Rapid Identification of Infants for Antiretroviral Therapy in a Resource Poor Setting: The Kenya Experience

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Summary

In Kenya, HIV diagnosis is not routinely carried out in infants, and yet rapid diagnosis could improve access to lifesaving interventions. A cheap and readily accessible service can resolve this problem, if feasible. In this pilot study the feasibility and costs of provision of an infant HIV diagnosis service in Kenya are evaluated. Dried blood spots (DBS) were collected from infants exposed to HIV, sent to a central testing laboratory and tested using the Roche Amplicor v.1.5 DNA PCR kit. The results were then dispatched to health facilities within a week. A total of 15.4% of the samples tested HIV+ despite the widespread access to prevention of mother to child transmission (PMTCT) programs in Kenya. The cost per test at 21.50 USD is prohibitive and will limit access to diagnosis. It remains to be seen whether the increase in testing will immediately lead to an increase in access to antiretroviral therapy (ART) services for infants.

Key words: dried blood spots, Roche amplicor DNA PCR, infant HIV diagnosis.

Introduction

Mother-to-child transmission of HIV in the developing world is estimated to be as high as 21–43% [1]. Anti-HIV antibodies passed from the infected mother can persist for as long as 18 months in the infant, and therefore diagnosis must rely on the use of other diagnostic modalities: HIV blood culture and HIV-specific-polymerase chain reaction (PCR) [2].

Early Infant Diagnosis (EID) of HIV using dried blood spots is widespread in the developed world [3–5]. Success stories in resource poor settings are becoming common, mainly in Southern Africa [6]. With a sensitive and specific diagnosis, infected infants can be identified quickly and put on the medication they need. Further, the success of prevention of mother to child transmission (PMTCT) programs can only be evaluated fully if diagnosis of HIV in infants is assured and routinely provided. Dried blood spots are easy to transport, and pose minimal infection risks. Blood on filter papers is biologically stable. DBS are very convenient to use in resource-limited settings such as Kenya.

The National AIDS Control Council of Kenya reported in August 2007 that more than 102,000 children are infected with HIV-1. Yet, this was at best an estimate, since the cost of infant diagnosis is at least 70 USD or more, hence out of reach for most children.

The Roche Amplicor® HIV-1 DNA PCR kit (Roche Molecular Systems, Inc, Branchburg, NJ) is a sensitive and specific test, especially for testing for HIV among infants [7]. It has been reported to have a sensitivity of 99.1% and a specificity of 100% [8]. It has been widely used elsewhere in the world, with excellent results [9]. Advantages of this assay include the potential to automate for large-scale testing and the ability to produce results within a day [7]. Despite this, the feasibility of the technology, and the fiscal implications for national programs, is not widely reported.

Here, we discuss the institutional framework and cost implications of a pilot EID program, and describe the procedures optimized for Pediatric HIV testing in a resource poor context. The possible impact of PMTCT interventions in Kenya is explored. We also propose future considerations for providing a reliable,
sustainable EID service in Kenya and other resource-poor settings.

Materials and Methods

Institutional context
This pilot project was set as a collaborative effort between the Clinton Foundation and the Kenya Medical Research Institute (KEMRI).

Ethical considerations
The KEMRI/National Ethical Review Committee, SSC No. 1066, granted authorization for the study. Samples collected from infants were given anonymous identifiers at blood draw.

Selection of sites and study population
All centers involved in the pilot study, ranging from private health centers to government clinics and public healthcare institutions, offered pre- and post-test counseling and a medical follow-up program for HIV-exposed children. A total of 190 centers countrywide were using the service by December 2007. Most samples were drawn at PMTCT and Maternal and Child Health (MCH) clinics.

Training of phlebotomy personnel
A 3-day training curriculum was assembled on Good Clinical Practice and Good Phlebotomy Practice. PMTCT and pediatric HIV care personnel from five of the eight Kenyan Provinces were trained. The project also took advantage of specific training offered by other stakeholders at the district level.

Algorithm for HIV testing for infants less than 18 months old using PCR
The HIV testing algorithm developed by the National AIDS and STDs Control Program was adopted, with slight modifications. Briefly, infants exposed to HIV who presented at the clinic at 6 weeks for immunization qualified for the test. Although the national algorithm recommends a repeat test at the age of one year, this was not possible due to logistical difficulties of follow-up facing clinicians in the sites.

