Indian Internet Journal of Forensic Medicine & Toxicology
Vol. 9, No. 4, October-December 2011, 82-85

IndianJournals.com
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Original Research

# Spectrophotometric Study of Yield of DNA from Fresh Blood and Dried Blood Samples

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**ABSTRACT**

DNA profiling has attained a pivotal role in forensic identification. It has assumed great significance in terms of exclusion as well as fixation of identity and is being relied upon by law enforcers and judiciary equally. A study was conducted to look at the effect of temperature on blood samples for isolation of DNA at MNL Medical College, Allahabad, U.P. With the increase in temperature yield of DNA decreases in both fresh and dried blood samples. Fresh samples stored at -20°C,0°C,4°C,40°C had 2.38% ,3.47%, 10.19%, 13.79% and dried blood samples had 0.78%, 0.96%, 2.56%, 5.90% decrease in DNA yield after one week.

**Keywords**: DNA isolation, Effect of temperature

## INTRODUCTION

DNA Fingerprinting or DNA typing (profiling) as it is now known was first described in 1985 by an English geneticist named Alec Jeffrey. Dr. Jeffrey found that certain regions of DNA contain sequences that were repeated over and over again next to each other. He also discovered that the number of repeated sections present in a sample could differ from one individual to other. By developing a technique to examine the length variation of these DNA repeat sequences Dr. Jeffrey created the ability to perform human identity tests. Only one-tenth of a single percent of DNA (about 3 million bases) differs from one person to the next. Scientists use these variable regions to generate a DNA profile of an individual, using samples from blood, bone, hair, and other body tissues and products[1].

DNA is present in every nucleated cell and is therefore present in biological material left at crime scenes. Some of the biological material that have been used for its isolation are blood and blood stains, semen and semen stains, bones ,teeth, hair with root, hair shaft, saliva ,urine, faeces, debris from fingernails, muscle tissue and others.[2]

DNA can provide insights into many aspects to identify potential suspects who's DNA may match evidence left at crime scenes, exonerate persons wrongly accused of crimes, identify crime and catastrophe victims, establish paternity and other family relationships.[3]

Evidences collected during criminal investigations are subjected tovarious types of environmental insults like, varying amount of humidity, temperature ranging from extreme cold to extreme heat, Ultraviolet light, soil contamination, bacterial contamination and so on. Not only these environmental insults but also collection (as fresh samples or dried form), transportation, storage affect the integrity of DNA. The importance of proper DNA evidence collection cannot be overemphasised. If DNA sample is contaminated from the start, obtaining unambiguous information becomes a challenge at test and an important investigation can be compromised. Samples for collection should be carefully chosen so that we can prevent needless redundancy in the evidence for a case.[4]

Considering these factors an attempt has been made in the present study to observe the effect of various environmental insults and the effect of method of collection on the yield of DNA from the samples.

## MATERIAL AND METHODS

Material for study comprised of blood samples collected from the patients referred to the outpatient and department of Pathology, Moti Lal Nehru Medical College, Allahabad during the period extending from September 2010 to February 2011.

A total of 48 blood samples were collected. They were divided in two groups of 24 each. From one half of 24 samples dried blood spots were created on Whatmann filter paper and second half was stored as fresh blood samples.

Fresh blood samples and dried blood spots were kept at temperatures ranging from -20°C to 40°C for a period of five weeks to study the effect of temperature, time and storage conditions on recovery of DNA.

DNA was isolated from the samples corresponding to zero time, one week, two weeks, three weeks, four weeks and five weeks. First extraction was done at zero time which was between 1 to 4 hrs from the time of sample collection.

Special coding system was devised by researcher so that samples were not mixed up. DNA isolation was done by using Qiagen QIAmp DNA Mini Kit for fresh and dried blood and optical density of isolated DNA was measured by spectrophotometer. The amount of blood taken for fresh blood samples was 200µl and for dried blood spots three punched out spots of size 3mm were taken for isolation of DNA. The samples were also run on 0.8% agarose gel in order to correlate the results obtained by spectrophotometer.

## METHODOLOGY

Fresh blood samples were collected in vacutainers containing an anticoagulant {EDTA}.It was mixed properly but gently for some time and was stored at 4°C before it reaches laboratory. Ice was used during transportation. Dried blood spots were prepared from fresh unclotted whole blood. The blood was dispensed in 200µl aliquot with a micropipette on to Whatmann filter paper. The prepared stains were dried at room temperature before storage.

For isolation of DNA from blood samples QIAGEN QIAmp DNA Mini Kit[5] was used according to manufacturer's instruction.

