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Isolation and Quantification of DNA from Blood Samples on Absorbent and Non-absorbent Surfaces

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Abstract Over the past twenty years, DNA analysis has revolutionized forensic science, and has become a dominant in law enforcement. Today, DNA evidence is a key to the conviction or exoneration of suspects of various types of crime, from theft to rape and murder.DNA is used in forensic laboratories for establishing origin if biological fluids found at the crime scene. A forensic analyst routinely encounters a variety of challenging biological samples, many of which contain DNA which has been exposed to environmental insults.As blood and blood stains are found at the crime scene, it important to isolate DNA from blood and blood stains. Isolation of DNA from anti-coagulated blood and blood stains were carried out using Bunce method and from clotted blood. Ideally blood sample obtained from suspect or victims should be placed immediately storage but in practice there will always be a deal between collection and sample processing. It is important to determine whether this deal introduces changes into the result obtained from the sample. Therefore, a first aim of this study was to assess the impact, on extracted DNA quality and quantity, from absorbent and non-absorbent surfaces.

In the present study it was observed that the collection of blood sample from absorbent and non-absorbent surfaces to isolate the DNA by using Swized method and scratched method. By using PCR method we can detect the quality and quantity of DNA obtained from the absorbent and non-absorbent surfaces. Collection and preservation of various biological materials from crime science thus plays an important role in the isolation of DNA from forensic samples for establishing the link between the crime suspects.

Keywords: Forensic science; Blood; Blood stains; DNA extraction.

Introduction

Forensic science is a science in the service of crime detection, law and justice. Its practice includes scientist of various discipline e.g. Chemists, physicists, biologist, serologists, firearm experts, toxicologists, document experts and others. Despite a wide overlap with the field of forensic medicine, forensic science as an integral component of the criminal justice system. In most of the crime scene blood and blood stains are found. So it is important to isolate DNA from the blood or blood stains on absorbents and non-absorbent surfaces. DNA is material that governs inheritance of eye color, hair color, stature, bone density and many other human and animal traits. DNA is a long, but narrow string-like object. A one foot long string or strand of DNA is normally packed into a space roughly equal to a cube 1/millionth of an inch on a side. This is possible only because DNA is a very thin string. Our body's cells each contain a complete sample of our DNA. One cell is roughly equal in size to the cube described in the previous paragraph. There are muscle cells, brain cells, liver cells, blood cells, sperm cells and others. Basically, every part of the body is made up of these tiny cells and each contains a sample or complement of DNA identical to that of every other cell within a given person. There are a few exceptions. For example, our red blood cells lack DNA. Blood itself can be typed because of the DNA contained in our white blood cells. Not only does the human body rely on DNA but so do most living things including plants, animals and bacteria. A strand of DNA is made up of tiny building-blocks. There are only four, different basic building-blocks. Scientists usually refer to these using four letters, A, T, G, and C. These four letters are short nicknames for more complicated building-block chemical names, but actually the letters (A, T, G and C) are used much more commonly than the chemical names so the latter will not be mentioned here. Another term for DNA's building blocks is the term, "bases." A, T, G and C are bases. This bases forms both coding and non-coding DNAs which may vary from one individual to another. These DNA variations can be used to identify people or at least distinguish one person from another. The following paper shows the method to isolate DNA from various sources, which will help to find out the criminal.

Materials and Methods

1. Blood DNA Isolation from Non-absorbent surface like Glass, Iron, Table top

100 µl of blood was taken on a glass slide with pipette and dried it for half an hour. Then with a spatula blood was collected into centrifuge tube and added 600 µl of lysis buffer. Centrifuged at 4000 rpm for 5min. The supernatant was discarded. Then added 2 volume of solution B and shaked for 1min. Incubated the sample at 370C for 30 min. Added 650 µl of ice cold chloroform and mixed it well. Centrifuged the solution at 4000 rpm for 10 min. Transfered supernatant into eppendrof tube and added equal amount of ice cold isopropanol. Then tube was incubated at 40C overnight and centrifuged the eppendrof tubes at 12000 rpm for 5min. Discard the supernatant and washed the pellet with 70% ethanol by centrifuging it at 12000 rpm for 5 min. discarded the supernatant and air dried the pellet. Dissolved the DNA pellet in 50-100µl of TE buffer.

