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Comparative Analysis of DNA Extraction Techniques and their Analysis from Blood Stained Clothes

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Abstract

The advancement of forensic DNA analysis has revolutionized criminal investigations, providing crucial evidence for identifying suspects and establishing their involvement in various crimes. The extraction of DNA from blood stains is a fundamental step in forensic investigations, enabling the profiling of individuals for identification and genetic analysis. The objective of this research is to evaluate and compare the DNA extraction methods on different fabric types, which is cotton, and silk. Blood stains were spotted in both the cloths and incubated for a certain period of time. After which, DNA extraction was done using phenol chloroform method. The quality and quantity of the extracted DNA are assessed using established techniques such as Gel electrophoresis and qPCR amplification. The results of the experiments indicate variations in the efficiency of DNA extraction methods depending on the fabric type. Ideally both the fabrics have shown similar pattern of DNA recovery but Cotton fabrics demonstrate higher DNA recovery rates, of DNA recovery but Cotton fabrics demonstrate higher DNA recovery rates, compared to silk fabric but this is non-significant. In conclusion, provides valuable insights into the challenges and opportunities associated with DNA extraction from blood stains on different fabrics for forensic investigation profiling. The research findings contribute to the advancement of forensic DNA analysis and offer practical guidance for forensic scientists to optimize their methods when handling blood-stained fabric evidence. Further research and development in this field will undoubtedly strengthen the forensic community's ability to solve crimes and administer justice with greater accuracy and reliability.

Keywords: DNA Extraction; Human DNA; Forensic DNA.

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INTRODUCTION

As forensic science works along the human body and attempts to establish to identify humans is a main theme to investigate and study.[1] In this way, forensic analysis is a specialty that studies psychological, physical, chemical, and biological phenomena that can affect humans including aspects of human identification, criminal, civil, labor, and administrative image examinations, forensic investigation etc.[2] Watson and Crick, identified the double helix structure of DNA. DNA can be found on clothes even by brief skin contact, clothing articles from crime scenes are frequently

submitted for trace DNA analysis to identify someone other than the wearer.[3] Numerous research has examined the intricacy of DNA transfer in recent years,[4] beginning with the active transfer of DNA from a human to an object. To the best of our knowledge, exposure to bodies of still or moving water was not one of these circumstances. Only tests using a washing machine have been used to evaluate whether epithelial or blood DNA remains after coming into contact with water.[7-10] For instance, it is necessary for DNA isolation from scientific crime scene materials to be efficient in eliminating a varied range of environmental impurities/inhibitors that impede subsequent PCR operations, such as denim dyes, humic acid, and tannins. The DNA extraction procedure is less complicated and may even be bypassed entirely for eg blood or buccal samples in order to save time.[11,12] Although, usefulness of a sample in criminological background is ultimately defined by the capacity to construct a DNA profile, DNA quantification is typically performed to control the efficacy of the extraction stage. To avoid overloading the PCR master mix with too much DNA, the information on the quantity of DNA is utilized to estimate the right amount of DNA to be added. Contrary to particular DNA-based hybridization tests, common quantification approaches employ photometric/ fluorometric assessment of the DNA amount, which are indifferent to the source of DNA.[13-15] The experiment is carried out using a device that can measure fluorescence at each PCR cycle straight through the cover of the reaction tube, allowing for online detection. In the annealing phase, the probe precisely binds to the product as the amplicon builds up during the reaction. Taq polymerase uses its 5′-exonuclease activity to cleave the probe during the extension phase, separating the quencher and fluorochrome molecules in the process.[16-19] Fluorescent signal is now being detected and released. By computing the actual template copy number from the calculated Ct value, this line can be used to determine the assay's active range and to quantify the amount of starting specified DNA from an unidentified sample.

