTS

In total, 3,039 HIV-exposed infants were screened at birth by the EIT Study from April 2015 through July 2016, and 15 (0.5%) were identified as HIV positive by Roche testing. All transmitting mothers had detectable HIV RNA at delivery (except for one mother who did not enroll in the treatment study and whose level is therefore unknown). Eleven (73%) of 15 transmitting mothers were on ART, and maternal HIV RNA levels ranged from as low as 67 copies/mL to as high as 125,093 copies/mL (Table 1). Median maternal CD4 cell count at delivery was 300 cells/mm³ (range 79 to 804 cells/mm³). The 15 PCR positive infants were screened at a median age of 19.2 hours after birth (range 6.6 to 44.8 hours). Per Botswana protocol, all HIV-exposed infants receive zidovudine (ZDV) and a single dose of nevirapine (sdNVP) at birth, which was documented to occur at a median of 5.6 hours after birth (except in 2 children, who received ZDV but did not have receipt of sdNVP documented). In 10 (71%) of 14 children, postexposure prophylaxis was administered prior to screening. Baseline HIV RNA for the HIV positive infants ranged from <40 copies/ml to >10,000,000 copies/ml, with a median of 2,403 copies/ml.

The 75 negative infants were screened at a median of Day 1 of life (range 0 to 3 days), which did not differ from transmitting pairs (exact hour of life was not available for the HIV negative group). Routine HIV RNA and CD4 testing at delivery was not available for mothers of HIV negative infants.

Fourteen of the 15 DNA PCR positive samples tested positive by Cepheid POC, yielding a sensitivity of 93.3% (95% CI: 68.1 – 99.8%). All of the 75 PCR-negative samples tested negative by Cepheid POC, yielding a specificity of 100% (95% CI: 96.1 – 100%).

The HIV RNA level for the infant with false negative POC testing was 1,661 copies/mL. Of note, one infant who was identified as “low-positive” by initial and repeat DNA PCR, and who had HIV RNA < 40 copies/mL at initial HIV RNA testing, was correctly identified as HIV positive by Cepheid POC testing. Another sample with a relatively low HIV RNA of 272 copies/mL was also identified as positive by Cepheid POC testing.

DISCUSSION

We evaluated the Cepheid POC test on DBS samples from infants within 48 hours of birth and found 93.3% sensitivity and 100% specificity. These results are similar to sensitivity and specificity testing that has been performed later in life,^{8–12} and do not appear to be impacted by concurrent use of ZDV and sdNVP infant prophylaxis immediately after birth.

Our results are consistent with reports of high sensitivity and specificity with the Cepheid platform among older children. A study in Zimbabwean infants with a median age of 6.9 weeks revealed a sensitivity of 97% and specificity of 100%, while another study in Malawi of infants at 1 and 6 months of age revealed 100% sensitivity and specificity.^{10,11}

Nevertheless, our slightly reduced sensitivity at birth to 93.3% is consistent with similarly reduced sensitivities of other POC platforms in children less than 3 days of age.^{15,16}

The extension of sensitivity and specificity results down to the first week of life in our study is important for two reasons: 1) this is the ideal time to use a POC test to rapidly identify and treat HIV positive children as early as possible and before they are lost to follow-up, and 2) it was previously unknown how this platform would perform in the setting of potentially lower viral loads either from very early infection or in the presence of two prophylactic antiretrovirals (ZDV and NVP) in the infant's circulation.

An interesting finding in our study was that the Cepheid platform detected two positive infants with very low HIV RNA levels (quantified within a few days of the screening test). One child had an HIV RNA of 272 copies/mL, and the other < 40 copies/mL. The Cepheid package insert reports a limit of detection of 668 copies/mL for DBS.⁷

The one discordant sample in our study was from an infant with a positive DNA PCR and an HIV RNA level of 1,661 copies/mL. The Cepheid assay for this sample did not detect any DNA; it was not a case where some DNA was detected but did not make the cycle threshold (Ct) cut-off, which is 42 for the Cepheid platform. Other platforms, such as the Alere q POC test, have shown that false negatives generally have higher Ct values than true positives with a possible rationale being lower levels of circulating virus during early infection.^{15,16}

The reason for this one false negative remains unknown. Of note, this was one of four children with a delay in the use of prophylactic ZDV and NVP until after screening, and therefore an antiretroviral effect was not an explanation. Possibilities include an insufficient DBS sample or interference from a high CD4 count. Furthermore, though greater than 99% of currently circulating HIV-1 in Botswana are subtype C viruses (BHP, unpublished data, February 2017), this child may have had a different subtype undetectable by the Cepheid platform. However, this is unlikely because the analytical reactivity of the Cepheid assay includes thirteen isolates representing various subtypes of HIV-1. More importantly, a DBS sample from the same infant taken two days later was positive upon testing with the Cepheid platform.

