Polymerase Chain Reaction for Diagnosis of Human Immunodeficiency Virus Infection in Infancy in Low Resource Settings

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Background: Diagnosis of human immunodeficiency virus (HIV) is essential for accessing treatment. Current HIV diagnostic protocols for infants require adaptation and validation before they can be implemented in the developing world. The timing and type of HIV assays will be dictated by country-specific circumstances and experience from similar settings. The performance of an HIV-1 DNA polymerase chain reaction (PCR) test, and in particular a single test at 6 weeks of age, in diagnosing HIV subtype C infection acquired in utero or peripartum was assessed.

Methods: A retrospective review of 1825 Amplicor HIV-1 DNA PCR version 1.5 tests performed between 2000 and 2004 in 2 laboratories in Johannesburg, South Africa on 769 effectively non-breast-fed infants from 3 clinically well characterized cohorts was undertaken. The HIV status of each infant was used as the standard against which the HIV PCR results were compared.

Results: The overall sensitivity and specificity of the HIV PCR test were 99.3 and 99.5% respectively. A single test was 98.8% sensitive and 99.4% specific in the 627 infants tested at 6 weeks of age (58 HIV-infected and 569 HIV-uninfected). Repeat testing of all positive HIV PCR tests minimized false positive results.

Conclusions: In resource-poor settings where HIV PCR testing in an environment of good laboratory practice is feasible, a single 6-week HIV DNA PCR test can increase identification of HIV-infected children substantially from current levels. Further operational research on how best to implement and monitor such a diagnostic protocol in specific local settings, especially in breast-fed infants, is necessary.

Key Words: human immunodeficiency virus, infant, diagnosis, polymerase chain reaction, developing country

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Now that human immunodeficiency virus (HIV) infection is being treated in resource-poor settings, the challenge is to adapt diagnostic, drug and monitoring protocols practiced in the developed world to conditions that prevail in the diverse settings of the developing world. HIV DNA polymerase chain reaction (PCR) testing for diagnosis of HIV infection in infancy has fundamentally been unavailable in the developing world until recently. HIV-exposed infants were first tested at 12 months of age with an HIV enzyme-linked immunosorbent assay (ELISA) test.[1] This practice led to high lost to follow-up rates exceeding 80% in Johannesburg, South Africa, resulting in an inability to identify and treat HIV-infected children.[1–3]

The Amplicor HIV-1 qualitative DNA PCR assay version 1.0 (Roche Diagnostic Systems, Inc., Branchburg, NJ) is considered a simplified, standardized format for PCR testing in the clinical laboratory.[4,5] Optimized to detect subtype B virus, the assay has been documented to yield false negative results as high as 25% of all non-B subtype samples tested.[4,6–12] This is problematic because most infected children reside in the developing world and are infected with HIV subtypes other than subtype B.[13]

Various methods to improve the sensitivity of the version 1.0 assay have been attempted,[5,10–12,14] but a definitive longer term solution is presented by the Amplicor HIV-1 DNA PCR version 1.5 assay (Roche Diagnostics, Inc., Alameda, CA) with universal primers SSK145 and SKCC1B for detection of all HIV subtypes.[6,8] This assay has been used for HIV diagnosis in infancy in South Africa and elsewhere since 2000, even though literature validating its use for this indication is scarce.[8,9,15–17] The assay was 100% sensitive and specific in detecting subtype C in 202 Zimbabwean adults and accurately detected subtypes A, C and D in 161 pregnant Tanzanian women.[8,9] The version 1.5 assay performed on dried blood spots yielded a sensitivity of 100% and a specificity of 98% from 139 children in Rwanda where subtype A is prevalent.[16] On dried blood spots from 288 six-week-old infants perinatally exposed to HIV subtype C, the assay had a sensitivity of 100% and a specificity of 99.6%.[17]

Because no definitive diagnostic test for HIV infection at birth exists, it is impossible to definitively distinguish transmission occurring in utero or at delivery from postnatal transmission.[18] HIV DNA PCR detects ~50% of vertically acquired HIV infection at birth and virtually 100% at 6 weeks

of age in non-breast-fed infants.[19–21] Six weeks is a useful time at which to test infants because it coincides with their first immunization visit after birth. In the developing world, the optimal timing and number of PCR assays required to diagnose HIV infection in infants is a balance between cost and accuracy. This report details the performance of the Amplicor HIV-1 qualitative (DNA) PCR version 1.5 assay in 2 laboratories in Johannesburg, South Africa when compared with the HIV status of 739 infants and demonstrates that a single test at 6 weeks of age is highly accurate in diagnosing HIV subtype C infection acquired in utero or peripartum.

