

COMPARISON OF HIV-1 DNA PCR RESULTS OBTAINED FROM FRESH AND STORED DRIED BLOOD SPOTS OF HIV-1 EXPOSED INFANTS.

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ABSTRACT

Purpose of the study: We compared the results of fresh dried blood spot (DBS) collected on filter paper (Whatman 903) and stored at room temperature for six months and one year. Basic Procedures: HIV testing was done using Roche Amplicor HIV-1 DNA Kit. The Optical Density (OD) values of fresh samples, stored samples at six months and one year were compared. Main Findings: Thirty-two of 400 DBS tested HIV positive. There was no significant difference between OD values of fresh DBS and stored DBS at six months and one year. Principal Conclusions: HIV-1 DNA was stable for one year on filter paper at room temperature.

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INTRODUCTION

World AIDS Day Report 2011 states that there were 2.7 million [2.4 million–2.9 million] new HIV infections in 2010 worldwide, including an estimated 390 000 [340 000–450 000] among children. [1] According to UNGASS India country report, National AIDS Control Organization (NACO) estimates that 57,000 children are infected at birth in India each year, but it is yet to finalize estimates of children living with HIV/AIDS. A total of 63,889 children living with HIV are registered, out of which, 18,763 are receiving antiretroviral therapy (ART) as on January 2010.[2]

In many developed countries, ART for pregnant women and successful PMTCT programs (Prevention of mother to child transmission of HIV) have resulted in marked reduction of pediatric HIV infection to almost nil. A study from Chennai, India suggested that a substantial proportion of HIV-1 positive children infected perinatally in India are rapid progressors and will die in infancy unless diagnosed and treated early. [3] In order to scale up HIV diagnosis in infants and children, the Government of India had launched the Early Infant Diagnosis (EID) program in 2010 using dried blood spots (DBS) and HIV-1 DNA PCR. DBS collected on filter paper is preferred as it is convenient for collection, transport and storage. [4,5] Virologic assays, including HIV-1 DNA or RNA assays, represent the gold standard for diagnostic testing of infants and children younger than 18 months [6] while the Roche DNA-PCR test was found to be sensitive for the diagnosis of HIV in infants when a standardized algorithm was used to define infection status since 1996.[7]

Limited data is available on the exposure of DBS to high room temperatures and high humidity. [8, 9, 10] The room temperature in Chennai (formerly known as Madras, in

South India) remains consistently hot and humid throughout the year. In summer, the temperature can rise to 42°C in the months of April to June and during winter, (November to February) the average temperature is around 24°C. The relative average humidity ranges from 25% in the morning and can go up to 80% at nights. The objective of this study was to compare the results of fresh DBS coated on filter paper and stored DBS at room temperature and tested after six months and one year.

EXPERIMENT WORK

This is a retrospective study where we have analyzed the stability of the DNA in the filter paper after storing for a period of one year at room temperature in Chennai. The department (designated as one of the regional reference laboratory for EID HIV-1 DNA PCR by NACO) received DBS samples for HIV testing from various hospitals in Tamilnadu, Andhra Pradesh, Karnataka and Mumbai. Whole blood of infants born to HIV positive mothers were coated on Whatman 903 filter paper. They were dried and placed in ziplock bags with silica desiccants and transported to the department laboratory for testing.

Sample Preparation: Two 4mm spots were punched (from the area that is well saturated with blood) and placed into a 2 ml sterile screw cap tube. 1000 µl of Specimen Wash Solution (WS) was added and incubated for 10 minutes at room temperature (air conditioned room: 22-25°C). In between the incubation period, the tube was inverted 10-15 times ensuring submersion of the DBS. After incubation, the sample was centrifuged at 14000rpm for 1-2 minutes. The supernatant was aspirated and discarded. For the complete removal of hemoglobin, 1000 µl of WS was added and the above procedure was repeated

