EVALUATION OF PHENOL CHLOROFORM DNA EXTRACTION METHOD FROM A SPECK BLOOD STAIN ON COTTON FABRIC

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EVALUATION OF PHENOL CHLOROFORM DNA EXTRACTION METHOD FROM A SPECK BLOOD STAIN ON COTTON FABRIC

by

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for the degree of
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CERTIFICATE

This is to certify that this dissertation, “Evaluation of Phenol Chloroform DNA Extraction Method from a Speck Blood Stain on Cotton Fabric” is sincerely recorded of research work done by Ms Nor Amalia Bt Nazri during the period February 2020 to September 2020 under my supervision. I have read this dissertation and that in my point of view it conforms to acceptable standards of scholarly presentation and is fully adequate. In scope and quality, as a dissertation to be submitted in partial fulfilment for the Master of Science (Forensic Science).

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DECLARATION

I hereby declare that this dissertation is the result of my own investigation, except where otherwise stated and duly acknowledged. I also declare that it has not been previously or concurrently submitted as a whole for any degrees at Universiti Sains Malaysia or any other institution. I grant Universiti Sains Malaysia the right to use the dessertation for teaching, research and promotional purposes.

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(NOR AMALIA BINTI NAZRI)

Date: __10/09/2020_____

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LIST OF ABBREVIATIONS

Bp	Base pair
DNA	Deoxyribonucleic acid
EDTA	Ethylediaminetetraacetic acid
LMW	Low molecular weight
HMW	High molecular weight
SDS	Sodium Dodecyl Sulphate
STR	Short tendem repeat
TBE	Tris Borate EDTA
STR	Short Tandem Repeat

LIST OF SYMBOLS

^{O}C	Degree Celcius
Hrs	Hours
g	Grams
µL	MicroLitre
M	Molar
mL	MilliLitre
mg/mL	Milligrams per milliLitre
ng/µL	Nanograms per microLitre
rpm	Revolutions per minute
%	Percent

PENILAIAN KAEDAH PENGEKSTRAKAN DNA PHENOL CHOLOFORM DARIPADA TOMPOKAN KECIL DARAH PADA KAIN KAPAS

ABSTRAK

Dalam kes forensik, pemulihan DNA dan kecekapan cara pengekstrakan DNA adalah salah satu kunci penting untuk penyiasat forensik untuk mengenalpasti individual yang terlibat dalam jenayah melalui analisis DNA. Kajian ini adalah bertujuan untuk menilai tiga parameter kaedah pengekstrakan fenol kloroform dari tompokan darah kecil pada kain kapas untuk mencari keadaan yang sesuai yang dapat memberikan pemulihan DNA yang tinggi. Nilai P yang sangat signifikan menunjukkan bahawa parameter yang dipilih iaitu waktu inkubasi, suhu inkubasi dan suhu penyimpanan mempengaruhi pemulihan DNA. Semua parameter menunjukkan nilai ketulenan yang diterima pada nisbah A260/A280 dan nilai ketulenan yang sangat rendah untuk A260/A230 dalam semua sampel yang diekstrak mungkin disebabkan oleh beberapa faktor. Kesimpulannya, keberkesanan melarutkan DNA didapati lebih baik apabila sampel di disimpan pada suhu 56^{O}C berdasarkan pada kaedah yang dikaji.

EVALUATION OF PHENOL CHOLOFORM DNA EXTRACTION METHOD FROM A SPECK BLOOD STAIN ON COTTON FABRIC

ABSTRACT

In forensic casework, DNA recovery and the efficiency of DNA extraction method is one of the important key for the forensic investigator to identify the individual involved with the crime through the DNA analysis. This study therefore is intended to evaluate three parameters of phenol chloroform extraction method from a tiny blood stain on fabric cotton in order to find suitable condition that can give a high DNA recovery. Highly significant of P value suggest that the selected parameters which is the incubation time, incubation temperature and storage temperature have influenced the DNA recovery. All parameters show accepted purity value at ratio A260/A280 and very low purity value for A260/A230 in all extracted samples which may be attributed by several factors. In summary, the efficiency to dissolve DNA was found to be good when sample was stored at 56^{O}C based on the tested method.

CHAPTER 1: INTRODUCTION

1.1 Background of study

Deoxyribonucleic acid (DNA) was first discovered by a young Swiss doctor Friedrich Miescher in 1860s when a precipitate of an unknown substances was observed during an experiment on the chemical composition of leukocytes in the laboratory of Felix Hoppe-Seyler at the University of Tübingen (Dahm, 2005). The named of DNA was ideally given by Felix since the unknown compound that he found was isolated from the nuclei cell (Dahm, 2008). This molecule contains the instructions necessary to create every type of cell in a person's body, with the exception of identical twins. Due to DNA's abundance in the body, biological fluids can be used as a source for DNA. Good sources of DNA include blood, saliva, and semen which is often visible to the naked eye.