The main limitation of our study was the small number of positive samples available, leading to a wide 95% confidence limit for the sensitivity of the birth POC testing. The possibility of sensitivity less than approximately 90% supports the need for additional studies among newborns to confirm our initial findings. Routine use of this assay outside of the study setting may lead to different results. An additional limitation of our study was that our EIT screening strategy excluded infants < 35 weeks of gestational age or < 2000 grams. However, we know of no biological reason why such infants would differ in test responses from those in this study.

The World Health Organization has prioritized the development of POC platforms for early infant diagnosis to improve treatment outcomes and prevent loss to follow-up.⁶ In 2015, the Botswana Ministry of Health reported that only 68% of all HIV-exposed infants were tested within 2 months after birth, and that nearly half of those tested did not receive the result.⁵ Our study provides support for use of Cepheid POC testing as an initial HIV screening test

to address these concerns. The testing can be performed in under 2 hours, requires minimal training, and can utilize either whole blood or DBS. The cost per test is currently about 30% less than DNA PCR testing; at the time of testing, each Cepheid cartridge was priced at 17.95 USD, whereas the supplier charged about 25 USD for each Taqman test. However, the added cost of additional POC testing at birth may be excessive for some PMTCT programs; thus, programs may choose to reduce costs by adding birth screening only for high-risk infants.

In conclusion, the Cepheid POC testing platform may be a useful approach for initial birth screening of HIV-exposed infants, even in the setting of extensive infant antiretroviral prophylaxis. Whether used for all HIV-exposed infants or selectively, POC testing at birth complements 6-week HIV DNA PCR testing, and allows the majority of *in utero* infected infants to start ART in the first week of life. This approach may be critical for reducing loss to follow-up, and for improving long-term treatment outcomes of HIV-infected children.

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Table 1

Maternal Characteristics by Transmitters/Non-Transmitters

	Transmitters (n=15) [*]	Non-Transmitters (n=75) [‡]
Mean Birth Weight, kg	3.0	3.0
Mean Gestational Age, weeks	38	38
Mean Maternal Age, years	26.7	30
Median Maternal CD4 (range), cells/mm ³ [∞]	300 (79, 804)	N/A
Median Maternal VL (range), copies/mL [∞]	10,321 (67, 125093)	N/A
Maternal ART Regimen		
Efavirenz/Tenofovir/Emtricitabine	9	37
Nevirapine/Lamivudine/Zidovudine	0	6
Lopinavir/ritonavir/Tenofovir/Emtricitabine	1	1
Abacavir/Lamivudine/Lopinavir/ritonavir	0	1
Nevirapine/Tenofovir/Emtricitabine	0	3
Tenofovir/Emtricitabine/Dolutegravir	1	0
Unspecified	0	1
None	4	0

^{*} Birth weight, gestational age, maternal CD4, and maternal VL not available for 1 out of 15 transmitters.

[‡] Birth weight, gestational age, maternal age, and ART regimen available for 49 of 75 non-transmitters.

[∞] CD4 and VL were not available for non-transmitters.

Table 2

Infant Characteristics and Cepheid POC Result for HIV-Positive Infants

Baby	Child Baseline CD4	Child Baseline VL	Age at first AZT/NVP dose (Hours) *	Age at Screening (Hours)	Point-of-Care Result
A	5,159	1,661	114.5	18.5	Negative
B	1,995	17,244	25.3	13.6	Positive
C	1,854	1,636	21.3	25.5	Positive
D	1,021	1,111,950	7.0	15.9	Positive
E	1,556	1,375	2.4	9.8	Positive
F	1,748	>10,000,000	29.9	6.6	Positive
G	1,634	<40	0.2 [‡]	39.2	Positive
H	1,950	60,247	3.5	19.9	Positive
I	1,671	3,145	4.2	40.1	Positive
J	2,616	1,005	1.6	44.8	Positive
K	2,177	272	23.0	36.5	Positive
L	1,066	1,314	0.2 ^{‡∞}	32.0	Positive
M	1,469	23,686	0.7 [∞]	6.6	Positive
N	1,601	20,291	27.3	14.2	Positive
O	---	---	---	---	Positive
Median	1,709.5	2,403	5.6	19.2	

* Single-dose NVP (10–15 mg PO) and daily AZT (4mg/kg PO twice daily)

[‡]Time estimated based on delivery[∞] Infant did not receive NVP