METHODOLOGY

Requirement

Reagents: Solution B, 20% SDS, Proteinase K enzyme, Phenol chloroform Isoamyl alcohol mixture (25:24:1), Sodium acetate (5M, pH 5.2), Chilled Isopropanol (IPA), 70% ethanol, Nuclease free water, Eppendorf tubes 2 ml, Micropipette. Agarose 1% agarose, 10X TAE buffer, Loading dye - 0.25% bromophenol blue, 0.4 M tris, 48.5g per L, Ethidium bromide (10mg/ml).

Equipment: AgaroseGel Electrophoresis, Real-Time Polymerase Chain Reaction.

Standard preparation

i. Solution B (pH 8.0): Dissolved in 15ml, 1M NaCl in 40ml, 1M trisHCl (pH 8), and added 10ml of 0.5 M EDTA in 95ml distilled water. Autoclaved the content and cooled it at room temperature. Then, 5ml of 20% SDS solution were added to this mixture and mixed it well.

ii. 20% SDS: Added 20g SDS in 100 ml of distilled water. Mixed it well and gently.

iii. Proteinase K: 10mg of Proteinase K was added in 1 ml autoclaved distilled water.

iv. Phenol–Chloroform Isoamyl Alcohol Mixture (PCI): 25 ml saturated phenol with 24 ml of chloroform and 1 ml of isoamyl alcohol.

v. 5M Sodium Acetate: 41g sodium acetate in 100 ml distilled water (pH 5.2).

vi. Isopropanol: Kept the IPA in -200C before use.

vii. 70% Ethanol: 70 ml absolute ethanol dissolved in 30 ml distilled water.

Sample collection: blood stains of 150 µl each were taken on silk and cotton cloth, collected from a single person. a total of 30 bloodstains were collected. The samples were stored at Room temperature (RT). Spots from silk cloth were coded VS1-VS15 and spots cotton were coded as VC1 – VC15. The samples were incubated at respective temperatures for 15 days before further processing.

Sample Pretreatment: 6 samples were processed every alternate day for DNA extraction.

Procedure

DNA extraction from blood stain on cloths:

i. Weighed the cloth and cut it in pieces.

ii. 1.5 ml Solution B, 50 µl 20% SDS and 5 µl Proteinase K was added to the cut cloth. prepared mixture incubated at RT overnight. After 24 hours again incubate the mixture 56°C for 45 mins.

iii. Transfer a fresh tube and 250 µl of Sodium acetate and 500 µl of PCI mixture were added

in the tube, and mixed it well.

iv. Centrifuged the mixture at 10,000 rpm for 15 minutes.

v. Three layers were formed. Carefully the upper layer which contains DNA was transferred in a fresh tube.

vi. 500 µl of chilled IPA was added to precipitate the DNA. Incubate the mixture at -20°C overnight.

vii. Centrifuged the mixture at 10000 RPM for 10 min. Discarded the supernatant.

viii. Pellet was washed with 500ul 70% ethanol by centrifuging the content at 10000 RPM for 5 mins. Supernatant was discarded and the pellet was air dried.

ix. After air dry the DNA pellet was dissolved in 15 µl of Nuclease free water.

x. The dissolved DNA was visualized under UV in agarose gel electrophoresis.

Agarose Gel Electrophoresis

i. Diluted 10X TAE buffer to 1X for gel formation. (Mixed 3 ml TAE with 27 ml d/w to make it 1X). Mixed 300 mg agarose with 30 ml 1X TAE buffer in a flask.

ii. Heated the flask and dissolved the agarose. Heat protecting gloves were used when heated the agarose.

iii. The agarose when dissolved, but not in a boil. Once fully melted and allowed it to cool without setting and the edges of a gel-casting tray was sealed with tape.

iv. 0.5 µl of ethidium bromide added to the agarose solution at about 60°C and gently mixed then a fine comb inserted into the casting tray.

v. Poured the agarose to a depth of about 1 cm and allowed it to solidify and removed the tape and 10µl of a 500µl DNA sample were taken 2.5 µl loading dye as added.

vi. 1µl of 1kb ladder (in refrigerator), 9µl water, and 2.5 µl loading dye was used as a marker.

vii. Filled the tank to adjust above the gel bed using 1X TAE buffer and gel was placed in the tank ensured that the gel was submerged.

viii. Samples wells were filled with samples and run at 80V for about an hour until the front dye reached the bottom of the gel.

ix. electrodes are bubbling indicated that the circuit was complete.

x. After the electrophoresis photographed the gel under UV trans-illumination.