In forensic investigation, DNA analysis plays an important role since it becomes an indispensable and routine part of modern forensic casework where suspects can be linked to the crime scene or can exonerate the suspects from the cases (Jobling and Gill, 2004). A number of researchers have reported that the common step of DNA analysis in forensic examination include examination of evidence, identification of body fluid, extraction of DNA, assessment of extracted DNA, amplification of target loci, detection of amplified products, analysis of data, and preparation of report. Recent developments in DNA extraction method have highlighted the need to produce high-quality of DNA yield to generate good quality of genotype profiles. (Lee and Shewale, 2017).

Extraction techniques that retrieve as much DNA as possible from a specimen are very importance in forensic science since many published techniques aimed for maximize

DNA yields to avoid co-extraction of polymerase chain reaction (PCR) inhibitors (Rohland and Hofreiter, 2007). Previous study has proven that DNA extraction technique which proposed in the late 1980s known as phenol-chloroform was successfully the first ancient genetic data due to minimal loss of DNA recovery (Campos and Gilbert, 2012). Phenol chloroform is a well establish DNA extraction procedure, however this method is laborious as well as not safe because of using toxic reagent such as phenol and chloroform (Köchl *et al*, 2005). Although, there are several DNA extraction based kits are available in the market, the used of conventional method phenol chloroform still become a choice for many researchers because the capability of producing of high yield of DNA.

Blood is one of the most common biological fluids that always found in the crime scene. This biological sample can be found deposited in varying substrates include wood, paper and fabric. The amount of deposited DNA can be limited due to many factors such the types of crime, types of DNA transfers and types of substrates (Meakin and Jamieson, 2013). In cases such as murder, sexual assault or hit and run, usually a few blood drops are frequently recovered from the scene of occurrence and this samples later was submitted to the laboratory for the DNA analysis (Kumar *et al*., 2019). Study has showed that obtaining DNA from clothing sample could be complicated and difficult due to the nature of the material deposited as well as the color of the fabric (Linacre *et al.,* 2010). However, the use of the conventional DNA extraction method to isolate DNA has showed good ability in isolating pure DNA without co-extraction of inhibitors or other components inhibitory to PCR include fabric dyes such as indigo from denim, heme from blood, humic acids from soil and melanin from hair samples (Akane *et al,* 1994; Stangegaard *et al*., 2013). Therefore, an effective method is required to isolate DNA from any biological samples to

be used for downstream applications especially for identification of individuals through DNA typing.

1.2 Significance of study

Biological sample found at the crime scene have an important role in forensic investigator to solve a crime by linking a sample to a suspect and to a crime, as well as can be used in the reconstruction of the crime scene. In common violent cases, bloodstain is the evidence that always encountered on different type on substrates such as on porous or non-porous surfaces. According to Wickenheiser, (2002) blood stain trace on the fabric material was frequently submitted for DNA analysis in forensic investigation. This type of substrate was classified as porous surface which is referred as solid material that have pores (Ishizaki *et al*, 2013). As we know, there are many types of fabrics available in the market and each of the fabric was made from different types of fibres such as natural and synthetic. Therefore, types of fabric is one of the factors that can effect the DNA yield. In this study, fabric was chosen as a substrate due to its high prevalence in everyday life. The decision to use a cotton fabric as a substrate material because this substrate has been used in many home decor items such as cushions, curtains, bedsheets and pillowcases as well as clothing materials.

Usually in most of the crime cases, a tiny spot of blood samples will be found and this much of samples will be used for DNA extraction. As a result, the success of DNA typing less or more is influenced by the DNA yield gained from a tiny spot. The goal of this study is therefore to evaluate certain parameters or variables used in phenol chloroform

DNA extraction method in order to find the best condition to obtain a high quantity and quality of DNA yield.

1.3 Objectives

1.3.1 Main objective

To evaluate the parameters of the phenol chloroform extraction method from the speck blood stain on cotton fabric

1.3.2 Specific objectives

i. To perform phenol chloroform DNA extraction at varying incubation time and temperature.

ii. To assess the suitable storage temperature to dissolve DNA from the phenol chloroform method

iii. To study the suitable parameters of the phenol chloroform method from the speck blood stain on cotton fabric based on DNA yield obtained.