2. Blood DNA Isolation from Absorbent Dried Filter Paper, Soil, Cotton

100µl of blood was put on filter paper and allowed to dry for an hour. After drying, the stained filter paper was cut into small piece and taken into centrifuge tube. 300µl of solution was added to it. Mixture was vortex vigorously for 2 min and incubated at room temperature for 5min. Sample was then centrifuged at 10000rpm for 5min. Supernatant was removed. 180µl of 2.5% SDS and 20µl 2X-prot K buffer was added and incubated at 560C for 1 hr. Chloroform and Isoamyl alcohol was added and centrifuged at 10000rpm for 4min. Aqueous phase was transferred to fresh tube and added 50µl of 3M sodium acetate with 40µl of isopropyl alcohol. Again tubes were centrifuged at 10000rpm for 4min. Pellet was washed with 70% ethanol. After air drying the tube 50-100µl of TE buffer was added and pellet was dissolved in it.

Quantitative Analysis of DNA By DPA Method

10lµ of DNA sample was taken in a test tube using a micropipette. To make up the volume to 2ml, 1990l distilled water was added. Blank was maintained with distilled water in the place of DNA. After making up the volume to 2ml, 3ml DPA reagent was added and kept in a water bath at boiling temp (1000C) for 15 min. Switch on the colorimeter and allow warming up. Set the wavelength to 595nm. Wash the cuvette with dist H2O. Dry it with tissue paper. Insert the cell containing 2000µl of TE into chamber as blank. Set the reading to zero. Set the wavelength to 595nm. Remove the cuvette from its compartment and discard the TE. Add the above prepared sample to the cuvette. Insert the cuvette into the sample compartment and cover it. Take the O.D value directly from the screen. Likewise take down the O.D values of the entire sample.

Results and Discussion

Samples were collected and processed to obtain DNA from blood and blood stains. To run the DNA sample 0.8% agarose gel was prepared. It was poured in a gel tray. After solidifying the combs were removed. The gel tray was then put into tank containing TAE buffer. 10µl of DNA sample was mixed well with 5 µl of loading dye on paraffin paper. Then the samples were loaded carefully on the gel by using micro pipette without disturbing the gel. After completion of loading samples run it at 100V for minimum one and half hour. After completion of agarose gel electrophoresis then the gel was placed in to UV transilluminator. It shows the bands of DNA samples. By watching UV transilluminator we can find the DNA bands. It will show orange color bands.

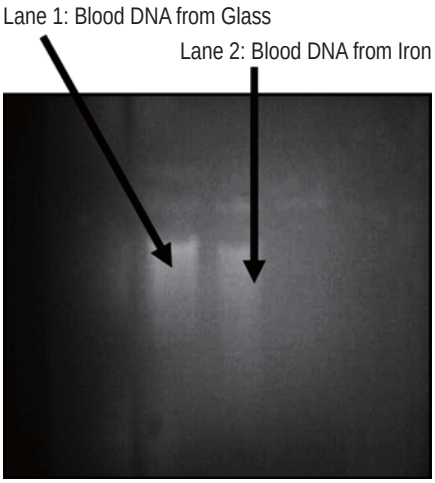

Fig. 1

Fig. 2

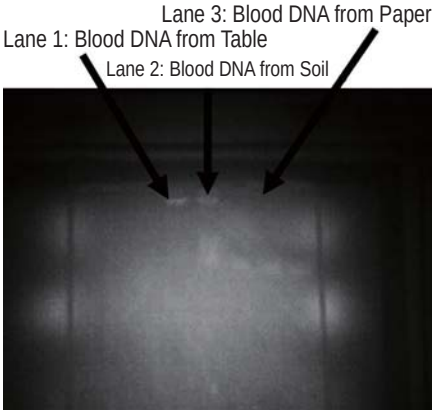

Fig. 3

Table 1.

