KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI, GHANA

COLLEGE OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

A COMPARATIVE STUDY OF DIFFERENT LABORATORY STORAGE CONDITIONS AND DNA EXTRACTION METHODS FOR ENHANCED FORENSIC ANALYSIS OF SOIL-HUMAN BLOOD MIXED SAMPLE

BY:

ALEXANDER BADU-BOATENG

JUNE, 2018

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY

KUMASI, GHANA

COLLEGE OF SCIENCE

DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY

A COMPARATIVE STUDY OF DIFFERENT LABORATORY STORAGE CONDITIONS AND DNA EXTRACTION METHODS FOR ENHANCED FORENSIC ANALYSIS OF SOIL-HUMAN BLOOD MIXED SAMPLE

THIS THESIS IS PRESENTED TO THE DEPARTMENT OF BIOCHEMISTRY AND BIOTECHNOLOGY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF AN MSc DEGREE IN FORENSIC SCIENCE

BY:

ALEXANDER BADU-BOATENG

JUNE, 2018

DECLARATION

I hereby declare that this thesis is the outcome of my own work and that, it has neither in part or whole, been presented for same or another degree in this university or elsewhere.

Alexander Badu-Boateng	…….	..……
Candidate's name (PG4428815)	Signature	Date

Certified by

Dr. Peter Twumasi	……	..…….
Supervisor	Signature	Date

Certified by

Dr. Pandam Salifu	…….	……...
Forensic Programme Coordinator	Signature	Date

Certified by

Dr. Peter Twumasi	……	….…….
Head of Department	Signature	Date

DEDICATION

This thesis is dedicated wholeheartedly to my late parents, my wife, and my daughter.

ACKNOWLEDGEMENTS

My upmost gratitude goes to God Almighty, the giver of life, for His protection. I am grateful to COP Joana Osei-Poku (Rtd) and my late parents for their support and contribution towards my education. I am also grateful to my wife Jessica for her understanding when I had to leave her most often just to make this work a success. I am grateful to Mr. Albert Aduhene and the entire staff of Jubilee Hospital, Akim-Oda for their contribution to this work.

My sincere gratitude goes to Dr. Peter Twumasi and Dr. Pandam Salifu for their guidance, suggestions and corrections throughout this project.

ABSTRACT

Crime scene investigation is an important step in the entire criminal investigation process because this is where evidence is gathered. Blood from the perpetrator or victim of a crime can be left at crime scenes or transferred to other materials such as clothing, knives and guns. Most often, this body fluid is contaminated with soil at outdoor crime scenes but this might be the only or the most important evidence in solving a crime. This study aimed at identifying the most appropriate method of storing crime scene soil-human blood mixed sample prior to analysis. The best DNA extraction method for this soil-blood mixed sample was also studied. Three commercial DNA extraction kits (PrepFiler Forensic DNA Extraction kit, Promega DNA IQ Kit, Blood Miniprep kit) that have been claimed by the manufacturers to be effective in extracting DNA from soil contaminated samples were used for the DNA extractions. Hemastix and Hexagon OBTI kits were used for the serological analysis in this study. Human blood was mixed with soil and stored at three different storage conditions (i.e., Room temperature/25℃, 4℃ and -20℃). Hemastix and Hexagon OBTI serological tests for blood and human blood, respectively were positive for soil-blood mixed samples at all storage conditions throughout the 12 week study period. Samples stored at room temperature saw significant reduction in DNA concentration as storage time increased (P=0.001 and 0.0055 for Prepfiler and DNA IQ extractions, respectively). Samples stored at 4℃ saw a drastic decrease in DNA concentration just after two weeks of storage. By the eighth week of storage at 4℃, there was no detectable DNA (P=0.000 for all extraction methods). Samples stored at -20℃ recorded no specific pattern in decrease or increase in DNA concentration for the entire 12 week storage (P=0.324 and 0.161 for PrepFiler and DNA IQ extractions respectively). The PrepFiler kit yielded more DNA than the DNA IQ and Blood Miniprep kits at all storage conditions with no significant difference between PrepFiler and DNA IQ (P=0.603). The PrepFiler kit and DNA IQ kit were successful at removing possible PCR inhibitors from the soil during DNA extraction with no significant difference (p=0.887). The Blood Miniprep kit performed poor in terms of removing possible PCR inhibitors. There were full STR Profiles generated for room temperature stored samples and -20℃ stored samples extracted with PrepFiler and DNA IQ kits throughout the study. There were no allele recorded for room temperature stored samples and -20℃ stored samples extracted with Blood Miniprep kit. There were full, partial and null Profiles generated for 4℃ stored samples extracted with PrepFiler and DNA IQ kits depending on the sample storage duration. There were no alleles recorded for 4℃ stored samples extracted with Blood Miniprep kit. In conclusion, the -20℃ and PrepFiler Forensic DNA extraction kit were identified as the best storage condition and extraction method, respectively.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

All incidence, be it a crime, accident, natural disaster, armed conflict, leaves traces of physical evidence at the scene **(**UNODC, 2009). The world of crime is a complex place. A crime can be committed everywhere such as workplace, schools, places of residence, vehicles, on the streets, on water bodies and even on the Internet in modern times **(**National Academy of Sciences, 2009).

Every forensic process starts from the crime scene in order to obtain evidence for analysis. The aim of an investigation that follows the commission of a crime is to interpret correctly the facts, reconstruct the events and know what happened. Physical evidence can take any form from clearly visible objects to microscopic objects, generated when a crime is committed and retrieved at the scene or at other locations. Comparing all other forms of evidence available to an investigator (e.g. confessions, testimonies, and video recordings), physical evidence **(**body fluids, guns, knives, fingerprints, etc.) plays an important and exceptional role. All other sources of evidence connected to a crime cannot be completely relied upon with the exception of physical evidence when it is recognized and properly collected because it provides objective and most reliable clue about the crime that has been committed **(**UNODC, 2009). Blood is the most common body fluid found at crime scenes (Tobe *et al.*, 2007; Legg, 2013; Vandewoestyne *et al.*, 2015) and hence bloodstains present at a crime scene help investigators to know if a connection exists amongst a suspect, victim and crime scene (Sharma *et al.*, 2011). Blood provides a good ground for the growth of microbes and these microbes secrete biochemicals which degrade or destroy DNA in the blood (Rudin and Inman, 2001).

Advances in molecular genetics techniques have had a beneficial impact in the field of forensic science (Legg, 2013), such as exonerating the innocent, identifying the perpetrator of a crime, and creation of criminal Deoxyribonucleic Acid (DNA) databases. DNA analysis is a robust process with high power of distinction and high reliability. The proper collection and analysis of biological evidence using DNA techniques give the criminal justice system a potent tool for identifying the guilty and exonerating the innocent. This is because DNA can be obtained from various materials, such as blood, saliva, buccal cells, cigarette butts, bone, teeth, tissues, organs, hair strands, semen, urine, feces, sweat and profile generated for each individual is unique (with the exception of monozygotic twins) (Lorente *et al.*, 2001; Carvalho *et al.*, 2010; McCord *et al.*, 2011). At outdoor violent-related crime scenes, many situations may cause human biological evidence from the suspect or victim to be deposited in the soil (Kasu and Shires, 2015). This evidence mixed with soil at outdoor crime scenes might be the only source of evidence available to the investigator. This evidence can be analyzed using DNA to link the suspect or victim to the crime scene.

Soil mixed evidence or samples often pose challenges to the analyst due to the presence of humic acid, which leads to the inhibition of DNA amplification by interfering with the activity of the DNA polymerase and subsequently result in unsuccessful DNA profiling. Humic acid are resistant to separation from DNA due to their chemical structure. It shows similar solubility characteristics to DNA. Eventually, traditional isolation methods, such as phenol-chloroform, and detergent and protease treatments are not able to remove this humic contaminant, thereby remaining in the final DNA elute (Bessetti, 2007; Lakay *et al.*, 2007; Sutlovic *et al.*, 2007; Shahzad *et al.*, 2009).

The effective removal of inhibitors from soil mixed samples is thus an important task to overcome when working on these important samples. Many attempts in the extraction of DNA from forensic evidence have been undertaken over the years to eliminate soil inhibitors. Some of these methods or techniques have been incorporated into forensic human DNA isolation kits (Kasu and Shires, 2015).

Sometimes, soil mixed biological evidence are often not collected at crime scenes because they are limited in quantity, are difficult to work with and have high failure rate. This is as a result of the samples been heavily degraded due to environmental factors. This can lead to loss of important evidence that can lead to the identification of victim or suspect. It is therefore important that the most appropriate method of isolation is chosen to ensure that the most information is acquired from each precious forensic sample. In the case of soil-mixed samples, this information is currently lacking (Kasu and Shires, 2015).