Collection and transport of specimens to laboratory
Blood samples were collected as DBS onto Grade 903 S&S filter paper cards (Whatman, Inc., Florham Park, NJ). Phlebotomists spotted at least two circles facing clinicians in the sites.

Selection of sites and study population
Phlebotomists spotted at least two circles on the DBS onto Grade 903 S&S filter paper cards (Whatman, Inc., Florham Park, NJ). Phlebotomists spotted at least two circles after the blood draw. DBS were coded, separated from one another with weighing paper, packed with desiccant and humidity indicator cards in impermeable plastic bags, and transported by courier to the HIV laboratory in KEMRI for testing. Coding was based on location, date and sample numbers collected. Samples were batched to reduce courier charges, and were sent to the lab weekly. On reception in the testing laboratory, the coding data and sample details were entered into a Microsoft Access database.

Laboratory analysis
Standard operating procedures were used to train technicians testing samples in the laboratory. Stringent acceptance and rejection criteria for DBS collection and testing were used. The Roche Amplicor® HIV DNA PCR kit was used for the PCR procedures, with some modifications. Briefly, a clean handheld punch (1/4 inch) was used to punch a disk (6 mm²) from the DBS into a 2 ml screw cap tube, and 1 ml of Roche Specimen Wash Buffer® added. DNA extraction, PCR amplification and analysis by ELISA was done as per the manufacturer’s recommendations [10]. Samples were considered unequivocally positive if they had an optical density (OD) of ≥0.8 and negative if they had an OD <0.2 using an A_450 filter. Samples that had ODs higher than 0.2 but less than 0.8 were considered indeterminate and retested. If these samples were still indeterminate, then fresh samples were requested from the Health Facilities. All positive samples were retested to confirm status; indeterminate samples were retested twice before ordering for a new sample. Quality was assured by retesting a batch of first 50 positive and negative samples every month. The lab subscribed to the Centers for Disease Control and Prevention proficiency panel testing program.

Results

Sample collection and scale up of service
Between May 2006 and January 2008, a total of 9922 samples from 190 health centers throughout Kenya were tested. The time between blood draw and delivery of sample to lab by courier ranged from 6 h to 3 weeks. The growth in demand for the service was exponential, with a large drop occurring in January 2008 owing to civil unrest in the country. Demand for the service in Nairobi was highest, with 47% of all samples coming from the city. This was expected, as the diagnostic laboratory is in Nairobi and awareness is high. Despite its distance from the testing laboratory, on average 600 km, Coast province provided 19% of all samples. Sometimes, service was interrupted by lack of test kits. High Quality DBS were collected almost always. Service scale-up is summarized in Fig. 1.

Qualitative PCR determination of HIV status using DBS
Of the 9922 samples that were analyzed successfully, 8061 (81%) tested were negative, while 1526 (15.4%) tested were positive for HIV-1. A total of 244 (2.5%) of the results were indeterminate. Three samples were rejected due to poor packaging and collection.
**Turnaround time**

Turnaround time was defined as the time between receipt of the specimen in the laboratory and dispatch of results from the laboratory. Between May 2006 and May 2007, the average turnaround time was 13.5 days, with a range of 6–20 days. In December 2007, the turnaround had improved to 4 days. However, the time between blood draw and presentation of results to the client continued to range from one to three months.

**Acceptability of the service**

EID received a high level of acceptance by medical staff and mothers as part of routine care.

**Cost analysis**

Each Roche Amplicor® DNA PCR test kit has a capacity to perform 90 tests and 6 controls at a negotiated cost of USD 960. The insurance, freight and tax charges were 4 USD per test. The cost of the filter papers and other laboratory reagents averaged an extra 6 USD per test, while the courier service charges were 0.50 USD per test. Labor was estimated to cost 1 USD per test. In total, each test costs approximately USD 21.50 (Table 1).

