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### Original Article

## Comparative study of venous liquid blood and dried blood for HIV infection in infants.

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#### ABSTRACT

Polymerase Chain Reaction (PCR) is the only tool to identify the HIV status in infants less than 18 months of age. The objective of the study was to compare the results obtained from venous liquid blood on tubes and dried blood on two different filter papers for HIV infection in infant diagnosis. A total of 100 infant samples were collected on EDTA tubes and coated onto the filter papers. DNA was extracted from all these samples using liquid blood, whatman 903 and FTA cards. Nested PCR was performed using gag genome and the positive bands were visualized by gel electrophoresis. Of 100, seven infants were found to be HIV positive and 93 were HIV negative. The results obtained from two types of filter papers were compared with the venous blood and the sensitivity and specificity was 100%. Seven HIV positive infants and sixteen HIV negative infants were repeated with the freshly collected venous samples and also the samples directly coated onto the filter paper from heel prick, PCR was performed and the results were concordant. The blood collected onto the filter papers are ideal for HIV testing in infants in resource limited countries.

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### 1. Introduction

Most of HIV infection results in children are due to mother to child transmission (MTCT). Transmission of HIV is most likely to occur late in pregnancy or during delivery. MTCT of HIV accounts for 14% of all new HIV infections worldwide, and may occur during pregnancy, labor and delivery or breastfeeding. In the absence of prevention, rates of MTCT are estimated to be 25-35 percent [1]. However, HIV-1 diagnosis of infants is often delayed, because maternal antibodies transferred to the infant preclude the use of routine serologic assays through the age of 18 months [2].

HIV-1 DNA PCR is considered the optimal test for diagnosing HIV-1 infection in infants [3]. Dried blood specimens are ideal for the diagnosis and evaluation of HIV-1 infection in infants in resource-limited regions; however, previous reports have shown a decline in the ability to detect HIV-1 antibodies in samples stored under hot, humid conditions [4]. In 2009, only 53% of pregnant women identified as HIV-infected worldwide received any ARVs for

PMTCT, resulting in approximately 370,000 new infant infections. Many of these women received the ARV regimens previously recommended as a "minimum" intervention by WHO: a single dose of nevirapine (sdNVP) to a pregnant woman in labor and her infant after birth [5], [6]. In 1987 Edward McCabe reported successful extraction of DNA from Dried Blood Spots (DBS) collected on filter paper [7]. The blood collected on filter paper reduces the supplies and less skill than blood collected on tubes.

The small amount of blood required, the ease of collection, storage, and transport of samples, and the low cost of the test make this assay ideal for HIV-1 testing of infants in the field or where resources are limited [8]. The objective of the study was to compare the results obtained from two different filter papers with the venous liquid blood samples for HIV infection in infants.

### 2. Material & Methods

The study was done for the period of April 2009 to March 2010 (11 months) at Prevention of Mother to Child Transmission (PMTCT) centre, Namakkal, India. A total of 100 samples were collected from the infants born to HIV seropositive mothers who had received sd NVP at the time of labour and the infants also received nevirapine syrup within 72 hours of age. Only HIV

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positive mother's infants were included in this study and the data were collected about their breast feeding practices and the informed consent was obtained from the mothers for this study.

One ml of blood was collected on EDTA tubes and these collected samples were coated on to two different filter papers: Whatman 903 cards and Flinders Technology Associates (FTA) filter paper (Whatman Bioscience, Florham Park, NJ) For whatman 903 cards 50 µl of blood was coated onto each circle (total five circles) card and then it was allowed to dry for 4 hours at air conditioned room or overnight at room temperature, and then the dried blood spots (DBS) was inserted in a zip locked cover with a desiccant and stored at room temperature for further processing. Using FTA cards, 125 µl of blood was coated onto each circle (total 2 circles) and it was allowed to dry at room temperature, the dried spots appeared darker than the fresh spotted ones. It was inserted in a zip locked bag with the desiccant. The positive samples were retested with another fresh sample on EDTA tubes and also from a heel prick which was directly spotted onto both the filter papers.