CHAPTER 2: LITERATURE REVIEW

2.1 Deoxyribonucleic acid (DNA)

DNA also called as blueprint containing the instructions that carries all the genetic information of an organism in production of proteins essential to cell function (Slagboom and Meulenbelt, 2002; Bello and Gbolagade, 2017). DNA is the double helix molecule form from two chains that coil together and carrying genetic instructions for the development, functioning, growth and reproduction (Anionye, 2017). It is condensed into chromosome and can be found inside the nucleus of the cell. In human, there are 23 chromosome pairs, which are 22 autosome pairs and one pair of sex chromosomes.

For the autosomes (chromosomes 1-22), it is inherited by means of one member from each pair comes from the father and the other one comes from the mother. Sex chromosomes will be different as male will have one X and one Y chromosome while female will have a pair of X chromosome. A coding region either for the messenger RNA that encodes amino acid sequence or a functional RNA molecule known as a gene can be found in DNA and it is the basic unit of heredity (Scitable, 2004). Genes can be found at specific location at the chromosome or known as locus. Each cell will receive two copies of the genes (one from the father and one from the mother), which influence the same trait but can either be identical or not. The variant forms of the same gene are known as alleles.

Human DNA mainly comprises coding and non-coding region which is 97% of human genomic DNA does not code the genetic information and only 3% of human genomic DNA provides genetic information (Zainuddin, 2004). It has been dominated that, every individual show 99.9% identical DNA sequences and less than 0.1% of DNA sequences are

differ among each other make the individual is unique (Dubey, 2014). This 0.1% is the main focus of forensic DNA investigations.

2.2 Trace DNA

DNA has become the "golden standard" for the identification of perpetrators at crime scenes. DNA can be found in large quantities throughout one's cells, however sometimes a trace amount of DNA may be deposited at a crime scene. This types of DNA is known as touch or trace DNA and is from an individual's epithelial cells or cell-free nucleic acid (Templeton *et al*., 2015). Other terms that are also used for trace DNA are low copy number (LCN) DNA, touch DNA and low-level DNA (Butler, 2006). Trace DNA is a source of DNA profiling that is becoming more common in the field of forensic science as it is deposited on surfaces that are contacted by the individual or objects (Hess, 2017).

Trace DNA is defined as a sample containing less than 200 pg of genomic DNA (Budowle *et al*., 2009). According to Locard's Exchange Principle, contact of objects results in an exchange between those objects (Miller, 2009). This principle applies when a person touches an object, possibly leaving fingerprints and touch DNA behind. Depositing of DNA occurs when cells slough off the surface of the skin. At this time, no established presumptive tests for trace DNA, making locating the evidence difficult. Additionally, due to the small amount of sample available, prevention of contamination and degradation of the DNA is critical (Butler, 2012 and Verdon *et al*, 2014).

2.3 Trace DNA on fabric surface

DNA analysis begins with the collection of the evidence that may harbor DNA. Studies show that as much as 86% of DNA deposited on a surface may remain uncollected and not analyzed. Trace DNA samples may be found on a variety of surfaces such as the clothing of a victim after an assault. According to Hess, (2017) epithelial cells adhere to porous substrates and natural fibers more commonly than nonporous substrates and synthetic fibers. Research has been conducted to determine either touch DNA can be transferred to clothing when washed or not, but the applicability has been limited by the number of participants and the collection methods (Gosch and Courts 2019).

As we know, fabrics are everywhere: from clothing and bedding to carpet and upholstery. Fabrics come in all colors, sizes, and textures imaginable. According to Dictionary.com, fabric is defined as a cloth made by weaving, knitting, or felting fibers. Because individual fibers are woven together, countless minute spaces are created. These spaces often trap molecules, including DNA, within the fabrics. The uneven surface of the fabric also creates an abrasive surface as skin brushes across the fibers (Sekulska *et al*, 2020). These features make fabric an excellent source of touch DNA; however, yield of DNA may be varied depend on types of fabrics.

2.3.1 Transfer of trace DNA

Material that contains DNA can be present on the surface by two means, direct and indirect transfers. Direct or primary transfer may not only due to touching, but it also can come from the activities that do not require contact such as speaking, sneezing or coughing (Meakin and Jamieson, 2013). For instance, Port *et al.* (2006) confirmed that oral DNA

could be expelled 184 cm ahead of a speaking individual. Indirect transfer of DNA may occur when DNA from an individual exist on a surface or item via an intermediary surface or medium. This process is known as secondary transfer as it involves one intermediary transfer medium between the individual and item in question.