Microbial activities in soil can degrade blood DNA after deposition making the DNA unusable for Profiling. Moreover, the co-extraction of microbial DNA, organic matter in soil as well as humic acid can interfere with DNA Profiling. When tissue samples are exposed to harsh environmental conditions, DNA degradation occurs rapidly to the extent that DNA becomes unrecoverable. Various problems can occur from the analysis of degraded DNA samples and these include signal loss, peak imbalance and allele dropout (McCord *et al.*, 2011), hence, crime scenes need to be processed as fast as possible and tissue samples properly stored.

1.2 Problem Statement

Bloodstains are often encountered on weapons, clothing and other materials and sometimes on the body of the victim. When these materials are discarded at outdoor locations, they may come

into contact with soil. Blood-mixed soil evidence may be of great value in crime investigation due to its potential presence at all outdoor crime scenes (Rohatgi and Kapoor, 2014). DNA obtained from blood, saliva or semen mixed with soil at an outdoor crime scene may become the main evidence to lead the investigation of a crime when a body is absent. However, biological sample mixed with soil is often contaminated with materials that pose a threat to the DNA profiling process (Kasu and Shires, 2015). It is estimated that 10 billion microbes can be found in a gram of soil and they comprise thousands of different species (knietsch *et al.*, 2003; Lakay *et al.*, 2007). These soil microbes destroy DNA in biological samples in a short period of time by fragmenting (Rudin and Inman, 2001) and together with inhibitors pose threat for successful DNA profiling. Inhibitors found in soil can interfere with the cell lysis process, interfere by nucleic acid degradation and also inhibit polymerase activity thus preventing enzymatic amplification of the template DNA (Wilson, 1997; Gryson, 2010; Butler, 2011). Most forensic scientists are faced with the problem of contamination and degradation when it comes to extraction of DNA from soil-mixed samples (Sutlovic *et al.*, 2007). According to Zhou *et al.*, 1996 and Sutlovic *et al.*, 2007, isolation of DNA from soils always ends in the co-isolation of humic materials which interfere with DNA detection and measurement.

It is important to note that, proper laboratory storage of soil-mixed blood samples prior to their processing has not been well documented. Microbes in the soil, organic matter and storage temperature can all affect the evidence and hence there is the need to study the best laboratory storage condition for these crime scene samples.

1.3 Main Objective

The primary objective of this project was to do a comparative evaluation of three laboratory storage conditions and DNA extraction methods on soil-blood mixed samples found at crime scenes using serological and DNA Analysis.

The specific objectives were:

- To investigate the DNA extraction potency of three commercial kits (PrepFiler Forensic kit, Promega DNA IQ Kit, Blood Miniprep kit) on soil-blood mixed sample.
- To investigate the effect of storage time on DNA yield and quality of soil-blood mixed sample at three different laboratory storage conditions (Room storage/25 ^{o}C, 4 ^{o}C and -20 ^{o}C).
- To investigate the effect of three laboratory storage conditions on DNA yield and quality of soil-blood mixed sample.

1.4 Justification

Blood mixed with soil may be of great interest in criminal investigation due to its potential presence at all outdoor crime scenes. Soil can provide valuable information to criminal investigations as transfer evidence because many criminal cases take place under circumstances such that soil transfers to a perpetrator or victim. Thus soil can be a valuable source of evidence especially in homicide cases when it is mixed with blood **(**Rohatgi and Kapoor, 2014). Crime scenes containing biological evidence are usually processed early to prevent loss or destruction of evidence. Evidence submitted to the laboratory from crime scenes are usually not processed immediately due to large number of pending cases, unavailability of analysts or unavailability of reagents. There is thus the need to investigate the proper way of storing these evidence at the

laboratory to prevent destruction prior to their processing. Extraction of DNA from soil-mixed sample will co-extract DNA from microbes in the soil, organic matters as well as presence of PCR inhibitors in the final elute, and thus, there is the need to investigate the best and appropriate method for the extraction of DNA from soil-mixed sample.

DNA IQ, Blood Miniprep Kit and Prepfiler Forensic DNA kit have been claimed by the manufacturers to be effective in extracting quality DNA from samples in the presence of inhibitors and contaminants (Bessetti , 2007; Brevnov *et al.*, 2009; Kasu and Shires, 2015; http://www.biopioneerinc.com/2016/molecular-biology/genomic-dna-isolation-kits/blood-genomic-dna-miniprep-kit-100-preps/ accessed 8th April, 2018).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Forensic Science

Over the past 20 years, advances in some forensic science disciplines, especially the use of DNA techniques, have shown that some branches of forensic science have great additional potential to help investigators identify criminals and exonerate the innocent. A lot of crimes that may have gone unsolved are now being solved because forensic science is aiding in the identification of the perpetrators (National Academy of Sciences, 2009).

Crime scene investigators process and package evidence found at crime scenes and transport them to the Forensic laboratory for scientific analysis. Experts at the laboratory use valid analytical techniques to give a credible forensic report which can help solve a case (National Academy of Sciences, 2009; National Academy of Sciences, 2015).

2.1.1 Forensic Science in Ghana

In Ghana, there is only one Forensic science laboratory and this is operated by the Ghana Police Service. The Forensic science laboratory is under the immediate supervision of a director. There are five sections that make up the laboratory. These are the Biology section (serology and DNA), Chemistry or Drug Analysis section, Photography section, Firearms examination section and Questioned documents examination section. There are also crime scene management teams at National Police Headquarters, each Police region and some few Divisional Police Headquarters. The various crime scene management teams feed the Forensic science laboratory with physical evidence needed for analysis. There also exist Cybercrime and Fingerprint Units at the National Police Headquarters. Other institutions that undertake some forensic work in Ghana include the

Military Police Unit of the Ghana Armed Forces, the Ghana Immigration Service, the Narcotics Control Board, the Ghana Standards Authority and the Customs, Excise, Preventive Service. Major hospitals in Ghana also help the Police in death and sexual assault investigations through autopsy and victim examination, respectively.

2.2 Collection, packaging, preservation and transport of biological evidence at crime scenes

Biological evidence found at crime scenes must be marked and photographed before they are collected and packaged. During evidence collection, the crime scene investigator or evidence collection technician must adhere to the rules governing biological evidence collection and use proper methods and safety precautions in order to prevent contracting potential diseases and also prevent contamination of evidence. Wearing of appropriate protective clothing such as latex gloves, protective overall coat, face and nose masks, head cover, etc. must all be done. Gloves must be changed for every evidence collected to prevent contamination. Each evidence collected must be packaged separately to prevent cross contamination. All evidence must be appropriately labeled with the case number, date of collection, name of collector and time of collection. Liquid evidence like bloodstains, semen stains, saliva stains must be air-dried before packaging. All dried biological evidence must be packaged in paper envelopes or bags and transported to the laboratory.

DNA in biological evidence at the crime scene may be degraded by harsh or adverse environmental conditions which breaks the DNA molecules into smaller fragments. Harsh environmental conditions that can degrade DNA include oxygen, water, UV, and nucleases that fragment DNA. Moreover, the activities of crime scene investigators can also pose challenge to analysis of biological evidence at the laboratory hence Proper DNA evidence collection and

packaging at the crime scene cannot be overlooked (Rudin and Inman, 2001; Butler, 2011). DNA evidence can be stored for longer periods as in its non-extracted form or as fully extracted DNA (Butler, 2011).

2.3 Brief History of Forensic Serology

Forensic serology started in 1901 through experiment by the Austrian scientist Karl Landsteiner. Landsteiner discovered that blood can be grouped into four types depending on the presence or absence of specific antigens and antibodies on their red cells. Leon Lattes used Landsteiner's work for forensic practice (Evans, 2009; Legg, 2013). Years before Landsteiner's work, physicians tried to transfuse blood from an individual to patient but their efforts failed because coagulation or clotting occurred as soon as blood from the donor entered the recipient. This resulted in death of the recipient. Landsteiner's work solved this problem because physicians got to know that blood from individuals were not the same (Hillyer, 2007). The German scientist Paul Uhlenhuth through experimentation in the same year also devised a test that differentiated blood from human and non-human sources based on antigen-antibody reactions. He called it the precipitin test (Evans, 2009)

2.3.1 Forensic Serology

Serology is used to describe a series of laboratory tests or experiments that makes use of antigen and antibody reactions popularly called immunologic tests (Butler, 2011; Conti and Buel, 2011). Some serological tests also make use of chemical reaction assays, enzyme activity assays, as well as microscopy based assays (Legg, 2013). These tests make use of body fluids and allow the scientist to identify which fluid is it and also confirm the source of the fluid (Williams, 2012; Claridge, 2016). Based on the amount of body fluid left at a scene of crime or based on the

material or substrate on which the fluid is deposited, it can be visible to the naked eye or invisible and hence will need enhancement to see it (Eckert, 1996).