#### DNA extraction from 903 cards

A 6 mm sized DBS sample was detached into the 2 ml screw capped tube and it was washed three times using nuclease free water ( P1195, Promega) and then suspended with 10% Chelex@100 Resin (Cat no. 143-2832, Bio Rad) . All the specimens were incubated at 56°C for 2 hours and then kept at 100 °C for 10 minutes and centrifuged at maximum speed for 3 minutes. The supernatant was collected and stored at -20°C for PCR amplification.

#### DNA extraction from FTA cards

A 2 mm sized DBS sample was detached into PCR amplification tube (0.2 ml), 200 µl of FTA purification reagent (Whatman Cat no. WB 120204) was added into the tube and it was incubated for 5 minutes at room temperature .After incubation the reagent in the tube was discarded and this process was repeated for twice, a total of three washes with FTA purification reagent.200 µl of TE (10 mM Tris- HCl, 0.1 mM EDTA) buffer was added into the tube and incubated for 5 minutes at room temperature, the spent TE was discarded and the same process with TE was repeated again. The disc in the PCR tube was dried for one hour and it was ready for PCR amplification.

#### DNA extraction from venous liquid blood on EDTA tubes

DNA extraction was performed from the venous blood using HiPura TM Blood Genomic DNA Mini prep Purification spin kit (Cat No.MB 504; HIMEDIA). DNA was extracted from 200 µl of the venous blood collected on EDTA tubes.

#### PCR amplification

Nested PCR was performed for the extracted DNA using two filter papers. The advantage of using this system is its sensitivity for low copy-number HIV-1 infection, achieved through nested PCR (Becket al., 2001).

Sequence of the gag primers1 & 2 for the first round is: 5'TCT CTC GAC GCA GGA CTC GGC TTG CTG3' and5' TAA CATTTG CAT GGC TGC TTG ATG TCC3'.The PCR reaction mixture contained 38.25 µl of nuclease free water, 5 µl of 10 x PCR buffer, 0.75 µl of 50 mM Mgcl<sub>2</sub>, 0.25 µl of Taq DNA polymerase (5U/µl), 0.25µl of dNTP mix,

0.25 µl of each primer and 5 µl of DNA template. The cycling parameters used were 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute for 2 cycles and 94°C for 15 seconds, 55°C for 45 seconds, 72°C for 1 minute for 33 cycles and the last cycle the extension prolonged to 72°C 5 minutes.

Sequence of the gag primers3 & 4 for the second round is: 5'CTA GAA GGA GAG AGA GAT GGG TGC GAG3' and 5'CTT GTG GGG TGG CTC CTT CTG ATA ATG3'. The PCR reaction mixture contained 41.25 µl of nuclease free water, 5 µl of 10 x PCR buffer, 0.75 µl of 50 mM Mgcl<sub>2</sub>, 0.25 µl of Taq DNA polymerase, 0.25 µl of dNTP mix, 0.25 µl of each primer and 2 µl of 1st round amplified product. The cycling parameters used were 94°C for 1 minute, 55°C for 1 minute, 72°C for 1 minute for 2 cycles and 94°C for 15 seconds, 65°C for 45 seconds, 72°C for 1 minute for 33 cycles and the last cycle the extension prolonged to 72°C 5 minutes.

For FTA cards the PCR reaction mixture (50 µl) was directly added onto the processed disc for the first round reaction. The final product is 650 bp, which was visualized by gel electrophoresis through 2% agarose with ethidium bromide staining.

### 3. Results

A total of 100 samples were tested in this study and the age range was between 2 weeks to six months. Seven (7%) samples were found to be HIV-1 positive, 4/7 were male and 3/7 were female infants, the age of the infants were (n=2) - 1 month, (n=1) - 4 months, (n=1) - 6 months and 93 (93%) samples were HIV-1 negative in whole blood.

The results obtained from both the filter papers were compared with the whole blood and it was found to be concordant, no false positive or false negative results were observed in this study. The sensitivity and specificity was 100% in both filter papers and also the Negative predictive value (NPV) and Positive Predictive value (PPV) was 100 %.