2.4 Factors affecting DNA on fabric surfaces

Recovery of DNA is a prime determine by the quality and quantity of DNA deposited on an objects or surfaces. The amount of DNA deposited is not constant since it was highly influenced by many factors such as the surfaces of the substrate, the time between deposition and recovery and the efficiency of DNA extraction (Alketbi, 2018).

2.4.1 Surface of DNA deposited on the substrates

DNA can be deposited either on porous or non-porous substrates after being touched individual. Previous study has showed DNA was significantly higher when primary substrate was porous material compared to non-porous material (Verdon *et al.*, 2013). The porous surface has higher ability to pick up more DNA than non-porous surfaces hence it transfers less DNA as a primary substrate (Verdon *et al*., 2013). Non porous surfaces includes glass, tiles, and plastic materials do not absorb water as well as do not traps DNA as good as porous substrate (Verdon *et al*., 2013). Porous surfaces such as paper, fabric, and untreated wood are defined as substance containing pores (Ishizaki *et al*., 2013). Study showed rough and porous surface able to retain DNA better than smooth and nonporous surfaces (Wickenheiser, 2002).

Higher recovery 85% was recorded from rough and porous substrates than smooth and non-porous surfaces 55% showing that porous substrates retain better DNA than non-porous substrates (Wood *et al*., 2017). This was supported by Goray *et al* (2010) where cotton substrate (rough surface) yielded higher DNA value than plastic (smooth surface). This indicates that higher DNA persistence on rough and porous substrate than smooth and non-porous substrate. However, Wickenheiser (2002) has argued that although it is true that more DNA was likely to be deposited on the rough and porous surface, but the amount of DNA that can be recovered from such surfaces was lower and this might be because of ineffective of recovery processes from the rough surface.

2.4.2 The time elapsed between deposition and recovery

Study has proved that by minimizing the time taken for the DNA collection able to improve DNA yield (Li and Harris, 2003). The deterioration rate of DNA was found to be advanced with time; however, it was highly influenced by the condition of touched object (Raymond *et al*., 2008). Increasing of exposed time also has a significant effect towards the quality of DNA profile generated (Raymond *et al*., 2009). DNA recovery from cotton was found to be reduced by 50% when the time was delay of 24 hours between deposition and recovery (Goray *et al*., 2010). As stated by Frégeau *et al*. (2010), fresh deposited touch gives more amount of DNA recovery compared to touch that has been stored over a long period of time.

2.4.3 Efficiency of DNA extraction from sample

There are several methods of DNA extraction can be selected for purification of DNA from varying samples types. The efficiency of extraction depends on the extraction process decided, with some methods offer more advantages than others. Frégeau *et al.* (2010), showed that several DNA extraction methods utilized by forensic scientists do not recover all of the collected DNA, with losses of up to 75% occurring from Chelex and organic extraction methods. Some of the loss is influenced by the substrate on which the sample is presented but the main is due to the methodology. For short tandem repeat (STR) profiling system, as much 0.1 – 0.5 ng was required and below that concentration, partial profiles will be generated. Thus, optimizing the DNA extraction method is necessary for trace DNA in order to avoid loss of DNA. Study by Alketbi (2018), showed higher DNA recovery was seen from QIAamp® DNA Investigator Kit, QIAsymphony® DNA Investigator® Kit, and DNA IQ™ compared to to Chelex®100 and QIAamp® DNA Blood Mini Kit. Other study revealed that the DNA extraction process can result in a loss of about 20% to 90% of the initial template amount depending on the extraction method used, as well as the accuracy of the quantification method (Balogh *et al.,* 2003; Ottens *et al.,* 2013).

CHAPTER 3: MATERIALS AND METHOD

3.1 Materials

3.1.1 The chemical, reagent and consumables

All the chemicals. Reagents and consumables used in this study were listed in the Table 3.1

3.1.2 Instrument and apparatus

The instrument and apparatus used in this study were listed in Table 3.2

3.1.3 Reagent preparations

3.1.3.1 Tris Hydrochloride 1 M (Tris – HCL) pH 8.0

A total of 32.657 g of Tris base was dissolved in 800 mL of distilled water. The pH was adjusted to 8.0 by adding 1 M HCL solution. Distilled was added to adjust the final volume of the solution to 250 mL. The solution was the autoclaved to sterile and the solution then were keep at room temperature for further use.

3.1.3.2 Ethylenediaminetetraacetic Acid (EDTA) 0.5 M, pH 8.0

A total of 45.359 g of ethylenediaminetetraacetic acid, disodium salt, dehydrate (Na_2 EDTA) powder was dissolved on a hot plate in 200 mL of distilled water. The solution was added approximately 20 g of sodium hydroxide (NaOH) pellet to adjust the pH to 8.0. The final volume was adjusted to 250 mL. The solution was then autoclaved and stored at room temperature.