Serological tests involve an initial preliminary or presumptive colour test, followed by a confirmatory test to determine the species origin of the body fluid in question **(**Conti and Buel, 2011). When the source of a crime scene sample such as semen, saliva or blood is confirmed as human sample, then DNA technology can be used on the sample **(**Prahlow, 2010). Serology plays an important role in forensic science but with the advent of modern DNA technology, it is of little use because most serological tests unlike DNA cannot pinpoint crime scene evidence to a single individual (Butler, 2011; Legg, 2013) yet without serological testing, a DNA Profile alone cannot tell you the type of biological fluid the profile originated from (Legg, 2013). In some instances like identical twins (same DNA Profiles), serological tests (Antibody profiles) can be used to individualize them (Sharma *et al.*, 2011).

2.3.2 What happens at the Forensic Serology Laboratory?

When a sample is received at the laboratory for serological testing, it is advisable for the analyst to ask and answer these questions

- Is the fluid or stain blood, semen, saliva, etc?
- Is it of animal or human source?
- Whose fluid is it? Eg. Blood grouping / DNA Analysis (Brown and Davenport, 2015).

The sample is first examined visually for physical characteristics. Normally the experience of the analyst is paramount to visually detect stains/fluids. Hidden stains can be visualized with alternate light source.

The sample then goes through presumptive test to see whether it is the suspected stain or not. Presumptive test does not tell the analyst whether the fluid is of human or animal origin. If a presumptive test is negative, serological testing ends. If it is positive, further serological confirmatory test is done to ascertain whether the fluid originated from human or animal source.

In some instances, confirmatory test is also performed to determine the species origin of a fluid, thus, to determine whether a particular fluid found at a crime scene originated from human, dog, monkey, fowl, goat, etc. After confirmatory identification of a body fluid/ stain, a serological report is written by the analyst or DNA Analysis is done to link the fluid to a particular individual.

2.4 Blood

Blood is made up of a fluid plasma and serum with some solid materials embedded in it. These solid materials consist of red blood cells or erythrocytes, white blood cells or leukocytes, and platelets or thrombocytes (Eckert, 1996; Butler, 2011; Legg, 2013).

Surrounding red blood cells is a layer containing antigens that can engage in particular antigen-antibody reactions (Eckert, 1996). At maturity, red blood cells do not have a nucleus and hence lacks DNA. White blood cells have nucleus and hence is the source of DNA in blood. A microliter of blood contains about 4000 white blood cells (Eckert, 1996). In forensic investigations, the preliminary method used for the identification of blood is called Presumptive blood test.

2.4.1 Presumptive tests for Blood

Presumptive tests for blood are fast, safe, inexpensive, very sensitive and easy to perform but do not identify the species origin of the blood in question. Most of these tests detect the presence of hemoglobin molecules which are found in the red blood cells and carry oxygen and carbon dioxide within the body (Shaler, 2002; Spalding, 2003; Tobe *et al*., 2007; Virkler and Lednev, 2009; Colotelo, 2009; Butler, 2011; Fisher and Fisher, 2012). Though the tests detect the presence of haemoglobin to produce a colour change, it is actually the peroxidase action of the haem group that causes the colour change (William, 2012). A positive presumptive test only indicates the possible presence of blood but it's not specific for blood because some plant materials that contain peroxidase can also give positive results (Dutelle, 2016). Presumptive tests should use only a small amount of the evidence (Fisher and Fisher, 2012) in order to get enough sample for subsequent confirmatory and DNA Testing.

2.4.1.1 Phenolphthalein (Kastle-Meyer test)

This test is used most often in many forensic laboratories to detect blood. It makes use of phenolphthalein and hydrogen peroxide to give a colour change to pink when exposed to blood (Spalding, 2003; Colotelo, 2009; Fisher and Fisher, 2012; Legg, 2013; Dutelle, 2016). It is simple, quick and cheap (Gunn, 2011).

2.4.1.2 Benzidine test

When a mixture of blood, Benzidine and hydrogen peroxide is made, an oxidation-reduction reaction occurs converting the Benzidine to diazo dye (a bluish-green product) (Newton, 2007). This test is very sensitive and can detect haemoglobin at very low concentration in blood (Anand

et al., 1994).The presences of impurities as well as instability of the hydrogen peroxide solution are major drawbacks to the Benzidine test (Ingham, 1932).

2.4.1.3 Leucomalachite green

This test makes use of Leucomalachite green powder, glacial acetic acid and distilled water. The entire mixture is diluted with hydrogen peroxide (Dutelle, 2016). When the mixture comes into contact with blood, iron in the blood changes the Leucomalachite green to blue-green (Brown and Davenport, 2015).

2.4.1.4 Luminol

Luminol is 5-amino- 2, 3-dihydro-1,4-phthalazinedione (Newton, 2007) and it is another presumptive test for the detection of blood (Butler, 2011; Brown and Davenport, 2015). The Luminol reagent is made by adding 3-amino-phthalhydrazide and sodium carbonate in distilled water (Gunn, 2011; Butler, 2011). By spraying Luminol at large areas, the presence of bloodstains can be detected (Butler, 2011; Brown and Davenport, 2015).

Luminol produces light or glows to indicate the presence of blood and the glow is best observed in a dark environment (Barni *et al.,* 2007; Stevens, 2009; Dutelle, 2016). The reaction or glow can quickly be photographed (Barni *et al.,* 2007; Fisher and Fisher, 2012; Dutelle, 2016). Using Luminol on crime scene samples do not interfere with subsequent DNA Profiling (James *et al.,* 2002; Colotelo, 2009; Gunn, 2011; Dutelle, 2016). Household bleach or sodium hypochlorite can give false-positive results to Luminol test (Fisher and Fisher, 2012).

2.4.1.5 Hemastix strips

Hemastix strips are plastic strips that have been treated with a special blood reagent (mixture of 2-methylaniline and hydrogen peroxide). The strips are moistened with distilled water and touched with the suspected blood stain. If blood is present in the substance being tested, the hemoglobin catalyzes the conversion of 2-methylaniline to a green product. In the case of a suspected liquid blood, there is no need to moisten the strip (Newton, 2007; Brown and Davenport, 2015). Hemastix strips were manufactured to detect blood in urine (Fisher and Fisher, 2012; Dutelle, 2016) but are used worldwide by crime scene investigators.

Hemastix doesn't interfere with subsequent DNA analysis (Dutelle, 2016).

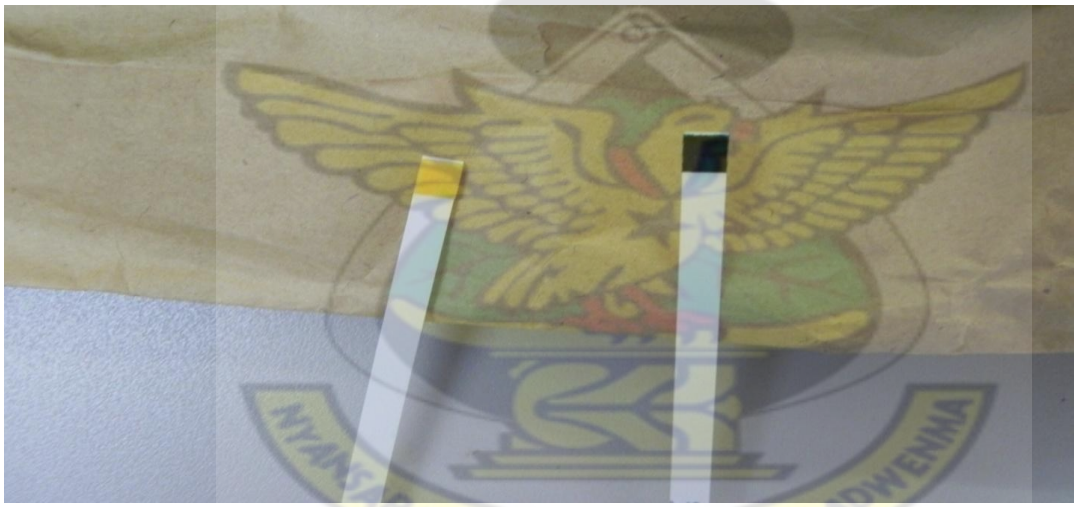


Figure 2.1: Hemastix strips showing positive (dark blue) and negative (yellow) for blood (Source: Ghana Police Forensic Science Lab)

2.4.2 Confirmatory tests for Blood

Positive presumptive test in forensic case analysis is usually followed with confirmatory test, which is more specific for a particular species.