METHODS

The performance of the HIV DNA PCR version 1.5 assay was studied in 3 cohorts of infants who had their HIV status determined prospectively according to current diagnostic guidelines.[22] All specimens were collected with informed consent under protocols approved by the Human Research Ethics Committee of the University of the Witwatersrand. The samples were processed in 2 routine HIV diagnostic laboratories in Johannesburg, South Africa at Johannesburg Hospital (JHB) and the National Institute for Communicable Diseases (NICD). Both form part of the National Health Laboratory Service and are affiliated with the University of the Witwatersrand. A retrospective review of the HIV DNA PCR results from both laboratories was performed in relation to 3 cohorts of infants with complete clinical data.

Cohort A: Infant Diagnostic Study. A cohort of 301 HIV-exposed infants attending the Prevention of Mother to Child Transmission (PMTCT) clinic in 2002 at Coronation Women and Children's Hospital was enrolled in a study to determine an affordable, accurate HIV diagnostic protocol. Study visits were at 6 weeks and 3, 4, 7 and 12 months of age, at which time age-appropriate HIV testing and clinical examinations were conducted.[3] HIV DNA PCR testing was performed prospectively at 6 weeks and 3 and 4 months of age. The 4-month PCR was performed only in instances where clinical features were discrepant with earlier PCR results. To demonstrate seroreversion, HIV ELISA tests were performed at 12 months of age. For infants with negative HIV PCR results who had not seroreverted by 12 months of age, an additional PCR was performed on stored samples at 12 months of age. If no stored sample was available at 12 months of age or if the infants failed to attend their 12-month follow-up, the additional PCR test was performed with stored plasma from the 7-month visit. The policy of the JHB laboratory is to check every positive HIV DNA PCR test result before releasing it. Tests are not run in duplicate. At 6 weeks of age, the cohort also underwent HIV-1 RNA PCR (Roche Amplicor Monitor assay version 1.5; Roche Diagnostic Systems, Inc.) testing on liquid blood and HIV DNA PCR testing on dried blood spots.[17]

Cohort B: Children's Homes Outreach Medical Program (CHOMP). HIV testing was conducted on abandoned children in residential care facilities to establish their HIV status as a component of their permanency planning in an operational research project aimed at improving the care of orphaned children. Although HIV DNA PCR testing is not advocated in South Africa unless HIV exposure had been documented, some children underwent simultaneous HIV ELISA and DNA PCR testing.[23] A negative HIV ELISA test, corroborated by a clinical examination to exclude signs of HIV, determined an HIV-negative status.[24] All PCR testing was conducted in the same laboratory as cohort A.

Cohort C: Infant Feeding Studies. A total of 443 infants born to HIV-infected women who had elected not to breast-feed were enrolled in 3 feeding studies between 2000 and 2004 by the Department of Pediatrics, University of the Witwatersrand in Johannesburg, South Africa. Infants were randomized to various milk formulas within the first week after birth and followed to 6 months of age. The main outcome measures of these studies were growth and tolerance of the formula feedings. All infants underwent HIV DNA PCR testing at 6 weeks with the second test done between 4 and 6 months of age, complying with the Centers for Disease Control and Prevention (CDC) guidelines for determining the HIV status of exposed infants.[22] If the 2 HIV PCR tests were discrepant, a third sample was submitted for HIV DNA and RNA PCR testing, and HIV RNA PCR tests were performed on stored samples where these were available. The HIV DNA PCR tests were performed at the NICD laboratory by testing all samples in duplicate on the same plate.

RESULTS

Cohort A: Infant Diagnostic Study. Single dose nevirapine therapy was administered to 97% of the mother-infant pairs in this cohort, only 2% of infants were breast-fed and the HIV transmission rate was <9%.[3] The HIV status of 26 HIV-infected and 245 HIV-uninfected infants could be determined according to CDC guidelines to serve as the standard against which the PCR test results could be assessed (Table 1).[22] In 30 (10%) cases, the HIV status could not be determined according to CDC standards because most of these infants were lost to follow-up. In these instances, the 6-week HIV RNA PCR and dried blood spot (DBS) HIV DNA PCR results were used as standards for comparison. HIV DNA PCR tests yielded concordant results at each age for every child except for a single false positive result at 6 weeks of age

TABLE 1. Determination of HIV Infection Status of 301 Infants in Cohort A

	HIV-Infected	HIV-Uninfected
≥2 positive PCR tests	25	
1 positive PCR test at 6 wk of age	1*	
2 negative PCR tests and seroreversion at 12 mo of age		124
≥2 negative PCR tests (final PCR at ≥4 mo of age)		121
2 negative PCR tests at 6 wk and 3 mo of age		24†
1 negative PCR test at 6 wk of age		6‡
Total no. of infants tested	26	275

*The child died before 3 mo of age but had clinical signs of HIV, and at 6 wk the HIV RNA PCR measured >750,000 copies per mL and the DBS DNA PCR was positive.