Table 3.1: List of chemical, reagents and consumables used in this study

Chemical/Reagents	**Supplier**	**Country**
Proteinase K	Sigma-Aldrich	USA
Sodium Hydroxide (NaOH) pellet	Sigma-Aldrich	USA
Hydrochloric Acid (HCL)	Merck	USA
Chloroform	Merck	USA
Sodium chloride (NaCl) powder	Sigma-Aldrich	USA
Disodium ethylenediaminetetraacetate dehydrate (Na_2EDTA) powder	1st BASE	Singapore
Ethylenediaminetetraacetatic acid (EDTA)	Sigma-Aldrich	USA
Sodium Dodecyl Sulfate (SDS)	Bio-Rid	USA
Glacial acetic acid, 100 %	Merck	USA
Isoamyl alcohol	HmbG Chemical	Germany
Tris-base	1st BASE	Singapore
Sodium acetate powder	Sigma-Aldrich	USA
Agarose powder	1ST BASE	Singapore
Ethidium Bromide	Sigma-Aldrich	USA
100 bp DNA ladder	Gene DireX	USA
Orange G dye	Sigma-Aldrich	USA
Consumables		
Pipette Tips	Greiner Bio One	Austria
1.5 mL Micro centrifuge tube	Bio-Rev	Singapore

Table 3.2: List of instruments and apparatus used in this study

Instrument	**Brand**	**Model**
10 µL, 20 µL, 100 µL, 200 µL and 1000 µL pipette	Gilson / Eppendoff	P10, P20, P100 and P100
Microwave Oven	Eiba, Malaysia	EMO-1706
Water Bath	Memmert	WB 29
Laminar Air Flow Cabinet	ERLA	CFM-4
Microprocessor pH meter	Henna Instrument	Ph 211
Gel Documentation system	Vilbert Lourmat/Quantum	ST4-1000/20
DNA Electropherosis system	Owl, Thermo Scientific	B2
Electropherosis power supply	Bio-Rad	Power-pac 3000 V
Analytical Balance	Shimadzu	ATX224
Refrigerated Centrifuge Benchtop	Hettich Universal	Micro 22R
Vortex Mixer	ERLA	EVM-6000
Micro Centrifuge	Labnet International	C1301-230 V
Spectrophotometer	Thermo Scientific	Nanodrop2000

3.1.3.3 Sodium Dodecyl Sulphate (SDS) 10%

Ten grams (10g) of SDS powder was dissolved on 90 mL of distilled water. The procedure was done on the hot plate to aid the dissolution process. The pH of the solution was adjusted to 7.2 by using 1 M HCl. Sterile distilled water were added to adjust the final volume to 100 mL.

3.1.3.4 Tris-EDTA Buffer (TE Buffer) pH 8.0

About 2.4 mL of 1 M Tris-HCl solution, 0.5 mL EDTA solution was added to prepare the buffer solution and the mixture was adjusted to 250 mL by adding distilled water. The solution was then aliquot into 100 mL and subjected to autoclave. The solution was then stored at room temperature.

3.1.3.5 Sodium Acetate (CH_3COONa) 3M

A total amount of 102.206 g sodium acetate powder was dissolved in 200 mL of distilled water. The pH was adjusted to 5.2 by adding glacial acetic acid. The final volume was adjusted to 250 mL by adding distilled water. The solution was the autoclaved and stored at room temperature.

3.1.3.6 Sodium Chloride (NaCl) 5M

A total of 73.05 g of sodium chloride was dissolved in 250 mL of distilled water. The solution was then autoclaved and stored at room temperature.

3.1.3.7 Tris-Borate-EDTA TBE Buffer (10 X)

A total of 26.95 g Tris base, 1.86 g of Na_2EDTA and 55 g of boric acid were mixed into 900 mL of distilled water. The volume of solution was made up to 1000 mL by adding distilled water. The solution was dissolved using magnetic stirrer. Then, the solution was autoclaved and stored at room temperature.

3.1.3.8 TBE Buffer (0.5 X)

A 50 mL of 10 X TBE buffer was mixed with 950 mL of distilled water. The solution was then stored at room temperature for further use.

3.1.3.9 Digestion Buffer

To prepare digestion buffer, added 1 mL of 1 M Tris-HCL pH (8.0), 4 mL of 0.5 M EDTA (pH 8.0), 40 mL of 10% SDS and 2 mL of 5 M NaCl solutions in a schott bottle. The mixture was then autoclaved and stored at room temperature.