2.4.2.1Teichmann test

This test involves heating dried blood (James *et al.*, 2002) to form crystals as a result of the chemical reaction between the iron portion of hemoglobin in the blood and Teichman reagent, a solution of potassium bromide, potassium chloride and potassium iodide in glacial acetic acid (Legg, 2013; Dutelle, 2016). The crystals formed are observed microscopically, and are usually brownish and rhombic (James *et al.*, 2002).

2.4.2.2 Precipitin test

This test is based on the principle of simple diffusion between 2 liquids in contact with each other. The antiserum of the species to be tested and the suspected bloodstain are the 2 liquids. When the antiserum (eg. human antiserum) is placed in a test tube and an extract of the suspected bloodstain is placed on it, the antigens and antibodies come into contact and a precipitate is formed. If the bloodstain is not from a human source, there will be no precipitate (James *et al*., 2002; Karmakar, 2003; James *et al.*, 2005; Newton, 2007).

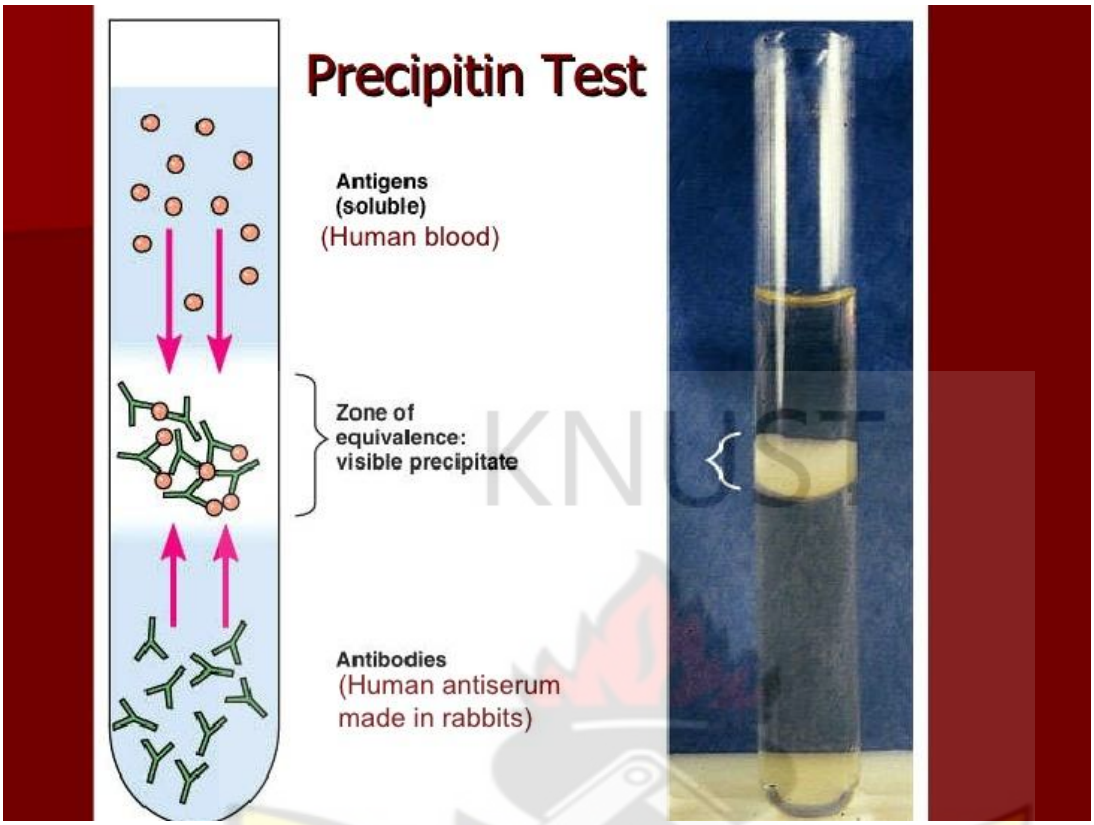


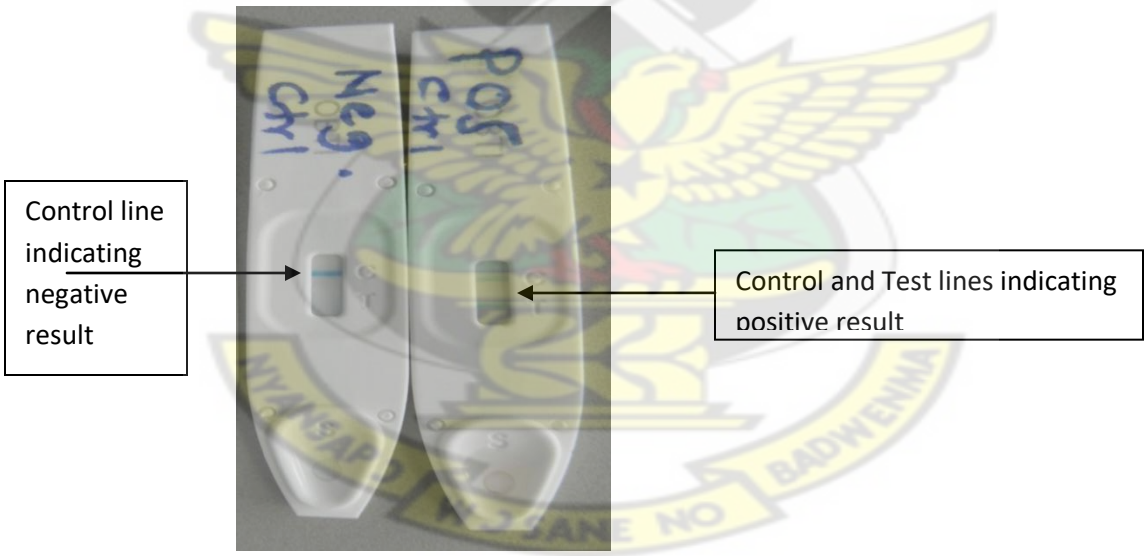
Figure 2.2: Precipitin test (Source: https://www.tes.com/lessons/cfjSt8FlQVcD1w/agglutination-and-precipitation accessed 1st March 2017).

2.4.2.3 Hexagon OBTI Kit

The hexagon OBTI is an immunochromatographic test kit for confirming the presence of human blood at a crime scene or the laboratory (Hermon *et al.*, 2003). The kit can detect minute quantities of hemoglobin. A test pouch contains a test cassette and a liquid collection medium (Tris buffer, pH 7.5). The test device comprises a conjugate consisting of blue particles and antibodies (anti-human Hb). When a blood sample is mixed with the Tris buffer and applied to the test cassette, human hemoglobin in the sample reacts with reagent comprising of blue materials and monoclonal anti-human hemoglobin antibodies. This immunocomplex moves in

the cassette to the T region where it is captured by an immobilized second antibody directed at human Haemoglobin forming a blue line at the T region on the cassette. This indicates the substance tested is human blood. Unreacted reagents migrate further and are bound in a second line by immobilized IgG antibodies. This is the control line on the test cassette and indicates correct function of the test kit (http://www.bluestar-forensic.com/pdf/en/instructions_hexagon_obti.pdf, accessed 21st February 2017). A visible blue line at the control region alone indicates a negative result for human blood. The Hexagon OBTI test is useful with aged and degraded material (James *et al*., 2002).

(a) Test cassette and liquid collection medium for the Hexagon OBTI test



(b) Hexagon OBTI test showing positive and negative results

Figure 2.3: Hexagon OBTI test kit for detecting human blood (Source: Ghana Police Forensic Science Lab)

2.5 Deoxyribonucleic Acid (DNA)

In the 1920s, Phoebus Levene discovered with his friends at the Rockefeller institute that cells contain nucleus which in turn contain nucleic acid (Alcamo, 1996; Evans, 2009). The average human body is made up of approximately 100 trillion cells and hence there is large quantity of Deoxyribonucleic acid or DNA in the human body (Kirby, 1992). DNA is the basic building block of a person's genetic makeup (Rudin and Inman, 2001; Kobilinsky *et al.,* 2007; James, 2012).

DNA is packed into 23 pairs of human chromosomes (long threadlike or rodlike structures in a person's body). One chromosome from each pair is inherited from an individual's mother and the other from an individual's father (National Research Council, 1992; Stevens, 2009; Gefrides and Welch, 2011). Due to its phosphate groups, DNA has a negative charge (Kobilinsky *et al.*, 2007; Elkins, 2012) and with the absence of mutation, DNA from any part of the body is the same (Kobilinsky *et al.,* 2007).