The concentrations of DNA samples were checked by the use of UV spectrophotometry. Optical density of isolated DNA was measured at 260 and 280 nm using TE buffer as the diluent. Then concentration of DNA was calculated using the formula

DNA Conc. (µg/ml) = OD 260/280 X 50 µg/ml X D.F. X Total Volume in ml

Conc. =Concentration OD=Optical Density

DF=Dilution Factor

50=constant as one OD 260 UNIT=50µg/ml for ds DNA

Total volume= Volume taken to dilute the sample in ml.

## RESULTS

The study was conducted in the Department of Forensic Medicine and Toxicology, Moti Lal Nehru Medical College, Allahabad. Blood samples were collected from outpatient department and department of pathology Moti Lal Nehru Medical College, Allahabad.

Extraction of DNA was done from 24 fresh and 24 dried blood samples stored at different temperatures for a period of five weeks.

Mean Optical density and DNA yield of fresh blood samples kept at room temperature at zero time was found to be 1.84±0.05 and 23.05±0.68µg/ml respectively. Minimum and maximum yield of DNA was 20.00µg/ml and 24.875µg/ml respectively.

Mean DNA yield of dried blood samples stored at room temperature at zero time was found to be 149±2.02ng. Minimum and maximum yield of DNA was 156ng and 148ng respectively. The yield of DNA of fresh and dried blood samples were also compared with agarose gel electrophoresis.

**Table1:_DNA yield of Fresh blood samples (FBS) stored at different temperatures (-20°C to 40°C ) during a period of five weeks**

| S.l.No. | Temp. | DNA yield of Fresh blood samples in µg/ml during a period of five weeks | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 wk | 1wk | 2wks | 3wks | 4wks | 5wks |
| 1. | -20°C | 23.05±0.68 | 22.50±1.15 (2.38%) | 21.55±0.41 (6.50%) | 20.45±0.38 (11.27%) | 19.87±0.197 (13.79%) | 19.75±0.10 (14.31%) |
| 2. | 0°C | 23.05±0.68 | 22.26±1.15 (3.47%) | 21.20±0.30 (8.02%) | 19.32±0.45 (16.18%) | 18.57±0.27 (19.43%) | 17.62±0.12 (23.55%) |
| 3. | 4°C | 23.05±0.68 | 20.70±0.42 (10.19%) | 18.25±0.53 (20.82%) | 17.02±0.78 (26.16%) | 14.85±0.77 (35.57%) | 13.50±0.51 (41.40%) |
| 4. | 40°C | 23.05±0.68 | 19.87±0.04 (13.79%) | 17.21±0.04 (31.45%) | 13.25±0.15 (42.51%) | 11.35±0.10 (50.75%) | 10.51±0.01 (54.40%) |

Table 1 shows the yield of DNA decreases uniformly in the fresh blood samples stored at -20°C and was 2.38%, 6.50%, 11.27%, 13.79% and 14.31% less at 1wk, 2wks, 3wks, 4wks and 5wks respectively than that of yield at zero time {23.05±0.68}.

Whereas in the fresh blood samples stored at 40°C the yield of DNA decreases more drastically and was 13.79%, 31.45%, 42.51%, 50.75%,54.40% less at 1wk, 2wks, 3wks, 4wks and 5wks respectively than that of yield at zero time {23.05±0.68}.

**Table 2:_DNA yield of Dried blood samples (DBS) stored at different temperatures (-20°C to 40°C ) during a period of five weeksa**

| S.l.No. | Temp. | DNA yield of Dried blood samples in ng during a period of five weeks | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 wk | 1wk | 2wks | 3wks | 4wks | 5wks |
| 1. | -20°C | 149±2.02 | 149±0.83 (0.78%) | 148±1.23 (1.17%) | 148±0.37 (1.74%) | 147±0.04 (2.62%) | 146±0.11 (3.24%) |
| 2. | 0°C | 149±2.02 | 149±0.56 (0.96%) | 148±0.96 1.35%) | 146±1.03 (2.63%) | 146±0.02 (3.2%) | 144±0.14 (4.54%) |
| 3. | 4°C | 149±2.02 | 147±0.13 (2.56%) | 145±0.04 (3.95%) | 141±1.56 (5.59%) | 138±0.16 (8.5%) | 133±0.31 (9.73%) |
| 4. | 40°C | 149±2.02 | 142±0.01 (5.90%) | 136±0.78 (9.42%) | 134±0.37 (11.01%) | 130±0.64 (13.48%) | 127±0.14 (15.80%) |

In dried blood samples Table 2 shows a very insignificant decrease in yield of DNA stored at -20°C and was 0.78%, 1.17%, 1.74%, 2.62% and 3.24% less at 1wk, 2wks, 3wks, 4wks and 5wks respectively than that of yield at zero time{149±2.02}.

