Dried Blood Spots Improve Access to HIV Diagnosis and Care for Infants in Low-Resource Settings

Gayle G. Sherman, MD, Gwynneth Stevens, PhD,* Stephanie A. Jones, MD,† Pamela Horsfield, MT,* and Wendy S. Stevens, MMed (Haem)**

Summary: Effective health care delivery to the majority of perinatally exposed infants worldwide, including those enrolled in prevention of mother-to-child transmission programs, is hampered by lack of access to an HIV diagnosis in infancy. Dried blood spot collection from young infants for centralized HIV polymerase chain reaction (PCR) testing is attainable in low-resource settings, provided PCR methodology suitable for routine laboratory service is available. The accuracy of the Roche Amplicor HIV-1 DNA test version 1.5 (Branchburg, NJ) performed on dried blood spots collected prospectively on ordinary Whatman filter paper from a cohort of 300 6-week-old infants born to HIV-infected women in Johannesburg, South Africa, was assessed. Anonymous analysis of the blood spots using a unique DNA extraction procedure was performed in a routine diagnostic laboratory and the results compared with HIV DNA and RNA PCR liquid blood tests at age 6 weeks, and the HIV status of the infant. Dried blood spots were available for 288 infants (96%) of whom 25 (8.7%) were HIV infected. The Roche Amplicor assay yielded a sensitivity of 100% and a specificity of 99.6%. HIV DNA PCR tests on dried blood spots have the potential to improve health care delivery to HIV-affected children in low-resource settings right now.

Key Words: dried blood spots, HIV, prevention of mother-to-child transmission, infant diagnosis, polymerase chain reaction, low-resource setting

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The South African antenatal clinic HIV prevalence rate of 29.5% in 2002 translates into vertical exposure of approximately 280,000 infants annually.[1] South African prevention of mother-to-child transmission (PMTCT) guidelines, like those in other low-resource settings, require all HIV-exposed children to be followed to 12 months of age or older before their HIV infection status can be determined using an HIV antibody test. The lack of capacity to achieve clinical follow-up of these children is a universal frustration of PMTCT programs in low-resource settings.[2,3] Over a 2-year period, only 426 (19%) of 2243 vertically exposed children born at Coronation Women and Children's Hospital (CWCH) in Johannesburg presented for HIV testing at 12 months of age. The result is a failure to identify HIV-infected children and improve their quality of life with basic medical care such as prophylactic medication, treatment of intercurrent infections, and nutritional and emotional support. The imminent provision of antiretroviral therapy and the need to measure HIV transmission rates in infants (to evaluate and improve PMTCT programs) provide additional impetus for identifying HIV-infected children. Early diagnosis of HIV infection status in exposed children has been identified as the first step to improving pediatric HIV/AIDS care in developing countries.[4,5] Infant HIV diagnostic protocols are considered too costly and complex for low-resource settings largely because of polymerase chain reaction (PCR) testing and the expertise required to venesect babies.[6] HIV DNA PCR tests for diagnosing HIV in infants have become technically easier, more robust, and less costly over time. Dried blood spots (DBS) can overcome the blood sampling and logistical obstacles that limit access to infant diagnosis in low-resource settings. This prospective cohort study illustrates that a routinely used and commercially available HIV DNA PCR test performed on blood collected on unmodified filter paper at 6 weeks of age yields an accurate diagnosis of HIV infection status.

METHODS

Collection of DBS at 6 weeks of age was undertaken as part of a cohort study to establish an accurate but affordable infant HIV diagnostic protocol for low-resource settings.[7] Six weeks is the earliest age at which HIV PCR is expected to detect virtually all perinatally transmitted HIV infection.[8,9] Approval was obtained from the University of the Witwatersrand's Ethics Committee. All infants born at CWCH to HIV-infected pregnant women who were telephonically contactable and gave written informed consent were eligible to participate. At 6 weeks of age, infants had HIV DNA and RNA PCR tests performed on liquid blood samples collected in EDTA using version 1.5 of the Roche Amplicor HIV-1 DNA and Amplicor Monitor assays, respectively (Roche Diagnostic Systems, Inc., Branchburg, NJ). Infants were considered HIV-infected if they tested PCR positive for HIV DNA on 2 occasions.[6] HIV-negative infants had 2 negative PCR tests for HIV DNA at

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From the *Department of Molecular Medicine and Haematology, National Health Laboratory Service and University of the Witwatersrand, Johannesburg, South Africa; and †Wits Paediatric HIV Working Group, University of the Witwatersrand, Johannesburg, South Africa.
Reprints: Gayle G. Sherman, PO Box 79722, Senderwood, 2145, Johannesburg, South Africa (e-mail: gayle.sherman@nhls.ac.za).

6 weeks and 3 months of age and were followed to 12 months of age to document seroreversion. At 6 weeks of age, infants had 4 spots of blood of variable size and thickness collected on a sheet of blank filter paper (no. 1, Whatman) either from a syringe or directly from the venepuncture site. The filter paper was air dried and stored at room temperature in separate, zip-locked plastic bags to prevent cross-contamination. In July 2003, 9–19 months after collection, the DBS were submitted for anonymous testing using the same PCR assay for HIV DNA performed on the liquid blood specimens. Analysis was performed in the routine molecular laboratory according to the laboratory's standard algorithm, which requires retesting of equivocal and positive results on newly extracted DNA. Optical density readings between 0.25–1.00 defined an equivocal test. A 0.5-cm diameter circle was cut from each filter paper spot using a fine-point scissors flame sterilized between each specimen. A slightly modified Roche Amplicor extraction procedure was performed. Briefly, the excised filter paper was washed twice with at least 500 μL Specimen Wash Solution for 10 minutes at room temperature without centrifugation, ensuring submersion of the entire paper. The solution was aspirated, 200 μL of Roche extraction reagent added, and DNA released by consecutive 15-minute incubations at 60 and then 100°C. The filter paper was removed from the tube and 50 μL of the extract was amplified and detected according to manufacturer's instructions.