3.1.3.10 Chloroform: Isoamyl Alcohol (24:1)

A total of 24 mL of chloroform was mixed with 1 mL of isoamyl alcohol in an amber bottle. The mixture was then stored at room temperature.

3.1.3.11 Proteinase K (20 mg/mL)

A total of 20 mg of proteinase K powder was dissolved in 1 mL of sterile distilled water. The solution was then stored at -20^{O}C.

3.1.3.12 Orange G Loading Dye

A total of 0.125 g of orange G powder was added to 15 g of glycerol solution and the total volume was adjusted to 50 mL by adding sterile distilled water. The solution was aliquoted into 1.5 mL microcentrifuge tube and stored at -20^{O}C.

3.1.3.13 70 % Ethanol

An approximately, 350 mL of absolute ethanol was mixed with 150 mL of deionized water.

3.2 Methods

3.2.1 Sample collection

After obtaining an ethical approval from the Ethic Committee for Research Involving Human Subject, USM (USM/JEPEM/19020156) (Appendix A). A 2 mL of venous blood was collected from one volunteer female individual from USM Health Campus. This was done by a certified phlebotomist. The collected blood were stored at -20^{O}C until further used.

3.2.2 Sampling method

Selection of individual was done through verbally interviewing before accepted as subject for this research. The selected participant was asked to fill the informed consent form as a part of procedure for sampling. The following criteria were applied for samples selection:

Inclusion criterions:

i) Healthy individual

ii) Age above 18 years old

Exclusion criterions:

i) Having diseases based on medical record in the past

3.2.3 Sample deposition

A 15 µL of the whole blood was spotted on the cotton fabric (Figure 3.1). The cotton fabric was cut into 1 cm X 1 cm as described by Verdon *et al*., (2013). The deposited blood was exposed at ambient temperature and to minimize the effect of environment the humidity of the area was controlled. A dry deposited sample then was used for DNA extraction using organic phenol chloroform method.

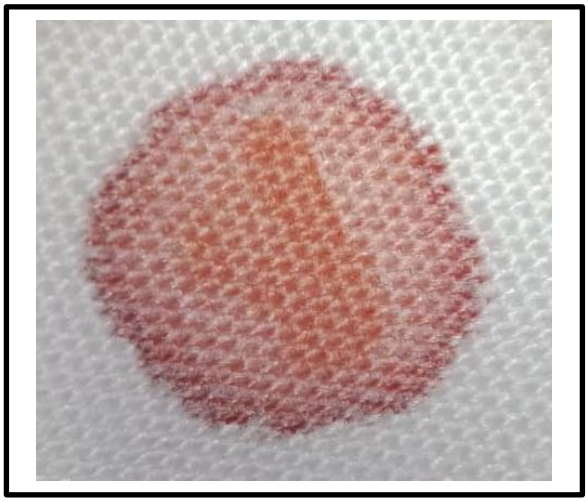

Figure 3.1: Blood deposition on cotton fabric

3.3 DNA extraction from blood stain

In this study, phenol chloroform method was used to extract DNA from the deposited blood on cotton fabric. Three parameters of phenol chloroform DNA extraction was evaluated by using triplicates samples (Table 3.3 to Table 3.5). The first parameter to be assessed was the incubation time after adding the digestion buffer and proteinase K (Table 3.3). For this parameter, samples were incubated at two different durations 4 hours and 18 hours. Second parameter, incubation temperature for sample lysis (Table 3.4).

Samples were incubated at three different temperatures, 37^{O}C, 56^{O}C and 65^{O}C. The last parameter to be evaluated was the storage temperature for dissolving DNA. At this parameter, samples were kept at 37^{O}C and 56^{O}C (Table 3.5).

The fabric containing the blood stain was cut into small pieces and placed into 1.5 mL micro centrifuge tube. A total of 500 µL of digestion buffer and 12 µL of proteinase K (20 mg/mL) were added and incubated at different temperature 37 ^{O}C, 56 ^{O}C and 65 ^{O}C for different durations (4 hrs and 18 hrs). Each of the tested parameter was carried out by triplicates samples. On the next day, 120 µL of buffered phenol was added and was mixed by vortexing. The solution was centrifuge at 8000 g for 3 minutes and the supernatant was transferred into a new 1.5 mL micro centrifuge tube.

