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THE USE OF DRIED BLOOD SPOTS ON FILTER PAPER FOR THE DIAGNOSIS OF HIV-1 IN INFANTS BORN TO HIV SEROPOSITIVE WOMEN

S Mini Jacob, D Anitha, R Vishwanath, S Parameshwari, *NM Samuel

Abstract

Polymerase chain reaction (PCR) is the most sensitive test to diagnose HIV-1 infection among infants born to HIV seropositive mothers. The purpose of this study was to evaluate the use of dried blood spot (DBS) specimens for PCR and to compare it with whole-blood stored in tubes for HIV-1 DNA PCR. Five hundred and seventy-seven whole-blood infant samples were tested using HIV-1 qualitative in-house nested DNA PCR. Three hundred and fifty-nine samples were from infants at 48 hours of birth and 218 samples at second month. All positive samples tested from whole-blood and every fifth negative sample were coated onto filter paper. DNA was extracted from the filter paper and was amplified using in-house nested PCR. Among the whole-blood samples tested using HIV-1 DNA PCR, 19 of 359 (5.29%) samples were HIV-1 positive and 340 (94.7%) were negative at 48 hours of birth. At second month, 19 (8.7%) of the 218 samples were positive and 199 (91.2%) were negative. Using dried filter paper, 18 samples (95%) tested positive from 19 positive samples (using whole-blood) and 1 tested negative at 48 hours of birth. The 68 negative samples tested using whole-blood were also negative in the DBS test (sensitivity 95% and specificity 100%). At second month, 19 were positive and 40 samples (every fifth sample of 199) were negative (sensitivity and specificity, 100%). PCR performed using DNA extracted from filter paper permits the diagnosis of HIV-1 infection among infants born to HIV-1 seropositive mothers. This assay is simple, rapid, sensitive and specific and can be used in resource limited settings.

Key words: Dried blood spots, HIV-1 DNA PCR, HIV infection in infants, MTCT

HIV-1 transmission from an infected mother to her infant is estimated to be 21-43% in less-developed countries. Simple and inexpensive assays are necessary for the diagnosis of HIV-1 infection in infants. However, early diagnosis of HIV-1 infection in infants cannot be accomplished with conventional antibody tests due to maternal antibodies for up to 18 months after birth.

Polymerase chain reaction (PCR) is the preferred method to determine the HIV-1 infection status in infants born to HIV-1 seropositive women. PCR is commercially available and has high sensitivity and specificity. An independently validated qualitative HIV-1 DNA nested PCR of gag gene has been used on specimens from infants. Advantages of this assay include the ability to adapt assay reagents to local reagents, the potential to automate for large-scale testing and the ability to produce the results within a day.

Blood spotted onto filter paper facilitate the collection, transport and storage of blood samples for laboratory use. In 1963, Guthrie first published data demonstrating the feasibility of collecting neonatal blood samples onto filter paper for phenylketonuria testing of newborns. In 1987, Edward McCabe reported successful extraction of DNA from dried blood spots (DBS) collected on filter paper.

Once dried, the blood specimens on filter paper are no longer infectious.

The objective of this study was to evaluate the use of DBS specimens for PCR and to compare the specimens with whole-blood stored in tubes for HIV-1 DNA PCR.

Materials and Methods

Between January 2005 and May 2006, 577 infant blood samples in EDTA collection tubes were received in our department from various institutions in South India. Samples were collected from infants at 48 hours of birth (n = 359) and second month after birth (n = 218). These samples were stored as whole-blood at −20 °C until assayed. An HIV-1 qualitative in-house nested DNA PCR was performed from whole-blood for all infant samples. Positive samples tested from whole-blood along with every fifth negative sample were coated onto the filter paper.

Specimens were thawed by placing at room temperature and mixed by pipetting; then DNA extraction was performed from the whole-blood using QIAamp® DNA blood mini kit (Catalog no. 51104; Qiagen GmbH, Germany). According to the kit protocol, 200 µL of whole-blood was used for DNA extraction and the extracted DNA was stored at −20 °C for the PCR amplification.

Aliquots of the thawed whole-blood were spotted onto Isocode Stix (Schleicher and Schuell, Keene, NH, USA). Each Isocode Stix (Figure) contains four triangular sample collection areas, perforated for easy removal of the
Five microlitres of first round PCR product was used in the 95 µL master mix composition. The time program was same as first round PCR except the annealing temperature, which was changed from 55 to 65 °C to increase the specificity. One confirmed positive sample and one confirmed negative control were included in every assay.

To determine if the PCR is positive, 5 µL from each nested PCR was loaded onto an 1% agarose gel along with DNA ladder (1 kb). After electrophoresis, the gel was stained with ethidium bromide. The PCR products at 650 base pairs were visualized using a UV transilluminator.

### Results

**Qualitative HIV-1 DNA PCR using whole-blood frozen in tubes**

During the study period, 577 infant samples were tested for HIV-1 infection by the qualitative in-house nested DNA PCR. A total of 359 (62%) samples were collected from infants at 48 hours of birth and 218 at second month after birth (38%). At 48 hours of birth, 19 of the 359 (5.29%) samples were HIV-1 positive and 340 (94.7%) samples were negative for HIV-1 DNA.

