

Effect of temperature and ageing on recovery of DNA in forensic blood samples

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Abstract

Blood Samples were kept at different temperature for a period of 3 months to know the amount of quantitative DNA recovery from these samples in different conditions of temperature and ageing [Time]. The DNA isolation was done by standard phenol-chloroform method and optical density of the isolated DNA was measured by spectrophotometer. Recoverable DNA was found in all samples even after 3 months of study however the amount of DNA recovery decreased with time which is more marked in the initial phase followed by a slow and uniform decrease in DNA yield. Mean DNA yield at zero time at room temperature from fresh blood samples was found to be 1027 ± 361 $\mu\text{g/ml}$. Minimum and maximum yield was found to be 432.7 $\mu\text{g/ml}$ and 1655 $\mu\text{g/ml}$ respectively.

Keywords: Forensic, DNA, Blood, Recovery, Time, Temperature

Introduction

The present study was conducted in the Department of Forensic Medicine and Toxicology, Jawaharlal Nehru Medical College, Aligarh Muslim University, Aligarh, INDIA. Blood samples were collected from out-patient and the Department of Pathology, J.N. Medical College Hospital, Aligarh Muslim University, Aligarh, INDIA.

To observe the effect of temperature and time on DNA recovery the blood samples were kept in conditions similar to that of Indian environment simulated in Forensic DNA laboratory and mortuary of Department of Forensic Medicine and Toxicology. A total of twenty four samples were taken and kept in different conditions of temperature for three months. DNA was isolated from the samples which were kept at different temperature and environmental conditions. Zero Time is the time when the first isolation was done which can be same or different from time of sample collection from the subject. Fresh blood sample and anticoagulated blood samples were collected and stored for 1, 2, 4, 8 and 12 weeks duration the temperature ranging from -20°C to $+50^{\circ}\text{C}$ to study the yield of intact DNA.

Material and Methods

Fresh samples were collected in plain as well as EDTA/ Citrate vials. Blood stored in the blood bank [anticoagulant used was CPDA, Creatinine Phosphate Dextrose Adenine] was also taken for

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the study. Record of defrosting and temperature of deep freezer was kept routinely.

The DNA isolation was done from the same sample corresponding to zero time, one week, two weeks, four weeks, eight weeks and twelve weeks.

The DNA isolation was done by standard phenol-chloroform method and optical density of the isolated DNA was measured by spectrophotometer. The amount of blood taken is 2.5 ml of whole blood. The samples were also run on 0.8% agarose gel in order to correlate the results obtained by spectrophotometer.

The study was conducted in the following selected samples

1. Blood
 - A. Fresh Blood
 - B. Stored Blood

Volume of Blood Taken: 2.5 ml

The concentration of DNA was calculated by the given formula

$$\text{Conc. of DNA } (\mu\text{g/ml}) = \text{OD}_{260} \times 50\mu\text{g/ml} \times \text{D.F.} \times \text{Total Vol. in ml}$$

Conc. = Concentration OD = Optical Density

Nm = Nano-Meter D.F. = Dilution Factor

Formula: $\text{OD}_{260} \times \text{conversion factor} = \mu\text{g/ml of nucleic acid}$

$\text{OD}_{260} \text{ Unit} = 50\mu\text{g/ml for dsDNA}$

Results:

DNA Yield at zero time at room temperature

Mean DNA yield at zero time at room temperature from fresh blood samples was found to be $1027 \pm 361 \mu\text{g/ml}$. Minimum and maximum yield was found to be $432.7 \mu\text{g/ml}$ and $1655 \mu\text{g/ml}$ respectively

In blood samples kept at -20°C recovery was decreased with time but we were able to recover good amount of DNA even after a period of 90 day from the time of start of experimentation. It was almost similar to that at fresh samples [decreased only 13.24% after 12 weeks].

In the blood samples which were kept at 4°C recovery was decreased with time [decreased 28.6% after 3 months] but we were able to recover DNA after a period of 90 day from the time of start of experimentation. Which was less as compared to the yield at -20°C but more as compared to yield at room temperature [71%] and 50°C [87%].

Table 1: Showing yield of DeoxyriboNucleic Acid [DNA] blood samples at minus 20°C over a period of twelve weeks time.

	Time	Mean DNA Yield in $\mu\text{g/ml}$ [Mean \pm S.D.]	Percent Decrease in DNA Yield
1	Zero	1027.35 \pm 361	—
2	One Week	1013 \pm 255	1.36
3	Two Weeks	974 \pm 157	5.16
4	Four Weeks	949 \pm 120	7.6
5	8-10 Weeks	923.5 \pm 352	10.1
6	11-12 Weeks	891 \pm 552	13.24

Table 2: Showing yield of DeoxyriboNucleic Acid [DNA] blood samples at 4 °C over a period of twelve weeks time.

	Time	Mean DNA Yield in $\mu\text{g/ml}$ [Mean \pm S.D.]	Percent Decrease in DNA Yield
1	Zero	1027.35 \pm 361	
2	One Week	915.35 \pm 303	11
3	Two Weeks	875.2 \pm 177	15
4	Four Weeks	747 \pm 115	27.3
5	8-10 Weeks	690 \pm 201	33
6	11- 12 Weeks	596 \pm 67.5	42

In the blood samples which were kept at room temperature. Recovery was decreased with time but we are able to recover DNA after a period of 90 day from the date of start of experimentation.

In the blood samples which were kept at 50 °C. Recovery was decreased with time. Recovery was decreased drastically after one to two weeks time. The recovery was poor after a period of 90 day from the date of start of experimentation.

Discussion

In forensic situations the collection, transportation, isolation and storage of DNA is different due to availability of samples from varied situations of collection which is affected by time of collection

Table 3: Showing yield of DeoxyriboNucleic Acid [DNA] blood samples at Room temperature over a period of twelve weeks time.

