Overseas processing of dried blood spots for timely diagnosis of HIV in Haitian infants

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ABSTRACT

Objective. *To determine the feasibility of sending dried blood spots (DBS) to an overseas processing center for the diagnosis of HIV infection in infants in rural Haiti.*

Methods. *The program took place in the Central Department of Haiti. Children under 18 months of age who were born to an HIV-infected mother or who had a positive HIV antibody test had blood collected on filter paper. Once dry, specimens were labeled with a unique identifying number, placed in sealed gas-impermeable envelopes containing a desiccant, stored at room temperature, and mailed to a commercial laboratory in The Netherlands, where blood was eluted from the filter paper and analyzed by the Retina™ rainbow HIV-1 RNA assay. Infants were tested at 1 month of age and again at 4 months of age.*

Results. *The DBS protocol was easily scaled up. During the study period, 138 infants had HIV status confirmed; 15 of them were found to be HIV infected and were enrolled in appropriate HIV care, and 123 were confirmed to be HIV uninfected, avoiding unnecessary prophylactic antibiotics and providing reassurance to caregivers.*

Conclusion. *Central, overseas processing of DBS is a feasible solution for the timely diagnosis of HIV infection in infants where local capacity is unavailable. Regional processing centers for DBS could improve the access of millions of children in Latin America and the Caribbean to timely diagnosis of HIV infection.*

Key words Blood specimen collection, HIV serodiagnosis, poverty areas, Haiti.

In 2006, an estimated 2.3 million children under the age of 15 years were living with HIV, including 22 000 in the Caribbean region alone (1). Globally, less than 5% of infected children who need antiretroviral treatment (ART) receive it (2), in part because of delayed confirmation of the serostatus in HIV-exposed infants. Passive transmission of maternal antibody means that an infant's HIV antibody test may be positive until 18 months of age even if the child is uninfected. This delay in diagnosis causes postponement of the initiation of ART, a prolonged period of concern for the child's parents or guardians, unnecessary use of antibiotic prophylaxis, and potentially the death of the child. Because 15% of HIV-infected children progress to AIDS or death in the first 12 months of life without appropriate therapy, it is important to diagnose them and treat the condition early (3). Recent data suggest that early (less than 12 weeks of age) commencement of ART in infants with HIV infection is associated with up to 75% reduction in early mortality compared with delayed start (4).

HIV DNA or RNA assays, including polymerase chain reaction (PCR) and nucleic acid sequence-based amplification (NASBA), offer definitive HIV di-

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agnosis in almost all infants by 3–4 months of age and have been the standard of care in developed countries for more than 14 years (5). Studies have demonstrated the sensitivity and specificity of these methods for diagnosing HIV in exposed infants (6–15). NASBA and PCR tests have been difficult to perform in resource-poor settings, however, because processing each test requires specialized facilities and materials, highly trained laboratory technicians, and dedicated laboratory space. Even when these conditions and resources are available in developing countries, transportation of blood or plasma is often challenging because of poor roads or lack of refrigeration. Using dried blood spots (DBS) on filter paper eliminates many concerns about transporting blood samples because they are not an infectious risk, do not need refrigeration, and can be safely sent by mail, allowing for safe transport to an off-site laboratory (13). Although potential exists for degradation of viral RNA during storage (16), particularly for dried plasma spots (17, 18), a number of studies have shown the stability of HIV RNA on whole blood spots dried on filter paper specimens stored for as long as weeks to up to 1 year at room temperature (11, 12). This paper describes the feasibility of using a centralized overseas laboratory to test DBS from HIV-exposed infants in central Haiti as a model pilot program to improve the timeliness of HIV diagnosis.

MATERIALS AND METHODS

The pilot program took place in the Central Department of Haiti, a rural impoverished area. In this region, the nongovernmental organization Partners In Health (PIH), in collaboration with the Ministry of Health, cares for more than 10 000 HIV-positive persons, 3 000 of whom are on ART, and performs more than 50 000 HIV antibody tests per year. With HIV antibody testing alone, definitive diagnosis of HIV in infants had previously been delayed until 18 months of age. This uncertainty often delayed the initiation of ART in children who were HIV positive.

In November 2004, PIH began using DBS specimens to diagnose HIV infection in infants. Children under 18 months of age who were either born to HIV-infected mothers or had a positive HIV antibody test had blood collected on filter paper by a trained nurse or laboratory technician using a heel prick, finger stick, or venipuncture. Once dry, specimens were labeled with a unique identifying number, placed in sealed gas-impermeable envelopes containing a desiccant, stored at room temperature, and mailed to a commercial laboratory in The Netherlands (Primagen Holding B.V.). This technique has previously been reported to be 100% sensitive and specific for the detection of HIV-1 RNA (19). To minimize viral RNA degradation during storage, specimens were scheduled to be shipped every 2 weeks. To our knowledge, no local or regional laboratory offered this testing commercially.