2.5.1 Sources of DNA

DNA can be found in the nucleus of a cell and hence can be extracted from all biological evidence left at crime scenes (Luftig and Richey, 2000; Butler, 2011; Gefrides and Welch, 2011). Blood on weapons, blood in soil, semen on clothing, vaginal swabs, buccal swabs from suspects and victims, bones, teeth, nails, etc., all contain DNA. Even latent prints containing few cells can be a good source of DNA (Butler, 2011). In the cytoplasm of a cell, there are special organelles called mitochondria which serve as power-house of the cell and these organelles also contain DNA (Kobilinsky *et al*., 2007).

2.5.2 Types of DNA

2.5.2.1 Nuclear DNA

This is the type of DNA that is extracted from the nucleus of a cell and hence can be found in all nucleated cells. This is commonly used for human identification because it is inherited from both parents and individually specific except in the case of identical twins. Nuclear DNA is linear in shape.

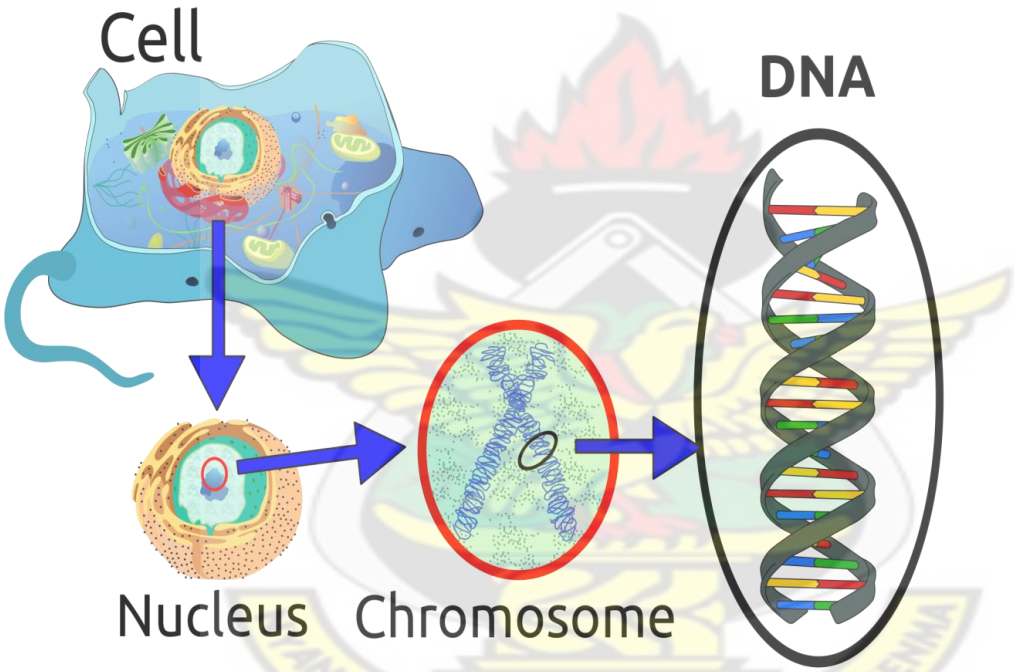


Figure 2.4: Location of eukaryotic nuclear DNA in a cell (Source: https://en.wikipedia.org/wiki/DNA accessed 7th September 2016).

2.5.2.2 Mitochondria DNA

This is the type of DNA found in an organelle called mitochondria. There are hundreds to thousands of mitochondria DNA in a single cell compared to nuclear DNA. Mitochondria DNA

is maternally inherited, meaning, all maternally related individuals have the same mitochondria DNA (Kobilinsky *et al*., 2007; Gunn, 2011; Elkins, 2012; Fisher and Fisher, 2012). This is a drawback to the use of mitochondria DNA in human identification. Mitochondria DNA is circular in shape (Gunn, 2011; Fisher and Fisher, 2012).

2.5.3 Effect of Ethylenediaminetetraacetic acid (EDTA) on blood DNA

Nucleases need magnesium to work properly so one of the measures to prevent them from digesting DNA in blood is the addition of a preservative known as EDTA. EDTA chelates, or binds most of the free magnesium and thus helps prevent the nucleases from destroying the DNA in the collected blood sample (Butler, 2011). Study by Khosravinia and Ramesha in 2007 showed that EDTA added to collected blood samples had no impact on quantity and quality of DNA as well as PCR.

2.6 DNA Profiling

In 1943, Oswald Avery through experimentation arrived at a conclusion that DNA is the material responsible for the inheritance of genetic traits from generation to generation (Rudin and Inman, 2001; Evans, 2009). Based on this, scientists got the hope that analysis of DNA might be useful in forensic science one day (Evans, 2009).

In 1984, Professor Sir Alec Jeffreys developed genetic markers on an X-ray film and compared it with other samples (Evans, 2009). Use of DNA in forensic investigation can be said to be an expansion of forensic serology which dates back as far as more than 50 years **(**National Research Council, 1992).

2.6.1 DNA Profiling Process

The sequencing of a person's DNA is known as DNA Profiling (Gunn, 2011). Most sequences on a DNA molecule are the same for all individuals. Due to the advances in molecular biology, it is possible to study the individual differences in the small percentage of sections or sequences of DNA that do not code for proteins. These non-coding sections of the DNA are highly variable in length and these are where forensic scientists analyze and use for human identification or DNA Profiling **(**National Research Council, 1992; Eckert, 1996; Gefrides and Welch, 2011). By analyzing these non-coding sections or markers, a forensic laboratory can develop a DNA profile from crime scene samples to be used in solving cases (Eckert, 1996; James, 2012). Like other forensic science tests, a reference sample from the victim or suspect is needed by the laboratory to compare with the crime scene evidence **(**Eckert, 1996; Prahlow, 2010; Butler, 2011). Where there is no reference sample, the DNA profile from the crime scene sample is compared to a DNA database to see if there would be a match. When there is no match from the database, the crime scene profile is added to the DNA database for any future hit or match (Butler, 2011).

With the exception of cases where crime scene evidence do not match sample from a suspect or victim, it is always necessary to show the significance of DNA result using population allelic frequencies **(**National Research Council, 1992). There exist frequencies of each allele in a particular population. From these allele frequencies, genotype frequencies for each marker used in the DNA profiling process of the crime scene sample can be calculated and the combined genotype frequency for the entire DNA profile also calculated. Based on this genotype frequency, a statement can be made as to whether a particular person can be included or excluded as a possible source of the crime scene sample **(**Petricevic, http://nzic.org.nz/ChemProcesses/biotech/12D.pdf, accessed 7th June 2016).

Modern Forensic DNA Profiling involves a number of steps:

- DNA extraction from reference and crime scene samples
- DNA quantity and quality determination
- Multiplex PCR amplification of target sequences
- Capillary electrophoresis for separation of amplified DNA fragments
- Fragment analysis with software and allele calls
- Comparison of electropherograms from reference and crime scene samples
- Statistical calculations to determine inclusion or exclusion status.

2.6.1.1 DNA Extraction

The first step in forensic DNA analysis is the removal of the biological material from its substrate and subsequent removal and purification of the DNA from the cells. This process is called extraction or isolation (Rudin and Inman, 2001; Gefrides and Welch, 2011; Butler, 2011). There exist several extraction methods and they all work to:

- Separate the biological material from any substrate
- Break the cell to bring the DNA and all other cellular contents into solution
- Separate the DNA from other cellular contents (proteins, Lipids, etc) and inhibitors in order to get a purified DNA (Newton, 2007; Gefrides and Welch, 2011; Butler, 2011; Taupin, 2013).
- Purify and elute the DNA for use or storage.

An ideal forensic DNA extraction process should prevent further degradation of the DNA (Butler, 2011) and also remove inhibitors that prevent or interfere with polymerase chain reaction (Bessetti, 2007; Butler, 2011). The main idea behind DNA extraction is to get a high

molecular weight DNA devoid of contaminants (Sambrook *et al*., 1989; Kirby, 1992). Isolated DNA is normally stored at -20 °C or -80 °C, to prevent nuclease destruction of the DNA (Butler, 2011).

Figure 2.5: DNA Extraction workflow (source: http://www.epigentek.com/catalog/fitamp-general-tissue-section-dna-isolation-kit-p-31.html accessed 7th September 2016).

2.6.1.1.1 Promega DNA IQ Extraction kit

The IQ means isolation and quantification. This is a quick extraction commercial kit which can deal with a lot of challenged forensic samples and remove inhibitors (Bessetti, 2007; Kasu and Shires, 2015). Amount of DNA is bound to a tube using paramagnetic resin. The resin has a limit for bound DNA and binds only a certain quantity of DNA. DNA quantity from this extraction method is stable within one sample type but changes from different samples (Promega, 2016).