However, the yield of DNA of dried blood samples stored at 40°C decreases uniformly and was 5.9%, 9.42%, 11.01%, 13.48%, and 15.80% less at 1wk, 2wks, 3wks, 4wks and 5wks respectively than that of yield at zero time {149±2.02}.

## DISCUSSION

Antony Hassall (2008) found that the quality of DNA extracted from liquid or dried blood was not adversely affected by storage at 48° C for up to 24 hrs. He found that good amounts of DNA can be extracted using the QIAmp kit (up to 17 µg) with no significant differences between t=0 and t=24 time point. The amounts of DNA extracted from him in the three different papers were FTA 120ng, BIOSPOT- 120ng, Whatmann 903- 75ng.[6] In our study we came with similar observation that the DNA yield was not very much affected in both fresh and dried blood samples stored at 40°C for a period of 1 week. The amount of DNA extracted was up to 19µg(FBS) and 142 ng(DBS).In our study yield is more because the sample amount taken for DNA extraction is more.

Caroline Mitchell (2008) analysed 916 DBS reactions including 100 DBS stored at baseline, 418 stored at -20°C, and 398 at 37°C for HIV DNA detection. He found that HIV-1 DNA yield from dried blood spots was diminished after storage in a humid incubator at 37°C compared to -20°C. Samples stored at -20°C showed little change in the probability of detecting HIV-1 DNA over time; odds ratio (OR) 0.93 after storage for one year. Samples stored at 37°C demonstrated a significant change in detection for one year (OR 0.29).[7]In this study we found that dried blood samples stored at -20°C for a period of five weeks showed very less(3.24%) decrease in the yield of DNA as compared to those stored at 40°C(15.80%).

Mc Nally L. (1988) analysed the effect of environmental conditions including the exposure of DBS to varying amount of relative humidity (0, 33, 67 and 98%), heat (37°C) , ultraviolet light for a period of 5 days and the effect of drying over a four day period in whole blood collected with and without EDTA and found that under these conditions the integrity of DNA is not altered such that false RFLP patterns are obtained. The only effect observed was that the overall RFLP pattern became weaker but individual RFLP fragments are neither created nor destroyed.[8] Similar results were obtained in our study, DNA was isolated from blood for five weeks but the quantity decreases.

Bomjen (1996) extracted and quantitated DNA using spectrophotometer from blood samples which were stored at room temperature, 4°C, -20°C, and -70°C for different duration varying from 1, 2, 4 and 8 weeks with or without anticoagulant/preservative (EDTA or Heparin). His result suggested that higher amount of genomic DNA could be recovered from blood samples stored at temperature 4°C or below in the presence of EDTA or heparin.[9] In our study we found that the highest amount of DNA can be recovered from the samples stored at -20°C.

Nederhand (2003) found that blood storage up to 28 days frozen at 4°C and at room temperature did not influence the amount or quality of DNA to an unsatisfactory level. However, the amount of extracted DNA was decreased in frozen samples and in samples stored for > 7 days at room temperature. Similar findings were seen in our study. We were able to extract DNA for a period of 35 days from samples stored at 4°C and 40°C.

Sabri I. (2003) found that recoverable DNA was found in all fresh blood samples even after 3 months of storage however the amount of DNA recovery decreases with time which is more marked in the initial phase followed by a slow and uniform decrease in DNA yield. The DNA isolation was done by standard phenol-chloroform method and optical density of the isolated DNA was measured by spectrophotometer. Similar results were obtained in our study.

## CONCLUSION

With the increasing incidence of criminal activities in present time identification of culprit became a very important challenge to us. In such a situation DNA fingerprinting came out to be a very important method for identification. However, the type and time of sample collection from the scene of crime for DNA extraction influence the results in many ways. In our study we found that:-

- DNA was recoverable from both fresh and dried blood samples kept for a period of five weeks.
- The yield of DNA decreased with time in both fresh and dried blood samples. There was uniform decrease of 10.19%,20.82%,26.16%,35.57%,41.40% in case of fresh blood samples whereas in dried blood samples it was 2.56%,3.95%,5.59%,8.50%,9.73% after 1wk, 2wks 3wks, 4wks and 5wks of storage at 4°C.
- With the increase in temperature yield of DNA decreases in both fresh and dried blood samples. Fresh samples stored at -20°C,0°C,4°C,40°C had 2.38% ,3.47%, 10.19%, 13.79% and dried blood samples had 0.78%, 0.96%, 2.56%, 5.90% decrease in DNA yield after one week.
- The quality and quantity of DNA extracted from dried blood samples was better as compared to fresh blood samples. Stored at different temperatures for a period of five weeks.

Hence, we can conclude that recovery of DNA from dried blood samples is better so we can take advantage of storing specimens in dried blood form for various criminal investigations during visit to the scene of crime.

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