At the laboratory in The Netherlands, blood was eluted from the filter paper and analyzed by the Retina™ rainbow HIV-1 RNA assay. The assay is based on real-time NASBA amplification technology and detects and quantifies HIV-1 RNA of all HIV subtypes from groups M, N, and O from 50 to 50 000 000 copies per milliliter (mL) (20). It requires a specimen volume of only 200 microliters and has been used in other settings as a method of monitoring HIV-1 viral load (20–22). Sensitivity and specificity of the technique for detecting viral loads greater than 500 copies/mL have both been reported as 100%. Once the assays were performed, coded data were sent electronically from The Netherlands to a PIH data manager who matched data with patient names at the individual sites in Haiti and forwarded test results to each site.

Infants were tested for HIV RNA at 1 month of age and again at 4 months of age. If mothers chose to breast-feed or presented to the clinic already breast-feeding, a PCR test was performed 3 months after the last breast feed. A newborn was considered HIV infected if two positive tests were obtained according to the protocol. Discordant results were settled with a third test and a careful review of the clinical case. Although ordinarily it is standard to confirm a positive virologic test on a second specimen, in our clinical practice infants over 4 months of age who had been exposed to HIV and who presented with clinical signs or symptoms of infection were considered to have sufficient evidence of infection if one PCR test was positive. This practice is consistent with World Health Organization guidelines on early diagnostic testing for pediatric HIV (23). Antibody testing was later used to confirm positive status at 18 months of age, but clinicians did not wait for the 18-month test results to initiate antiretroviral therapy if the single PCR test and clinical evaluation warranted it. We report results from the first 138 infants tested prospectively by the DBS method.

RESULTS

Between November 2004 and January 2006, 138 children exposed to HIV in utero or found to have a positive HIV antibody test had their HIV status tested by the protocol described. Median time from blood collection to the results reaching the team in Haiti was 1.7 months. Fifteen children were confirmed to be HIV positive and 123 were confirmed to be HIV negative. Among children who tested positive, HIV status was later confirmed by a second positive PCR test (n = 4), a confirmatory antibody test at 18 months of age (n = 7), or other clinical markers of HIV infection such as opportunistic infection or low CD4 percentage (n = 4). The median age at which HIV status was confirmed was 7.3 months. See Table 1 for the stratification of tests by HIV status and timing of confirmed diagnosis.

In terms of clinical characteristics, most of the women received ART (86%) (either single or triple therapy) (see Table 2); the remaining women presented to our clinics after delivery of their infant. Mean antenatal CD4 cell count before delivery (where available)

TABLE 1. HIV test results based on DBS method (*n* = 138), Haiti, November 2004–January 2006

Test results	No.	%
Positive results		
Two positive PCR tests (at 1 month and ≥ 4 months of age)	4	3
One positive PCR test (≥ 4 months of age) plus confirmatory antibody ≥ 18 months	7	5[a]
One positive PCR test (≥ 4 months of age) plus clinical signs/symptoms of HIV infection	4	3[a]
Negative results		
Two negative tests (at 1 month and ≥ 4 months of age)	51	37
One negative test (≥ 4 months of age)	72	52[a]

[a] For those infants initially at 4 months of age or after (*n* = 83), 29 (35%) had a concordant confirmatory PCR test. Among these 83 children, mean age at time of initial test was 8.2 months (range, 4.1 to 17.6 months).

TABLE 2. Clinical characteristics (*n* = 138),[a] Haiti, November 2004–January 2006

Characteristic	No.	%
Infant HIV status		
Positive	15	11
Negative	123	89
Breast-feeding (*n* = 80)		
Yes	14	17
No	66	83
Mother received ART[b] during antenatal period (*n* = 79)		
Yes	68	86
Mother antenatal CD4 cell count (*n* = 65)		
Mean (range)	482	(25 to 1 358)

[a] Sample size is 138 unless otherwise indicated due to missing data.
[b] ART, antiretroviral therapy—single drug or combination triple drug therapy.

was 482 cells per cubic millimeter of blood (range, 25 to 1 358). Most of the women did not breast-feed (83%). Preliminary results suggest a low transmission rate among those infants whose mothers presented for care at our clinics during pregnancy and who therefore received full supportive services including antenatal ART as well as supplies, support, and formula allowing them not to breast-feed; these results will be forthcoming upon analysis of the entire study cohort.

The 15 HIV-infected children are enrolled in chronic HIV care and followed regularly in clinic; decisions about their enrollment in ART were based on standard clinical and laboratory evaluation. Of the 15 children with HIV, all cases were the result of perinatal transmission, and most were born to mothers who had not benefited from antiretroviral therapy before delivery. All 123 children confirmed to be negative stopped cotrimoxazole prophylaxis.

DISCUSSION

Our study demonstrates two important principles. First, use of a central overseas processing center is feasible in rural resource-poor settings that lack laboratory capacity. The DBS method has been used in pilot study settings elsewhere (24–29) and recently national programs in Africa are beginning to incorporate DBS PCR as a standard procedure for infant HIV diagnosis (30). Batch shipping for centralized processing was feasible in this rural field-based study.