**Discussion**

In this pilot project, we sought to determine the feasibility and the costs involved in setting up a pediatric diagnostics service in a resource-limited setting. It is evident that EID of HIV by DNA PCR is feasible from a technical and infrastructural standpoint even in a poor country such as Kenya. The costs per test are prohibitive, and could easily be unaffordable for the majority of the population where there is no financial support.

The inconsistent supply of testing kits is a major challenge due to a poor commodity supply chain. Robust documentation and a strong supply chain management system are essential in the success of a testing program.

In most cases, without intervention, up to 35% or more of all children born to HIV positive mothers will become infected [11]. Our preliminary findings suggest that the infection rate in the Kenyan setup is less than half of this figure, probably a promising indicator of the success of PMTCT programs and comparable to other areas where non-pharmaceutical interventions are used [12], although the ideal situation would reduce the rate to single digits.

*Fig. 1. Scale up of the early infant diagnosis service.*

| **TABLE 1** | 
| --- | --- |
| **The cost of EID per test** | 
| Item | Cost per test ($) |
| Cost of test CIF Nairobi | 10 |
| Clearing and 2.75% IDF fee | 4 |
| Laboratory consumables | 4 |
| Filter paper and packaging | 2 |
| Transportation | 0.50 |
| Labour costs | 1 |
| Total cost per test | 21.50 |
Very few samples were rejected due to poor collection techniques. This confirms that training is an important aspect to provision the service. Training also facilitated an easy countrypide rollout of the service with a high success rate.

It is estimated that 1,361,000 children were born in 2005 in Kenya. The HIV prevalence among women in Kenya was 6.1% during the same period; at least 83,000 newborns were exposed to HIV. It can be assumed that every year at least this number of HIV tests would be required to determine the status of these children. If those currently living with HIV were initially all tested, then the annual burden for testing newly born children who need it would be 1.8 million USD. In 2007, Kenya received USD 300 million from the Global Fund for TB, HIV and Malaria. Clearly, even though 1.8 Million USD is a lot of money by any standards, it is still only 0.3% of this fund. Further, the HIV prevalence rate in Kenya is falling steadily, and whereas initially more and more children will be born with HIV as infected parents live longer, eventually this number will fall as fewer and fewer parents continue to live with HIV.

In Kenya, as is the case in many countries, HIV test kits are exempted from import duties. Unfortunately, the process of getting this exemption is tortuous, imprecise and unpredictable. In our experience reagents can pass their expiry date awaiting exemption, at the point of entry, this should be avoided by paying the duty whose rate as of 2006–07 was 2.75%. In spite of this shortcoming, the costs incurred per test are considerably lower than those available in the private sector. This could be partly explained by the lower comparative cost of the test kit that was used, and the non-profit nature of the service.

Quite clearly, some centers are too far from a centralized laboratory. For instance, one centre, Kilifi District Hospital, is 560 km away and the fastest courier service takes at least 18 h. To cut down on costs, samples have to be dispatched in batches once a week. There is a need to strengthen laboratory systems and infrastructure in all parts of a country that intend to offer HIV diagnostic services. This in turn would lead to improved access to quality of the service as was evident in this study. Centralized testing laboratories need to be capacitated with specialized equipment like safety cabinets, PCR thermocyclers and separate laboratories for sample reception, DNA extraction, PCR amplification and analysis rooms, as well as the technical know-how. These facilities are not available in district hospitals or health centers.

The turnaround time for PCR HIV diagnostic testing in this service was below a week. The turnaround time can be reduced to three days by bringing the testing center close to the PMTCT and related sites. This is an ideal time for making the necessary interventions that are needed, i.e. prevention of mother to child transmission of HIV or putting the infants on the appropriate medication on time if they turn out to be HIV-positive. The turnaround time between DBS collection and reception of the results at the health centre can and must be improved.

This study clearly indicates that the co-ordinated training of health workers, rapid testing from a central laboratory and quick dispatch of results can minimize the complexities of PCR testing. In areas where e-mail services are available, results can be received at the health centers countrywide immediately they are processed.

Early Infant Diagnosis services can be marketed as one means by which the success of PMTCT programs can be evaluated. But, what happens to those who test positive through this service? How many infants actually receive the result of the test? How many eventually benefit from care and treatment service? Should a CD4 test be offered freely to all who test positive? These are questions that need to be answered as the service expands.

References