†All infants with 2 negative PCR tests had either undetectable viral loads and or negative DBS DNA PCR tests at 6 weeks.

‡All infants with a single PCR result had undetectable viral loads and negative DBS HIV PCR results.

TABLE 2. HIV DNA PCR Results According to the HIV Infection Status of 326 Infants in Cohort C

	HIV-Infected	HIV-Uninfected
2 negative PCR tests at 6 wk and 4–6 mo of age		287
2 positive PCR tests at 6 wk and 4–6 mo of age	31	
Negative PCR at 6 wk; positive PCR at 4–6 mo	1*	4†
Positive PCR at 6 wk; negative PCR at 4–6 mo		3†
Total no. of infants tested	32	294

*One false negative result.
†Seven false positive results.

in an HIV-uninfected infant that repeatedly tested negative on multiple subsequent checks of the same sample. Repeat analysis of 2 equivocal HIV DNA PCR tests at 3 months of age and 1 at 6 weeks of age yielded negative results consistent with the HIV status of the respective infants. All 6-week HIV RNA PCR results (n = 266 negative and n = 26 positive) concurred with the HIV status of the infants except for 1 low false positive result of 109 copies/mL, a finding that has previously been documented.[12] The 288 DBS DNA PCR results yielded a single false positive result in an HIV-uninfected infant, which also tested negative on multiple checks of the same sample.[17] No false negative results were obtained.

Cohort B: Children's Homes Outreach Medical Program (CHOMP). Between 2001 and 2004, 142 children with negative HIV ELISA tests (AxSYM System; Abbott) had HIV DNA PCR tests performed simultaneously which were all negative. Their estimated ages, available for 121 (85%) children, ranged from younger than 1 month to 26 months with a median of 2.8 months. Although perinatal HIV exposure cannot be excluded in the older children, it is likely that the 94 infants (66%) tested before 6 months of age were never exposed.

Cohort C: Infant Feeding Studies. Single dose nevirapine therapy to mother and infant became routine practice after the first year of the feeding studies. As a result, only some of the initial enrollees received prophylaxis. The overall HIV transmission rate of the cohort was 10.8%. The HIV status of 326 (74%) infants was determined on the basis of at least 2 concordant HIV DNA PCR results. The remaining infants were not followed long enough for their HIV status to be determined. Of those followed up, 294 were HIV-uninfected and 32 were HIV-infected (Table 2). Five infants whose initial test was negative at 6 weeks of age tested positive at 4–6 months, but only 1 was proved to be HIV-infected on subsequent testing. A further 3 infants tested PCR-positive at 6 weeks with subsequent negative tests and were all shown to be HIV-uninfected. Thus in this cohort 7 false positive PCR results were obtained, and 1 HIV-infected infant had a negative DNA PCR test at 6 weeks. It was not possible to determine conclusively the reason for this false negative result because no 6-week sample was available for further testing. Possible reasons for the negative PCR result at 6 weeks include a technical or human error, that the PCR failed to detect a peripartum infection or that the infant was infected postnatally. However, no known risk factors for postpartum HIV infection could be elicited.

The HIV status of 739 (96%) infants, determined according to developed world criteria, was used as the standard against which the performance of the HIV DNA PCR version 1.5 assay was assessed. In the remaining 30 HIV-uninfected infants, 1 HIV PCR result or more and clinical information were used for corroboration (Table 1). When the infant was 6 weeks of age, the HIV DNA PCR version 1.5 assay was 98.8% sensitive in that it detected all but 1 of 58 HIV-infected children (Table 3). Five false positive results were recorded in 832 tests performed on 569 HIV-uninfected 6-week-old infants yielding a specificity of 99.4%. The standard practice of the JHB laboratory, to repeat all positive PCR results on the same sample, resulted in the 2 false positive results reported being detected before the result was issued. The sensitivity and specificity for the 1173 (64%) assays performed in this laboratory was effectively 100% (Table 3).

DISCUSSION

This study is the first to assess the performance of the HIV DNA PCR version 1.5 assay in infant diagnosis and at 6 weeks of age in particular, in comparison with the true HIV status of infants in a developing country where subtype C accounts for 99% of HIV infections.[25] The information is vital to inform fledgling infant diagnostic programs emerging in sub-Saharan Africa and elsewhere.