At second month after birth, 19 (8.7%) of the 218 samples were positive and 199 (91.2%) were negative (Table 1).

**Qualitative HIV-1 DNA PCR using dried blood spots**

All of the specimens testing positive from the whole-blood samples and every fifth negative sample were coated on the filter paper. DNA was extracted from the DBS on filter paper and was amplified using in-house nested PCR.

Of 19 positive samples, 18 (95%) tested positive and 1 tested negative. The 68 specimens that tested negative when whole-blood was assayed were also negative in the DBS test. Therefore, the sensitivity of the test is 95% and the specificity 100%.

At second month after birth, 19 were positive and 40 negative samples (every fifth sample of 199) were negative (Table 2). Therefore, the sensitivity and specificity was 100% (Table 3).

### Table 1: Results of in-house nested polymerase chain reaction using whole-blood

<table>
<thead>
<tr>
<th>Infant samples</th>
<th>Whole-blood PCR results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total</td>
</tr>
<tr>
<td>At birth</td>
<td>359</td>
</tr>
<tr>
<td>Second month</td>
<td>218</td>
</tr>
</tbody>
</table>
Table 2: Results of in-house nested polymerase chain reaction using dried blood spots

<table>
<thead>
<tr>
<th>Infant samples</th>
<th>Dried blood spots PCR results</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>At birth</td>
<td>86</td>
<td>18</td>
<td>68</td>
<td></td>
</tr>
<tr>
<td>Second month</td>
<td>59</td>
<td>19</td>
<td>40</td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Results of specimens assayed by both assays

<table>
<thead>
<tr>
<th>Infant samples</th>
<th>No. of samples tested</th>
<th>Positive</th>
<th>Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole-blood at birth</td>
<td>359</td>
<td>19</td>
<td>340</td>
</tr>
<tr>
<td>Whole-blood at second month</td>
<td>218</td>
<td>19</td>
<td>199</td>
</tr>
<tr>
<td>DBS at birth</td>
<td>86</td>
<td>18</td>
<td>68</td>
</tr>
<tr>
<td>DBS at second month</td>
<td>59</td>
<td>19</td>
<td>40</td>
</tr>
</tbody>
</table>

Discussion

In the present study, we have performed in-house qualitative HIV-1 DNA PCR for infant samples (at birth and at second month after birth). HIV-1 DNA PCR was performed for whole-blood samples and for DBS coated on filter paper. This study performed a total of 722 DNA PCR tests. Nineteen infant samples were found to be HIV-1 positive using whole-blood and 18 of these were positive using DBS. Three hundred and forty infant samples were HIV-1 negative using whole-blood. Every fifth negative sample was coated on the filter paper; therefore, 68 negative samples were tested using HIV-1 DNA PCR and the results were concordant.

However in the second month, the results were concordant for 19 positive infant samples tested by PCR using whole-blood and DBS. Two hundred and eighteen infant samples were negative using whole-blood and 40 samples (every fifth sample of 218) coated on the filter paper were negative.

HIV-1 DNA PCR assay using DBS offers a sensitive and specific test appropriate for the diagnosis of HIV-1 in infants. In this study, the sensitivity and specificity of HIV-1 DNA PCR using DBS is 95 and 100% in birth samples. In a previous study, using Isocode cards, the sensitivity and specificity of the PCR using DBS were 90 and >98%, respectively. [6]

According to the National AIDS Control Organization in India, with approximately 27 million pregnancies each year and an overall estimated 0.3% prevalence rate of HIV infection among pregnant women, it is estimated that around 100,000 HIV-infected women deliver every year. [7]

Early diagnosis of HIV-1 infection in infants cannot be accomplished with conventional antibody tests due to the persistence of passively transferred maternal antibodies for up to 18 months after birth. [8]

Detection of HIV-1 DNA by PCR is an established method for determining infection status in children born to HIV-1-seropositive mothers. [9] PCR testing at birth is to obtain the earliest possible identification of infants infected in utero; and at two months is to identify all perinatally infected infants. DNA PCR sensitivity increases dramatically by two weeks and reaches 96% by one month of age. [10]

Two positive HIV-1 DNA PCRs at any time confirms HIV-1 infection. Once infection is confirmed, further HIV-1 DNA PCR testing is not required.

There are many advantages of the use of HIV-1 DNA PCR with dried blood specimens over HIV-1 DNA PCR with whole-blood specimens. [11] Whole-blood can easily be coated on the filter paper from heel stick or finger punctures in infants; thus avoiding the use of syringes and vaccutainer tubes. Blood coated on filter paper lyses the cells and binds the DNA. Therefore, the sample centrifugation and extraction procedures are reduced. Dried blood on filter paper appears biologically stable [12] and can be stored at room temperature. It can be transported easily and therefore it is convenient to use the DBS in resource limited settings.

In conclusion, qualitative in-house nested PCR using DNA extracted from filter paper permits the diagnosis of HIV-1 infection among infants born to HIV-1 seropositive mothers. This assay is simple, rapid, sensitive and specific and can be used in resource limited settings.

Further studies need to be evaluated using the filter paper directly coated with whole-blood from finger or heel stick in infants.

References


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Source of Support: Nil, Conflict of Interest: None declared.