2 times. Then 200 µl of Working HIV-1 Extraction Reagent was added to the filter spot and incubated at 60°C for 60 minutes and thereafter at 100°C for 30 minutes. The samples were vortexed gently and micro centrifuged at 5000rpm for 20-30 seconds. Fifty microliter of supernatant of the samples was added to appropriate reaction tubes which contained 50 µl of the Working Master Mix (previously added in to 200µl PCR reaction tubes). PCR and detection of the amplicons procedures were as per the manufacturer's instructions (Roche Amplicor HIV-1 DNA test, version 1.5 assay). Optical density (OD) values were noted. The samples, which were found HIV-1 positive, were stored in zip lock covers with desiccants and stored at room temperature and retested at six months and the same was retested at one year. The OD values of fresh samples, samples at six months and one year of storage was compared and statistically analyzed using paired t- test.

RESULTS

Four hundred infants blood samples coated onto filter paper were tested. Thirty-two infants DBS samples were found to be HIV-1 positive. The age range of the HIV-1 positive infants were between one month to seventeen months. Twelve infants were in the age group of 1-5 months and 20 were between 6-17 months. The mean OD values of the fresh DBS samples were 2.27 and of the DBS samples that were stored for six months in room temperature were 2.39. The mean OD value of the DBS stored for one year was 2.31. Therefore, there was no significant difference in the OD values of HIV positive samples at baseline and at 6 months and 12 months of storage at room temperature (Table 1).

Table 1: Mean OD values of HIV positive samples.

| DBS | Mean | Std. Error Mean | 95% Confidence Interval | P value |
|-------------------|-------|-----------------|-------------------------|---------|
| Fresh - 6 months | .0094 | .1755 | -.3674 -.3486 | .958 |
| Fresh - 1year | .0969 | .1783 | -.2668-.4606 | .591 |
| 6 months - 1 year | .1062 | .1150 | -.1283-.3408 | .363 |

DISCUSSION

In this study, the stability of the DNA did not diminish even after one year of storage in extreme high temperatures and humidity. This is suggestive of optimal storage of the DBS at room temperature at least for the 1st one year. This is in contrast to the study where the authors concluded that there was diminished HIV-1 yield from DBS (coated on Whatman 903) after storage in a humid incubator at 37° compared to -20° C [8]. In 2004, a study from University of Washington, Seattle had suggested the stability of HIV DNA in whole blood collected on filter paper (FTA cards) and after more than 4 years of storage at room temperature in the dark detected virus at a similar rate to that of their initial test suggesting long term HIV-1 DNA stability. [10]

Knowledge on degradation of DNA in DBS is important as many countries opt for DBS testing. As Whatman 903 paper is cost effective, many developing countries use this filter paper for HIV-1 DBS testing including India. Added to this, for DBS testing, lesser volumes of blood are collected, dried, stored at room temperature and transported easily as the dried blood samples are not infectious. HIV RNA stability and RNA copies decreased with storage for up to 2 weeks for dried plasma spots [11] or 28 days on dried blood spots. [12]

Limited data is available on the DNA stability using Watman 903 paper and storing the DBS for a longer time and at high temperatures and humidity. In one study, DNA was detected on 903 filter paper after storing it for 15 weeks at 22 ° C. [9] In our study, the filter papers with DBS were stored with silica gels at high temperature and humidity. This kept away growth of fungus and molds. However, like other studies we noticed impaired lysis of the red blood cells on the 903 papers on long-term storage. This red color could not be washed off the paper fully. [8] However, the DNA stability was not compromised unlike what they observed in the IMPAACT Network study. [8] There was not much difference in the OD values of the IC (internal control) when used at six months and one year samples when compared with fresh DBS samples.

The limitation of our study was that the number of HIV-1 positive sample size. Similar studies need to be conducted with increased number of HIV-1 positive samples in order to suggest the optimal recovery of HIV-1 DNA from Whatman 903 paper in higher temperature, humidity and over time. Nonetheless, our study suggests the recovery of HIV-1 DNA did not diminish even after one year of storage in extreme high temperatures and humidity.

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