Buffered phenol and chloroform:isoamyl (24:1) were added in equal volume of supernatant. The solution was again centrifuged at 8000 g for 3 minutes. The supernatant was then transferred into a new 1.5 mL tubes and 250 µL of chloroform:isoamyl (24:1) was added into the tube and centrifuged at 8000 g for 5 minutes. Again the supernatant was transferred into a new 1.5 mL micro centrifuge tube and 500 µL of chilled ethanol and 50 µL of 2 M sodium acetate was added. The mixture was then mixed by inverting the tube and centrifuged at 8000 g for 5 minutes. The supernatant was discarded and 500 µL of 70% ethanol was added and centrifuged at 8000 g for 3 minutes. All the supernatant again was discarded and the DNA pellet that formed at the bottom of the tube was let dry at room temperature. To dissolve the DNA, 30 µL of TE buffer was added and incubated for overnight at 37 ^{O}C and 56 ^{O}C. Then, the extracted DNA was stored at -20 ^{O}C until further use.

Table 3.3: The changes parameters of incubation time

Changes parameter (incubation time)	Incubation temperature	Storage temperature
4 hours	56^{O}C	37^{O}C
18 hours	56^{O}C	37^{O}C

Table 3.4 The changes parameters of incubation temperature

Changes parameter (incubation temperature)	Incubation time	Storage temperature
37^{O}C	4 hours	37^{O}C
56^{O}C	4 hours	37^{O}C
65^{O}C	4 hours	37^{O}C

Table 3.5: The changes parameters of storage temperature

Changes parameter (Storage temperature)	Incubation temperature	Incubation time
37^{O}C	56^{O}C	4 hours
65^{O}C	56^{O}C	4 hours

3.4 Assessment of Genomic DNA quality

The NanoDrop^{TM} 2000 Spectrophotometer was used to determine the quality and quantity of the extracted genomic DNA. A 2 µL of ddH_2O was loaded on the lower pedestal using a micropipette and the sampling arm was brought down to wash the upper and lower pedestal. Both of the surfaces, upper and lower pedestal were cleaned with Kim wipes and then 2 µL of 1 X TE buffer was loaded to measure a blank reading. Then, 3 µL of DNA sample was loaded on the lower pedestal and the measurement was made after lowering the sampling arm. The result was transferred from the software to be observed.

3.5 Agarose gel electrophoresis of genomic DNA

Agarose gel electrophoresis was used to confirm the presence of the high molecular weight DNA (HMW-DNA). One gram (1 g) of agarose gel powder was mixed with 100 mL of 0.5 X TBE buffer and heated in microwave for 3 minutes. The solution was allowed to cool under the running tab water to 60 ^{O}C and 1.5 µL of ethidium dibromide (10 mg/mL) was added into the solution. The agarose gel solution was poured into horizontal electrophoresis unit and was left to solidify for 30 minutes. A total of 2 µL of DNA was mixed with 1.5 µL of Orange G dye and loaded into the well. The electrophoresis was performed at 90 V for 60 minutes. The presence of high molecular weight (HMW) DNA was observed under UV light using gel documentation system (Vilbert Lourmat/Quantum).

CHAPTER 4: RESULT

4.1 Fibre examination

4.1.1 Microscopic examination by using stereomicroscope

Microscopic examination of cotton fabric was performed using stereomicroscope to reveal the weave pattern of the cloth. Using this technique, the weave pattern of the fabric can be seen and recorded through magnification 4.0X (Figure 4.1). As shown in Figure 4.1, cotton fabric weave pattern can be classified as plain weave. This type of weave pattern explained by yarns (weft) was alternatively passed over under the vertical yarns (warp). Figure 4.2 shows the characteristic of cotton fibre under the light microscope.