	Time	Mean DNA Yield in $\mu\text{g/ml}$ [Mean \pm S.D.]	Percent Decrease in DNA Yield
1	Zero	1027.35 \pm 361	
2	One Week	686 \pm 309	33
3	Two Weeks	608 \pm 158	41
4	Four Weeks	385 \pm 151.5	62.5
5	8-10 Weeks	378 \pm 147.75	63.2
6	11- 12 Weeks	299 \pm 59.58	71

Table 4: Showing yield of DeoxyriboNucleic Acid [DNA] blood samples at 50°C over a period of twelve weeks time.

	Time	Mean DNA Yield in $\mu\text{g/ml}$ [Mean \pm S.D.]	Percent Decrease in DNA Yield
1	Zero	1027.35 \pm 361	
2	One Week	450.5 \pm 135	56
3	Two Weeks	244.3 \pm 79.5	76
4	Four Weeks	238 \pm 31.19	77
5	8-10 Weeks	182.5 \pm 27.5	82.3
6	11- 12 Weeks	137 \pm 26.5	87

of samples, temperature, humidity, and contaminations etc of samples, which adversely affect the yield of required amount of intact DNA for identification studies. Onward transmission of the samples from the site of collection to the laboratories and the analysis in the laboratory is also affected by the time and temperature of storage of the isolated DNA. The study was performed on blood samples which constituted a major chunk of the forensic samples to know the effect of time and temperature on the isolation of intact DNA.

The mean DNA yield at -20°C after one week was $1013 \pm 255 \mu\text{g/ml}$ and was less as compared to mean yield of DNA at zero time [$1027 \pm 361 \mu\text{g/ml}$]. The yield of DNA decreased uniformly in all the blood samples kept in this condition. The mean DNA yield at -20°C after twelve weeks was $891 \mu\text{g/ml} \pm 552$ and the minimum and maximum yield was found to be $391 \mu\text{g/ml}$ and $1434 \mu\text{g/ml}$ respectively.

The mean DNA yield at 4°C after one week was 915 ± 303 µg/ml and was less as compared to mean yield of DNA at zero time [1027 ± 361 µg/ml]. The yield was also less as compared with the yield at -20°C.

The mean DNA yield at room temperature after one week was 686 ± 309 µg/ml and was less as compared to mean yield of DNA at zero time [1027 ± 361 µg/ml]. The yield of DNA decreased uniformly in all the blood samples kept in this condition. The yield was also less as compared with the yield at -20°C and room temperature.

The mean DNA yield at 50°C after one week was 450.5 ± 135 µg/ml and was less as compared to mean yield of DNA at zero time [1027 ± 361 µg/ml]. The yield of DNA decreased drastically within first month in all the blood samples kept in this condition. The yield was also less as compared with the yield at -20°C, room temperature and 50°C.

Yield of DNA decreases in all the samples which were kept in different conditions of temperature and time. In the samples kept at -20°C the in DNA yield was decrease uniformly at a slower rate as compared to the decrease in yield at 4°C and more temperature. As the temperature increases the e.g. in case 50°C there was as initial drastic decrease in DNA yield followed by uniform decrease. This pattern was seen in all the samples which were kept in different conditions.

The decrease in DNA yield was more in samples kept at higher temperature. The reason for an initial drastic decrease in samples kept at 50°C and room temperature [22°C- 32°C] may be initial fast destruction of DNA as a result of degradation by enzymes and contamination followed by slow destruction afterwards.

Samples stored at -70°C have significantly more DNA [900 micrograms/10 ml] as compared to the samples with no storage [Polakova et al 1989] similar pattern was seen in our study.

There was no considerable recovery of DNA after two weeks in samples stored at room temperature in case of bomjen et al 1996 however we were able to recover good amount of DNA even after two to twelve weeks the reason may be due improved methodology and the large sample [2.5 ml] of whole blood in our case.

Summery and conclusion:

Yield of DNA decreases in all the samples which were kept in different conditions of temperature and time. In the samples kept at -20°C the in DNA yield was decrease uniformly at a slower rate as compared to the decrease in yield at 4°C and more temperature. As the temperature increases the e.g. in case 50°C there was as initial drastic decrease in DNA yield followed by uniform decrease. This pattern was seen in all the samples which were kept in different conditions. The decease in DNA yield was more in samples kept at higher temperature. The reason for an initial drastic decrease in samples kept at 50°C and room temperature [22°C- 32°C] may be initial fast destruction of DNA as a result of degradation by enzymes, environmental factors like heat and contamination followed by slow destruction afterwards.

It was concluded that the DNA recovery in blood decreased with time. Temperature also has an inverse effect on the amount of recovery. As the temperature increases the amount of recovery decreased. The reason may be due to degradation of DNA as a result of enzymatic action, contamination

and environmental factors like heat, light and humidity etc. Higher recovery of DNA was found in samples which were stored at low temperature.

After three months of storage the yield of DNA in blood samples was similar to the yield at room temperature when the samples were stored at -20°C [decreased only 13.24%] while there was a loss of 42% at 4°C, 71% at room temperature and 87% at 50°C therefore the storage was found to be ideal at -20°C as compared to 4°C. Though the yield at room temperature and 50°C was 29% and 13% it can be utilized for isolation of DNA from forensic samples collected from various sites in summer season where the temperature ranges from 35°C to 50°C.

Although the yield of DNA decreases with time but DNA can be recovered from these samples even after 90 days and even after [data not shown]. The study can be further continued in blood and seminal samples as well other samples like soft tissue, bones, hair etc.

The time frame can further be extended so as to extend the applicability of this pilot study. The temperature may also be increased or decreased in the same way.

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