Vial No.	Sample volume of DNA	DPA	Dist. H_2O		O.D value
1	10µl	3ml	1ml	Incubate the sample for 15 min	0.15
2	10µl	3ml	1ml		0.26
3	10µl	3ml	1ml		0.18
4	10µl	3ml	1ml		0.06
5	10µl	3ml	1ml		0.16
6	10µl	3ml	1ml		0.20

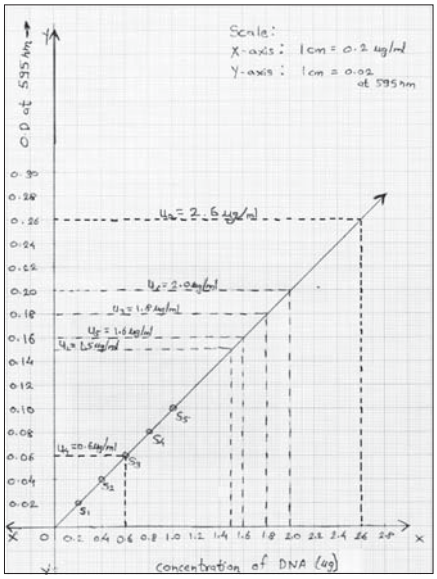

Fig. 4

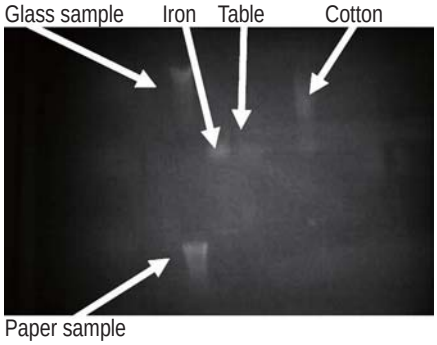

Fig. 5

Observation Table for Quantitative Analysis of DNA by DPA Method (Table 1)

Calculation of DNA concentration (by graph)

1. For Glass surface sample

Amount of DNA present in 10µl of sample is 1.5 µg. Hence concentration of DNA as obtained from Glass surface is 0.15 µg/ µl

2. For Iron surface Sample

Amount of DNA present in 10µl of sample is 2.6 µg. Hence concentration of DNA as obtained from Iron surface is 0.26 µg/ µl

3. For Table surface sample

Amount of DNA present in 10µl of sample is 1.8 µg. Hence concentration of DNA as obtained from Table surface is 0.18 µg/ µl

4. For Soil sample

Amount of DNA present in 10µl of sample is 0.6 µg. Hence concentration of DNA as obtained from Soil is 0.06 µg/ µl

5. For Cotton sample

Amount of DNA present in 10µl of sample is 1.6 µg. Hence concentration of DNA as obtained from Cotton is 0.16 µg/ µl

6. For filter paper sample

Amount of DNA present in 10µl of sample is 2µg. Hence concentration of DNA as obtained from Filter paper is 0.2 µg/ µl

Restriction Fragment Length Polymorphism was performed. Various bands patterns and smear was observed as in following fig.

Conclusion

In most of the crime scene blood or blood stains are found as evidence in more quantity. So isolation of DNA from blood on absorbent and non-absorbent surface was studied.

From the above experiment performed we can conclude that the concentration of DNA on non-absorbent surface is more as compared to absorbent surface. So the blood and blood stains on non-absorbent surfaces like floor, weapons, table, glass, non-absorbent wall etc. should be collected to get good quality and quantity of DNA, which further can reveal many queries regarding the crime scene.

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