Second, use of the DBS test has a clear clinical advantage over traditional antibody tests; 15 children under the age of 18 months in whom the diagnosis of HIV otherwise would not have been known were confirmed to be infected. This diagnosis allowed initiation of life-saving ART and aggressive treatment of opportunistic infections, as necessary. Definitive diagnosis of HIV also allows for proper diagnoses of tuberculosis, malnutrition, and other childhood diseases that often coexist in a patient and may have presentations similar to those of AIDS in children. Cotrimoxazole prophylaxis was suspended in infants who were not infected, lessening the risk of toxicity and reducing their risk of developing antimicrobial resistance. Early diagnosis with DBS also alleviated the anxiety of family members who otherwise would have had to wait up to 18 months to learn whether their child was infected.

The average processing time from blood draw to result was 1.7 months. This delay was in part because of local couriers' concerns that required the specimens to be hand-carried out of the country. This restriction has since been lifted with the authorization of the Haitian Ministry of Health allowing specimens to be directly shipped from Haiti to the processing laboratory. Further delay was introduced by shipping to Europe from the Caribbean. Even with this delay, however, the testing method still offered an advantage over waiting until the 18-month antibody test. If a regional Latin American or Caribbean laboratory offered the test, a significant decrease in time from blood draw to test result would be possible.

Given limited resources, the cost of DBS PCR testing is an important consideration. The cost per DBS PCR test was US$35–50 during the period of the study. This cost could have been reduced if there were higher-volume testing and also if the testing were provided by a national or regional referral laboratory that was not for profit rather than by a commercial entity. We did not perform a detailed cost analysis; however, a study in Botswana demonstrated that the marginal additional investment for the cost of DBS HIV

PCR testing versus antibody testing could triple the efficacy of prevention-of-mother-to-child-transmission programs and offer further societal benefits beyond economic settings (26).

Disadvantages of using the DBS method included the lag time needed to train clinical and laboratory staff in the new procedure; however, the team in Haiti was easily trained in the simple technique of spotting the blood on the filter paper and has provided positive feedback about the ease of use of the test. The introduction of off-site testing also required stringent laboratory tracking procedures to minimize clerical errors; however, once these administrative procedures were put in place they were used with minimal disruption to clinic or laboratory work flow.

Conclusion

Increased emphasis must be placed on identifying HIV infection in exposed infants in developing countries earlier than is possible with HIV antibody testing. This need is increasingly compelling as data emerge demonstrating the benefits of early ART among infants with HIV infection. Molecular diagnostic tools for HIV infection are the standard of care in wealthy countries and should be made available to resource-poor programs even when local processing labs are not available. The DBS method is sensitive and specific, convenient, feasible, and reliable even in the most adverse circumstances in rural Haiti. Regional Caribbean or Latin American laboratories processing batched shipments of DBS could improve the timeliness of diagnosis of HIV infection for thousands of infants in the region. As with other commercial products such as ART, volume of consumption and advocacy by international groups will ultimately reduce the price of PCR testing and make this test increasingly available locally to all who need it.

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RESUMEN

Diagnóstico oportuno del VIH en niños haitianos mediante el procesamiento en el extranjero de muestras de sangre seca

Objetivo. Determinar la factibilidad de enviar muestras de sangre seca (MSS) a un centro en el extranjero para el diagnóstico de la infección por el VIH en niños de zonas rurales de Haití.

Métodos. El programa se realizó en el Departamento Central de Haití. Se tomó una muestra de sangre en papel de filtro de los niños menores de 18 meses nacidos de madres infectadas con el VIH o que tuvieran una prueba positiva de anticuerpos contra el VIH. Una vez secas, las muestras se etiquetaron con un número de identificación único, se colocaron en sobres sellados impermeable a gases con desecante, se almacenaron a temperatura ambiente y se enviaron por correo a un laboratorio comercial en los Países Bajos, donde se eluyó la sangre del papel de filtro y se analizó mediante el sistema Retina™ Rainbow para la detección de ARN del VIH-1. Las pruebas se realizaron a los niños de 1 mes y se repitió a los 4 meses de edad.

Resultados. El procedimiento de MSS se llevó fácilmente a una escala mayor. En el período de estudio se confirmó el diagnóstico de 138 niños: 15 de ellos estaban infectados y recibieron los cuidados apropiados; 123 niños no tenían la infección, lo que evitó aplicar innecesariamente el tratamiento antibiótico profiláctico y el personal de salud sintió mayor confianza.

Conclusiones. El procesamiento centralizado de MSS en el extranjero es una solución factible para el diagnóstico oportuno de la infección por el VIH en niños cuando no hay capacidad local de diagnóstico. Centros regionales para el procesamiento de MSS podrían mejorar el acceso de millones de niños de América Latina y el Caribe al diagnóstico oportuno de esta infección.

Palabras clave Recolección de muestras de sangre, serodiagnóstico del sida, áreas de pobreza, Haití.