TABLE 3. Performance of the HIV DNA PCR Version 1.5 Assay in Each Laboratory and Combined Results Depicting Overall Performance of the Assay During Infancy and at 6 Weeks of Age

	JHB Laboratory	NICD Laboratory	Overall	Overall (6 wk)
Cohort	A and B	C	A, B and C	A, B and C
No. of infants tested	443 (26 HIV+)	326 (32 HIV+)	769 (58 HIV+)	627 (58 HIV+)
No. of PCR tests performed	1173 (81 on HIV+)	652 (63 on HIV+)	1825 (144 on HIV+)	912 (80 on HIV+)
HIV prevalence (%)	5.8	9.8	7.5	9.2
Sensitivity (%)	100	98.4	99.3 [98.9, 99.7]*	98.8 [98.0, 99.5]
Specificity (%)	99.8	98.8	99.5 [99.1, 99.8]	99.4 [98.9, 99.9]
Positive predictive value (%)	97.6	90.0	94.1 [93.0, 95.2]	94.1 [92.6, 95.6]
Negative predictive value (%)	100	99.8	99.9 [99.8, 100]	99.9 [99.7, 100]

*Numbers in brackets, 95% confidence intervals.
HIV+ indicates HIV-infected.

The Amplicor HIV DNA PCR version 1.5 assay was 99.3% sensitive and 99.5% specific in detecting HIV subtype C infection in 769 infants of whom 58 were HIV-infected. The assay performed equally well at 6 weeks of age in 623 of these infants comparing favorably with the performance of the HIV-1 DNA PCR version 1.0 assay at the same age in the developed world, where a sensitivity of 98% and specificity of 99.9% have been reported.[21] Because positive predictive value is dependent on the prevalence of HIV, prevalence rates higher than 9.2% likely to be encountered in the developing world, would increase the positive predictive value of 94%. Dried blood spots improve accessibility to infant HIV PCR testing in low resource settings because sample collection is simpler and centralized processing is facilitated.[17,26] Importantly the HIV DNA PCR version 1.5 assay maintains highly accurate results when performed on dried blood spots.[16,17]

With good laboratory technique, the sensitivity and specificity of qualitative HIV PCR approach 100%, but because of the most common pitfall, namely false positive results secondary to contamination in the laboratory, a second confirmatory specimen is recommended.[18,19,27] Submitting every positive result to a confirmatory PCR test on the same sample increases costs but was effective in reducing false positive rates in the JHB laboratory.

The reality of poorly resourced health care settings, not taken into account by this study, will undoubtedly have a negative impact on the performance of the HIV PCR assay. Samples were collected and processed under controlled research conditions, minimizing the effect of human error (eg, mislabeling of specimens) and assuming the availability of a laboratory proficient in PCR testing. Furthermore breastfeeding rates in these cohorts were far lower than would be expected in the majority of communities in the developing world complicating the timing of HIV diagnosis.

Notwithstanding these problems, if resources permitted only a single PCR test in the 6-week-old infant, the potential to improve access for children to an HIV diagnosis globally would be substantially increased from current levels. The challenge is to minimize the chances of misdiagnosis at the laboratory, clinical and community levels. When establishing PCR laboratory facilities with the objective of achieving consistently accurate results in a high throughput environment, attention must be paid to staff training and retention, quality assurance programs, good laboratory practice and the use of standardized test kits. Education of health care workers and counseling of parents regarding the fallibility of a single 6-week HIV PCR test is essential to ensure further HIV testing is sought particularly when there is a risk of postnatal transmission. Clinical examination is useful to corroborate HIV test results but is less helpful at 6 weeks than at 12 months of age.[28,29]

The recommendation to delay the second HIV PCR test in uninfected children to beyond 4 months of age stems from a concern that a minority (<3%) of HIV infections, particularly those occurring at the time of delivery, might not be detected by PCR until after 4 months of age.[20–22,30] In general, the PMTCT prophylaxis administered in the developing world primarily reduces peripartum transmission, effectively decreasing the small number of HIV-infected infants who might have false negative HIV PCR results at 6 weeks of age.

An HIV DNA PCR version 1.5 test is highly accurate in diagnosing HIV infection at 6 weeks of age in subtype C virus, but PCR might not be an option for every resource-poor setting. Current alternatives include the ultrasensitive p24 antigen assay in early infancy[31,32] or continued use of rapid and or laboratory-based HIV-1 antibody assays around 12 months of age on serum or oral fluid.[33,34] Once a diagnostic protocol suited to local circumstances is implemented, it is vital that ongoing monitoring, evaluation and validation of newer technologies occur to maximize accurate identification of HIV-infected children, the first step toward effective treatment.

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