2.6.1.1.2 PrepFiler Forensic DNA Extraction kit

This extraction method works on magnetic particle technique similar to Promega's DNA IQ (Applied Biosystems, 2008; Butler, 2011). This kit has magnetic particles which binds DNA

whiles PCR inhibitors are removed by washing the bound DNA, resulting in pure genomic DNA (Applied Biosystems, 2008; Brevnov *et al.*, 2009).

2.6.1.1.3 Blood Genomic DNA Miniprep kit

This kit is meant for quick extraction of genomic DNA from blood samples and other body fluids. A small starting material is sufficient to obtain about 30 µg of genomic DNA devoid of contaminants (http://www.biopioneerinc.com/2016/molecular-biology/genomic-dna-isolation-kits/blood-genomic-dna-miniprep-kit-100-preps/ accessed 31st August, 2016).

2.6.1.2 DNA Quantification

After extraction of DNA from human crime scene samples, it is possible to have other non-human DNA such as bacterial and fungal DNA in the final elutes (Butler, 2011). It is important to know the amount of total DNA as well as human DNA in the extracted sample and its integrity (Newton, 2007; Goodwin *et al.*, 2011; Butler, 2011). Modern real-time PCR quantification methods can help an analyst measure total human DNA in an extracted sample.

Measuring or knowing the amount of DNA in the extracted sample is called DNA quantification (Gefrides and Welch, 2011). DNA quantification uses a small amount of the extracted sample and compares it to DNA standard of known concentration (Taupin, 2013).

DNA degrades as time passes, thus, the DNA disintegrates into smaller fragments. It is therefore advisable to assess the integrity of extracted DNA because the type of subsequent analysis to be employed depends on the integrity and quantity of DNA extracted (Newton, 2007; Gefrides and Welch, 2011; Butler, 2011). There are different methods of quantifying DNA.

2.6.1.2.1 Spectrophotometry

Measuring absorbance of an extracted DNA sample at 260 nm using spectrophotometer is a method of determining the quantity of DNA (Sambrook *et al*., 1989; Kirby, 1992). However, quantification methods using absorbance techniques are not accurate, because contaminants like phenol and proteins in the extracted sample can give false concentrations (Butler, 2011). Moreover, very low concentrations of DNA cannot be measured with spectrophotometer.

Nucleic acids absorb most at 260 nm; hence, amount of light absorbed at this wavelength can be used to calculate the quantity of nucleic acids. Nucleotides, ssDNA, dsDNA and RNA all absorb at 260 nm so absorbance at 260 nm is not specific for one type of nucleic acid. Absorbance at 280 nm is used as a measure of protein concentration.

To determine protein contamination of a nucleic acid, the ratio of A260/A280 is measured. Pure DNA has A260/A280 ratio of about 1.8 whiles pure RNA has A260/A280 ratio of about 2.0. Any value lower than these is an indication of protein contamination of the nucleic acid sample.

2.6.1.2.2 Slot Blots

Slot blotting involves capturing DNA on nylon membrane followed by addition of a human-specific probe. Signal intensities resulting from chemiluminiscent of samples and standards are then compared (Butler, 2011). This method is time-consuming and can detect ssDNA and dsDNA of up to 150 pg concentration (Walsh *et al*., 1992; Butler, 2011).

2.6.1.2.3 Real-time Polymerase Chain Reaction (RT-PCR)

Real-time PCR (RT-PCR) is a quantification method in which the amplification process is monitored as it happens (Gefrides and Welch, 2011). Knowing the amount of DNA extracted is

very key in obtaining quality Short Tandem Repeat (STR) electrophoresis results. Forensic DNA analysts often use RT-PCR to quantify extracted samples because RT-PCR is very sensitive and targets only the human portion of the sample (Stevens, 2009; Seo *et al.,* 2012). Also, with real-time PCR, analysts can know the state or quality of the extracted sample as well as presence of inhibitors in the sample (Gefrides and Welch, 2011).

Figure 2.6: Applied Biosystem's 7500 Real-time PCR Machine (Source: Ghana Police Forensic Science Lab)

2.6.1.2.3.1 QuantiFiler Trio Kit

The QuantiFiler Trio Kit quantifies total human DNA and total human male DNA at the same time. Results from real time PCR with QuantiFiler trio can assist the analyst to know:

- If there is sufficient human DNA and/or human male DNA for subsequent STR analysis

- The quantity of sample to use for PCR amplification
- Ratio of male to female in mixed samples especially sexual assault samples.
- The quality of DNA (degradation and inhibition).

There are three targets of amplification in the QuantiFiler trio kit; small autosomal, large autosomal and Y target. The Small Autosomal and Y targets have short amplicons (75 to 80 bases) in order to maximize the chance of detecting degraded samples. The Large Autosomal has relatively longer amplicon (>200 bases) to aid an analyst know if the sample is degraded. The small autosomal quantity actually gives the concentration of the sample.

There is also an internal positive control which contains a synthetic template DNA. By assessing the internal positive control, an analyst can determine if a sample has zero concentration or contains inhibitors.

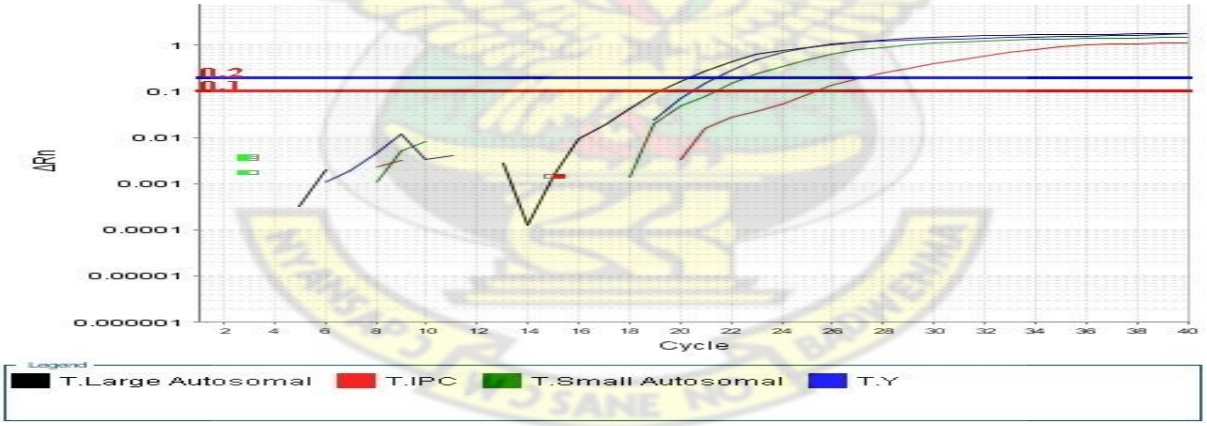


Figure 2.7: Real-time PCR Amplification plot for male sample using QuantiFiler trio kit (Source: Ghana Police Forensic Science Lab)

ΔRn means Rn minus the baseline. Rn means the fluorescence of the reporter dye divided by the fluorescence of a passive reference dye; thus, Rn is the reporter signal normalized to the fluorescence signal of Applied Biosystems ROX dye.

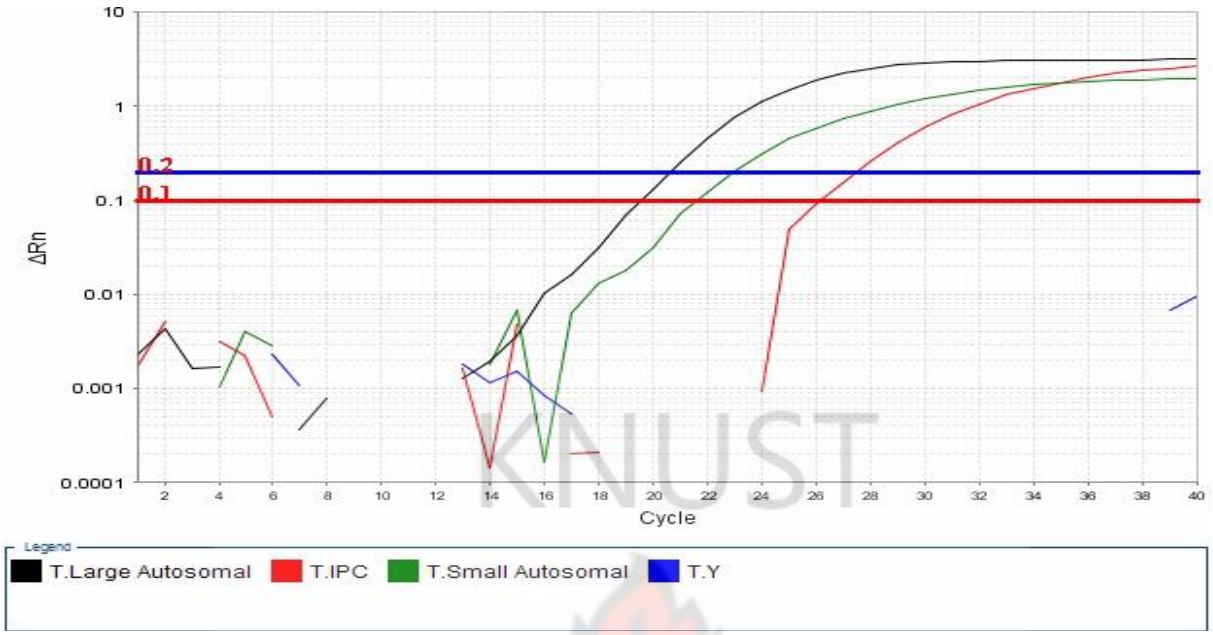


Figure 2.8: Real-time PCR amplification plot for female sample using QuantiFiler trio kit (Source: Applied Biosystems, 2016)