In this study the seven HIV-1 positive infants were retested with the freshly collected venous samples and samples directly spotted into the two types of filter papers from heel prick to confirm their HIV status of infection. Some negative infants (n= 16) also retested with freshly collected venous samples and samples directly spotted from heel prick to confirm the negative status and the data showed that the mothers were stopped the breast feeding. The results obtained from venous and heel prick samples were concordant.

**Table. 1: Results of venous blood and filter papers**

Sample type	Total no. of samples	Positive	Negative
Venous Blood	100	7 (7%)	93 (93%)
Whatman 903	100	7 (7%)	93 (93%)
FTA cards	100	7 (7%)	93 (93%)

Table 1 shows that the results of venous blood collected on EDTA tubes and the same sample coated onto filter papers.

**Table 2: Results of venous samples and heel prick samples**

Sample type	Total no. of samples	Positive	Negative
Venous Blood	23	7	16
Whatman 903	23	7	16
FTA cards	23	7	16

Table 2 shows that the results of venous blood on EDTA tubes and the samples directly spotted onto filter papers from heel prick.

### Discussion

In this study the infant samples (n=100) were tested for HIV infection using venous blood and the same was coated onto two different filter papers. The volume of the sample coated onto each filter paper was different. Multiple replicate testing of samples and specialized filter paper types have been used to improve the accuracy of the PCR testing for HIV DNA [9].

For whatman 903 card requires 50 µl per circle, filling of 2 circles are enough to perform the test (minimum volume of the sample to perform the test is 100 µl) DNA extraction from whatman 903 card is much easier than whole blood method, no other extra chemicals are required except chelex resin and the storage for the sample coated 903 paper is either at room temperature or for long term storage refrigerator or freezer. Optimal recovery of HIV-1 DNA from whatman 903 paper, DBS should be stored frozen. FTA filter paper is a treated filter paper for specimen collection that is designed to lyse proteins and bind nucleic acids, allowing stable storage of DNA at room temperature for >4 years [10]. FTA card contains 2 circles and 125 µl of sample required for each circle, the total volume is 250 µl.

The DNA present in the blood spot remains bound to the filter paper, while proteins and other cellular contaminants are degraded and later washed away. An added benefit is that there is no need to keep the specimen cool or frozen.

The simplicity of the assay is derived from the use of whole blood collected on a filter paper that lyses cells and binds DNA. Thus, the centrifugation and lysis procedures generally used prior to HIV-1 DNA PCR of liquid blood samples are not required [8].

The elution with Chelex-100 should eliminate the potential PCR inhibitors, since Chelex is a cation-chelating resin: the positively charged ions are captured by the resin, whereas the DNA, negatively charged, remains free in the solution [11].

In this study the results were compared between the dried blood samples on filter papers and the liquid venous samples on EDTA tubes, the results are concordant. Blood collected on filter paper proved reliable source for HIV-1 detection in infants. This method of collection is easier, requiring a smaller amount of blood about 50 µl of blood which is equivalent to one drop for whatman 903 cards and 2-3 drops for FTA cards for spotted the filter papers directly from heel prick when compared with 1 ml of blood by venipuncture. The use of DBS samples to detect HIV-1 infection by PCR was first demonstrated in 1991 [12].

DBS samples carry less of a biohazard risk than liquid samples and require minimal storage facilities, since the samples are stable at room temperature for prolonged periods and are easy to ship, facilitating centralized laboratory testing in developed regions [13].

Venipuncture of small infants is not always successful, once they have dried; blood samples on filter paper are no longer infectious and can be stored at room temperature

[14]. The filter papers either whatman 903 or FTA cards both are excellent in the field of infant diagnosis in HIV infection and the costs of these filter papers are: FTA cards are little expensive than whatman 903 cards.

In conclusion the study reveals that the samples can be directly coated onto the filter papers by heel prick instead of drawing blood from venipuncture. The method of collection is easier for health care workers and does not require any refrigeration for storage till the transportation to the central laboratory for testing especially in resource constrained settings.

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