Real-Time Polymerase Chain Reaction

q-PCR is a method for the quantitative detection of specific DNA or complementary DNA (cDNA) regions by selective amplification.

Preparation of master mix for q-PCR and running the qPCR: Reaction mixture was prepared for the total volume of 10µl.The amplification cycle was carried out for 45 cycles leading to the last stage of PCR which was melting.

Table 1: Reaction mixture for q-PCR

Content	Volume (µl)
2X qPCR Mix	5
Fw Primer (n65)	0.5
Rv Primer (n65)	0.5
RNAse free water	3.8
Sample (DNA)	0.2

Table 2: Steps in q-PCR

Contents	Temp(^{O}C)	Duration
Pre-denaturation	95	3 mint
Denaturation	95	10 sec
Annealing & Extension	60	20 sec
Melting	72	3 min

RESULTS AND DISCUSSIONS

From the results obtained after gel electrophoresis it can be clearly seen that the blood samples from both the cloths have yielded similar intensity of DNA. So, it can be inferred that DNA can be retrieved from the both types of cloths.

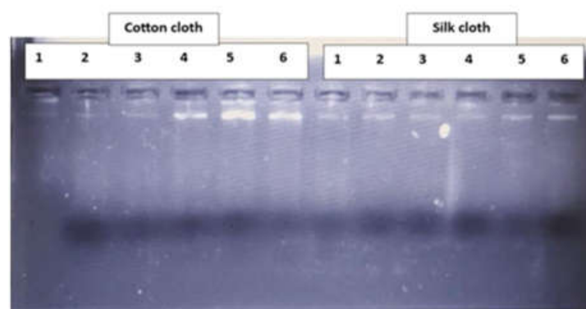

Fig. 1. Gel image of DNA bands under UV

qPCR Analysis: The technique most frequently used for DNA quantification and quality analysis in the past was spectrophotometry, which allows for the assessment of the amount and purity of DNA in a sample in respect to possible impurities like proteins.[20] The appropriate n65 primer was used to amplify the gDNA.

Table 3: CT value and fold change of n65

Well	CT Value (Mean)	Fold Change (Mean)
E1-E5	21.09	77.8
E6-E10	20.47	77.7

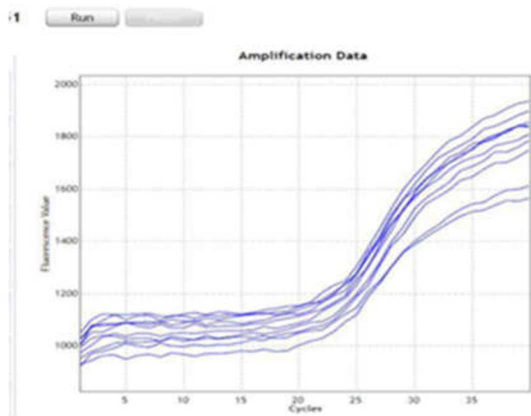

Fig. 2. Raw Data Plot of qPCR

Fig. 3. Melting curve of qPCR

The CT Value and Fold Change of first five samples were 21.09 and 77.8. The CT Value and Fold Change of next five samples were 20.47 and 77.7

CONCLUSION

The present study explored the extraction of DNA from two different cloth samples using phenol chloroform method. The results obtained through in study indicated that DNA can be successfully retrieved from both the cloths (cotton & Silk) during forensic investigations. Also, qPCR analysis has proved that the DNA extracted is functional and can be used for future forensics studies.

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