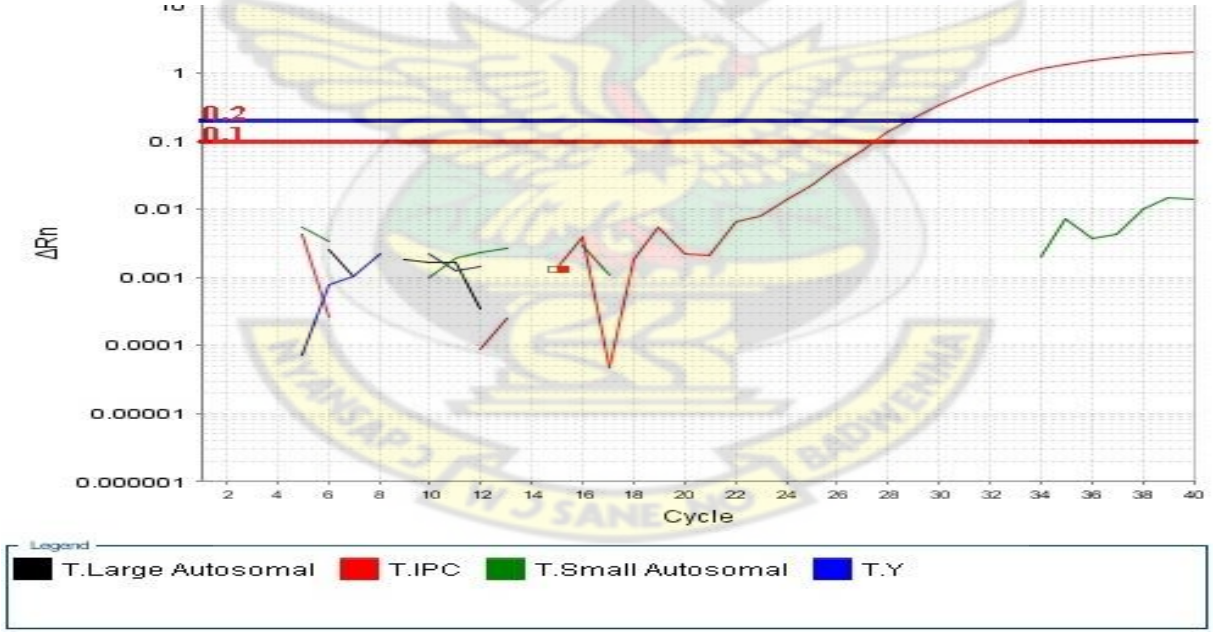


Figure 2.9: Real-time PCR amplification plot for no template (NTC) sample using QuantiFiler trio kit (Source: Ghana Police Forensic Science Lab)

Threshold cycle is where an amplification curve and a threshold line meet. When the Threshold cycle (C_T) value of a target is less than 40, then positive amplification has occurred. The Internal positive control (IPC) C_T value is relatively the same in normal reactions but presence of inhibitors in the sample and/or DNA quantities can increase the IPC C_T value compared to the average IPC C_T value of the standards on the same reaction plate. When PCR inhibitors are in large concentration in a sample such that subsequent analysis could be affected, the IPC C_T flag is triggered for that particular sample. Large Autosomal target may be affected by the increasing inhibitor concentration before the Small Autosomal target and before the IPC C_T flag is triggered. Small rise in value of degradation index may be as a result of degradation and/or presence of inhibitors (ThermoFisher Scientific, 2016).

2.6.1.3 DNA Amplification (Polymerase Chain Reaction)

The technique of polymerase chain reaction was developed by Kary Mullis in the mid 1980s and this technique has helped forensic DNA analysis and molecular biology in general (Houck and Siegel, 2009). DNA concentration and degradation or inhibitions are the most essential factors that can affect the success rate of PCR and the entire nuclear DNA profiling process (Taupin, 2013). Crime scene biological samples are usually limited in DNA quantity and quality but PCR has the ability to work on these samples (Butler, 2011).

PCR is a very sensitive technique that has the ability to make copies or amplify a particular sequence or target so that sequence can be detected by electrophoresis (Luftig and Richey, 2000; Rudin and Inman, 2001; Triggs *et al.*, 2004; Kobilinsky, 2007; Sutlovic *et al.*, 2007; Stevens, 2009; Butler, 2011; Gefrides and Welch, 2011; Fisher and Fisher, 2012; Schrader *et al.*, 2012; Taupin, 2013).

The PCR process employs a reaction buffer, a thermostable polymerase, the template DNA, Primers, $MgCl_2$, the nucleotides dATP, dCTP, dGTP, and dTTP, and a thermal cycler (Kirby, 1992). A PCR cycle is made up of three stages:

- Denaturing the template DNA
- Annealing of the primers to the single-stranded DNA
- Extension

Each PCR cycle results in a doubling of product (Eckert, 1996; Rudin and Inman, 2001; Kobilinsky *et al*., 2007; Stevens, 2009).

Figure 2.10: Applied Biosystem's GeneAmp 9700 thermal cycler (Source: Ghana Police Forensic Science Lab)

2.6.1.3.1 GlobalFiler PCR amplification kit

The GlobalFiler PCR Amplification Kit manufactured by Applied Biosystems uses a 6-dye, STR multiplex assay for amplifying human nuclear DNA. This kit amplifies: 21 autosomal STR loci (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338), 1 Y-STR (DYS391), 1 insertion/deletion polymorphic marker on the Y chromosome (Y indel) and Amelogenin (sex determining marker). This kit is very sensitive and can work in the midst of inhibitors (ThermoFisher Scientific, 2016).

2.6.1.4 Electrophoresis

2.6.1.4.1 Introduction

The movement of charged particles in an electric field through an appropriate buffer is called electrophoresis. This process separates DNA fragments produced after PCR into their individual sizes (James *et al.*, 2002; Stevens, 2009). It can either be traditional gel electrophoresis or modern capillary electrophoresis. During electrophoresis, negatively charged particles move to the anode and positively charged ones move to the cathode (James *et al.*, 2002).

2.6.1.4.2 Gel Electrophoresis

DNA sample can be run on an Agarose gel stained with ethidium bromide to determine its quality. A sample of high quality and high molecular weight runs on the agarose gel and is seen as a sharp band. Degraded DNA is seen as smear on the gel (Kirby, 1992; Butler, 2011; Goodwin *et al.*, 2011). During gel electrophoresis, smaller DNA fragments move faster in the gel matrix

than larger fragments. As a result, smaller fragments are always seen closer to the positive electrode than larger fragments (Eckert, 1996).

2.6.1.4.3 Capillary Electrophoresis

This is the method of detecting PCR products through capillary using a Genetic analyzer (Fisher and Fisher, 2012). The method makes use of a polymer, which is identical to gel in the traditional gel electrophoresis. Smaller fragments move faster through the polymer in the capillary than larger fragments (Taupin, 2013).