RESULTS

Between January and October 2002, 300 6-week-old infants were enrolled. DBS were available for 288 infants (145 male and 143 female) with a median age of 5.9 weeks. The overall HIV transmission in the cohort of 300 at 6 weeks and 3 months of age was <9%.[7] The HIV infection status of 23 of the 25 HIV-infected children was determined on the basis of positive PCR tests for HIV DNA at 6 weeks and 3 months of age. Two infants were considered infected on the basis of positive PCR tests for HIV DNA at 6 weeks and death due to AIDS-related illnesses prior to their 3-month visits. The negative HIV infection status of 231 infants was determined by negative PCR tests for HIV DNA at 6 weeks and 3 months of age and either seroreversion at 12 months or a 3rd negative PCR test at 7 or 12 months of age. Two negative PCR tests for HIV DNA with normal clinical assessments at 6 weeks and 3 months of age defined lack of HIV infection in 23 infants. Of the remaining 9 children included as HIV-uninfected children, 7 had negative PCR tests for HIV DNA and RNA at 6 weeks of age and were subsequently lost to follow-up, and 2 had no available 3-month HIV DNA or RNA PCR results but demonstrated seroreversion at 12 months. The DBS PCR results for HIV DNA could be compared with both HIV DNA and RNA PCR results on liquid blood in 280 cases (97%) at 6 weeks of age (Table 1). Seven cases had only PCR results for HIV DNA for comparison at 6 weeks. In 1 case with a negative DBS PCR result for HIV DNA, neither the DNA nor the RNA PCR result was available at 6 weeks of age but the infant was considered HIV uninfected on the basis of a negative PCR result for HIV DNA at 3 months and seroreversion at 12 months. DBS PCR testing for HIV DNA generated 8 equivocal results. Repeat testing yielded 1 positive and 7 negative results consistent with the reference standards in all cases. The 6-week DBS PCR test for HIV DNA was 100% sensitive and 99.6% specific when compared with all 3 reference standards viz. the 6-week liquid blood HIV DNA and RNA PCR results and the infant's HIV infection status at a year of age (Table 1). By following the laboratory's standard operating procedure of repeating all positive HIV DNA PCR tests, the single false-positive result was detected, increasing specificity to 100%.

TABLE 1. DBS HIV DNA PCR Test Results Compared to the 3 Reference Standards

	Child's Age	Positive Result	Negative Result	Total Tests
DBS HIV DNA PCR	6 weeks	26*	262	288
Liquid blood HIV DNA PCR	6 weeks	25	262	287
Liquid blood HIV RNA PCR	6 weeks	24	256	280
HIV Infection status of child	1 year	25	256	281

*Includes 1 false-positive test.

DISCUSSION

DBS collected in newborn screening programs have been used to determine maternal HIV seroprevalence rates, estimate maternal-infant HIV transmission rates at birth, measure viral load, subtype HIV, and perform drug resistance genotyping.[8,10–14] DBS carry less of a biohazard risk than liquid samples, require minimal storage facilities since the samples are stable at room temperature for prolonged periods, and are easier to ship, facilitating centralized laboratory testing.[15–19] Reliable detection of HIV-1 proviral DNA on DBS by PCR was first demonstrated in 1991.[16] Initial studies, often performed retrospectively on archived material, focused on technical issues such as PCR methods, the volume of blood required to achieve accurate results, and appropriate storage conditions.[10–17,19–23] Multiple replicate testing of samples and specialized filter paper have been advocated to improve the accuracy of the PCR testing for HIV DNA.[18,21,23] PCR tests on DBS for HIV DNA demonstrate sensitivities and specificities comparable to those achieved on liquid blood but use PCR methods unsuitable for high-throughput, routine laboratory service.[9,13,15,18,19,21,22] The Roche Amplicor HIV-1 DNA test version 1.5 is a standardized, commercially available assay that has been optimized to detect viral subtypes other than subtype B including subtype C, prevalent in South Africa. The Amplicor PCR assay performed on DBS achieved highly accurate results using standard filter paper and a simple, shortened, novel DNA extraction procedure.[13,14,18,22,23] The Amplicor assay has been successfully used on DBS collected on specialized filter paper in Rwanda, where HIV-1 subtype A predominates,[14] but the DNA extraction method described here is further simplified, quicker, and uses the extraction reagents supplied with the test kit (viz. the same reagents as for liquid blood testing), facilitating standardization of the test and rendering it suitable for high-throughput, routine laboratory use in poorly resourced settings. The expense of replicate testing was

avoided in 255 (89%) of 288 cases. The minimum input HIV DNA required per blood spot to ensure detection of the virus by PCR has been extensively studied but is difficult to validate in routine practice.[10,12,16–18,20,21] This study suggests that there is no need for stringent standardization of the volume of blood collected, probably because of high levels of virus in infected 6-week-old infants.[18,23] The skills required for venesection of babies are not available in the majority of PMTCT clinics. DBS collection is easier and requires less expensive supplies and little formal training.[8,17,18] The 6-week postpartum visit at a "well baby" clinic, when newborns undergo a general medical check and receive their first set of immunizations, is well entrenched in health care systems. These clinics hold the potential for extensive DBS collection by heelprick and earlier, more widespread detection of HIV-infected children. DBS PCR testing for HIV DNA is highly sensitive and specific and ready to enter the realm of routine practice.

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