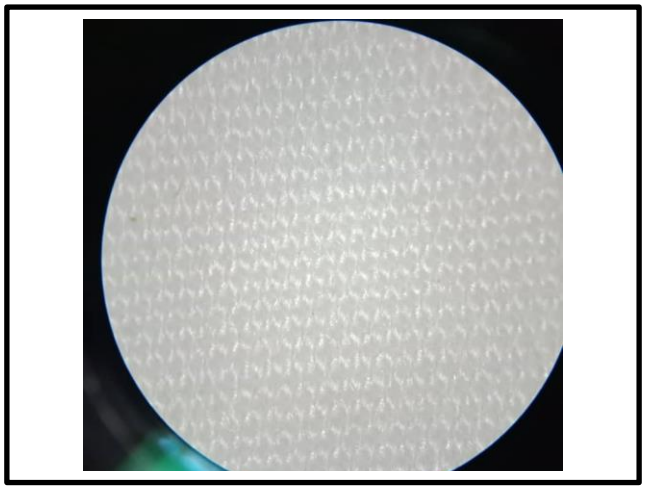

Figure 4.1: Cotton fabric under stereomicroscope with magnification 4.0X

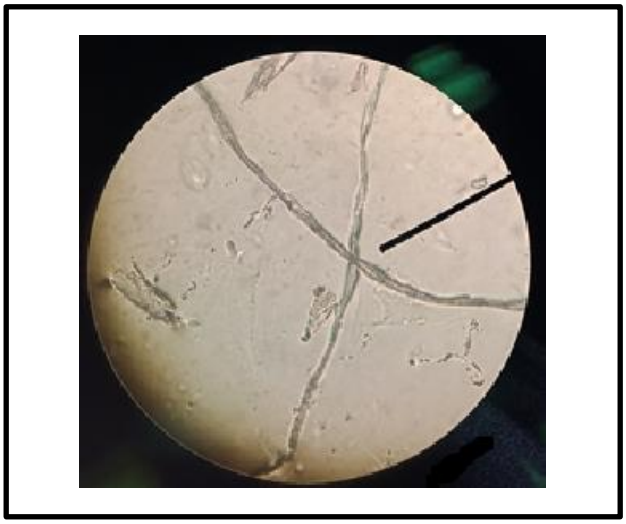

Figure 4.2: Cotton fabric under the light microscope with magnification 40X

4.2 Genomic DNA

The extracted DNA from the deposited blood on cotton fabric was visualized through agarose gel electrophoresis. A 1% agarose gel was used to run all the extracted samples. Figure 4.3 and Figure 4.4 were showed the extracted genomic DNA from the three different parameters. Each of the parameter represent by triplicates sample.

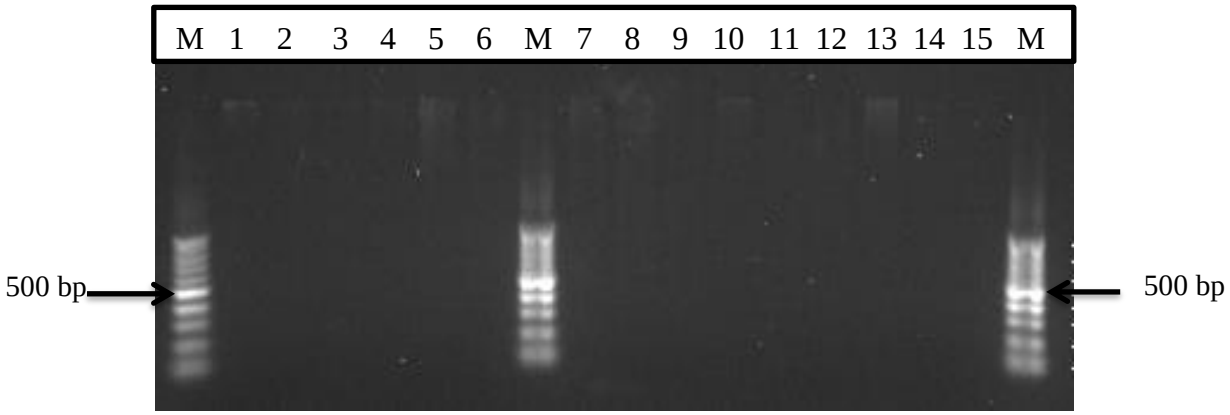

Figure 4.3: Agarose gel electrophoresis showing an extracted genomic DNA at varying incubation time and incubation temperature

Lane M: 100 bp DNA ladder

Lane 1: Incubation Time 4 Hours (IT4H1)

Lane 2: Incubation Time 4 Hours (IT4H2)

Lane 3: Incubation Time 4 Hours (IT4H3)

Lane 4: Incubation Time 18 Hours (IT18H1)

Lane 5: Incubation Time 18 Hours (IT18H2)

Lane 6: Incubation Time 18 Hours (IT18H3)

Lane 7: Incubation Temperature 37^{O}C (IT37^{O}C1)

Lane 8: Incubation Temperature 37^{O}C (IT37^{O}C2)

Lane 9: Incubation Temperature 37^{O}C (IT37^{O}C3)

Lane 10: Incubation Temperature 56^{O}C (IT56^{O}C1)

Lane 11: Incubation Temperature 56^{O}C (IT56^{O}C2)

Lane 12: Incubation Temperature 56^{O}C (IT37^{O}C3)

Lane 13: Incubation Temperature 65^{O}C (IT65^{O}C1)

Lane 14: Incubation Temperature 65^{O}C (IT65^{O}C2)

Lane 15: Incubation Temperature 65^{O}C (IT65^{O}C3)