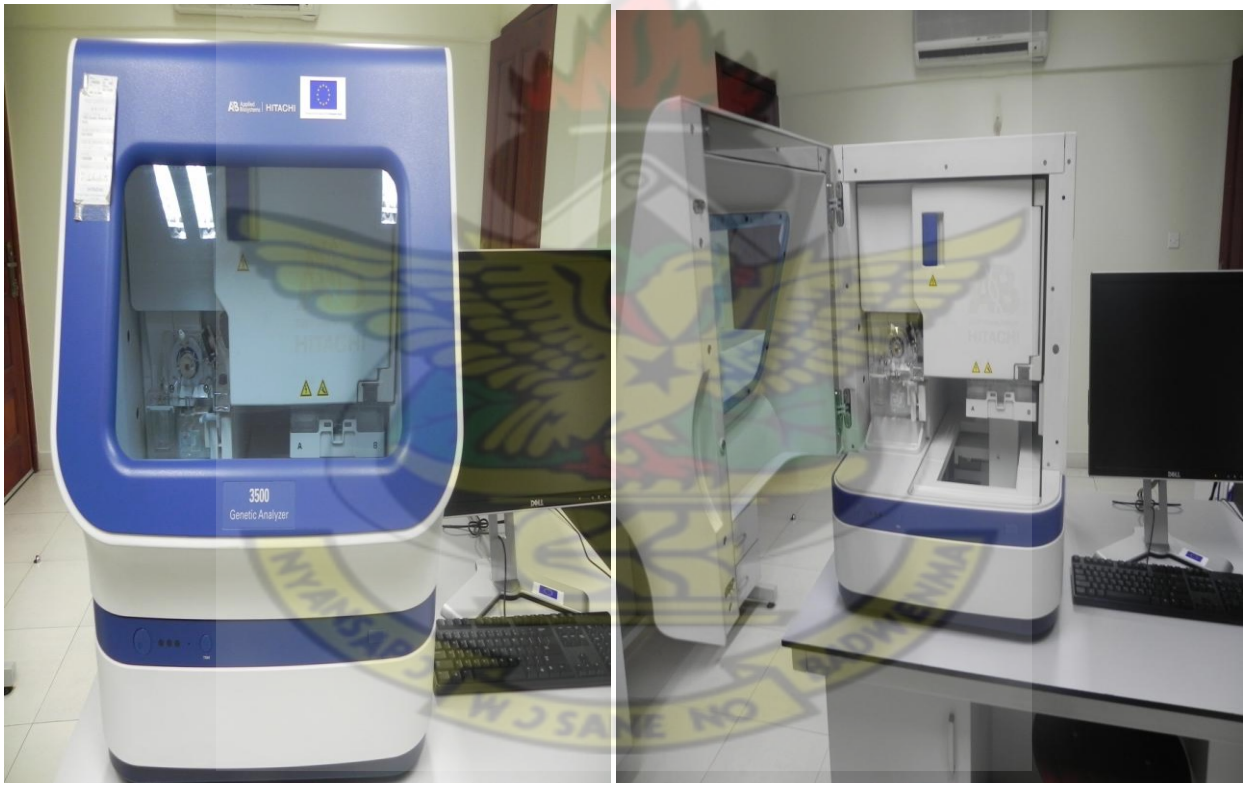


Figure 2.11: Applied Biosystem's 3500 Genetic analyzer (Source: Ghana Police Forensic Science Lab)

2.7 Soil

Different soil types differ in structure and constitution, chemical properties and composition, texture, colour as well as biological composition **(**Mitchell and Soga, 1976; Jenny, 1994; Lasota, 2014).

Soil can be grouped into sandy, silty and clay depending on soil particle size. However, soil can occur as a mixture of the three (loamy) (Mitchell and Soga, 1976; Lasota, 2014). Soil can be of great value in criminal investigation since it can connect a person or object to a crime scene (Dawson and Hillier, 2010).

2.7.1 PCR Inhibitors in soil and effects on DNA Profiling

Sometimes, some foreign substances remain in a DNA sample after extraction thereby preventing successful amplification (Akane *et al.,* 1994; Rådström *et al.,* 2004; Butler, 2011). These substances are called inhibitors and can be present in biological evidence collected from crime scenes.

Blood and semen on soil can be present at outdoor crime scenes and may contain inhibitors which may remain with the DNA after extraction **(**Bessetti, 2007; Schrader *et al.*, 2012). Humic acid is a major PCR inhibitor found in soil and the major organic component of soil. When microbes degrade plant and animal materials, humic acid is formed (Zipper *et al.*, 2003; Kasu and Shires, 2015).

In forensic DNA, humic substances are major cause of amplification failure because they chelate the magnesium ions needed by the DNA polymerase (Harry *et al.,* 1999; Fortin *et al.*, 2004; Lakay *et al.*, 2007; Buckwalter *et al.*, 2014; Lasota, 2014; Kasu and Shires, 2015). The chemical properties of humic acid is similar to that of double-stranded DNA (Buckwalter *et al.*, 2014;

Kasu and Shires, 2015). Amplification of DNA sample with inhibitors can result in partial profile or no profile at all (Butler, 2011).

2.7.2 Microbes in soil and impact on DNA

Soil is a major reservoir of microbes and these microbes degrade DNA in biological evidence when they come into contact (Robe *et al.*, 2003; Lakay *et al.*, 2007). A study conducted by the FBI showed that when DNA samples are exposed to soil for 5 days, they result in no profiles (Budowle, 1990; Kirby, 1992).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Ethical clearance

Ethical clearance for this study was obtained from the Committee of Human Research, Publications and Ethics of the Komfo Anokye Teaching Hospital and the School of Medical Sciences, Kwame Nkrumah University of Science and Technology (KNUST).

3.1.2 Blood sample

Fresh adult male whole blood sample from a single person in a tube with Ethylenediaminetetraacetic acid (EDTA) anticoagulant was obtained from Jubilee Hospital, an accredited private Hospital at Akim-Oda, Eastern Region of Ghana and transported on ice to the Forensic lab, Accra. Freshly collected whole blood from same source was also obtained from the same facility on same day without anticoagulant and sample preparation prior to storage done before been transported. Identity of donor isn't known and sample collection was done by a staff of the facility.

3.1.3 Soil sample

Dried black soil was collected in paper envelope from a garden at the compound of the Ghana Police Forensic Lab and brought to the lab.

3.1.4 Storage facilities

Room with an air condition of 25 °C temperature, fridge with temperature set to 4 °C and a freezer with -20 °C temperature served as the three sample storage conditions for this study.

3.1.5 Kits for serological tests

Hemastix test strips and Hexagon OBTI test cassettes were used for the serological aspect of this study.

3.1.6 Reagents and Instruments for DNA Profiling

PrepFiler Forensic DNA extraction kit, Promega DNA IQ Extraction kit and Blood Miniprep kit were used for the DNA extractions. QuantiFiler trio DNA quantification kit and 7500 real-time PCR machine manufactured by Applied Biosystem's were used for DNA Quantification. GlobalFiler PCR Amplification kit and 9700 Thermal cycler manufactured by Applied Biosystem's were used for DNA multiplex PCR amplification. Applied Biosystem's 3500 Genetic Analyzer was used for electrophoresis.

3.2 Study time

The study was conducted at Jubilee Hospital, Akim-Oda and the Ghana Police Forensic Science Laboratory, Accra, over a period of 12 weeks.

3.3. Sample preparation and storage

1 g of soil was mixed with 1 ml of whole blood sample containing Ethylenediaminetetraacetic acid (EDTA). In all, six soil-blood mixed samples containing EDTA were stored at each storage condition (Room temperature/25 °C, 4 °C and -20 °C). 1 g of soil was also mixed with 1 ml of

whole blood sample without EDTA. In all, three soil-blood mixed samples without EDTA were stored at each storage condition. Soil-blood mixed samples with EDTA were stored for 2, 4, 6, 8, 10 and 12 week periods. Soil-blood mixed samples without EDTA were stored for 4, 8 and 12 week periods. The samples were stored in capped tubes at 4 °C and -20 °C and in dried state on paper druggist folds at room temperature/25 °C.

3.4 Forensic Analysis of samples

3.4.1 Sample preparation prior to analysis

The soil-blood mixed sample stored under each condition was placed in appropriate tube and 4 ml of DNAse/RNAse free water added to it. It was allowed to stand for 30 minutes at room temperature for the blood to come into solution after which it was vortexed thoroughly to mix soil particles and blood. In order to minimize the amount of lysis buffer used for the extraction, it was important to get the blood cells into solution and separate them from the large soil particles. This also ensured most cells were lysed for maximum DNA yield and also soil particles if not removed can clog the wells of most serological test cassettes preventing the samples from running.

The tube was then centrifuged with Eppendorf centrifuge at 14000 rpm for 30 seconds. The supernatant was pippeted and 500 µl volume used for each extraction method and 20 µl for serological tests.

3.4.2 Serological and DNA tests controls for the study

Prior to soil-blood mixed samples storage at the three conditions, Hemastix and Hexagon OBTI test controls using clean blood with EDTA, clean blood without EDTA, soil-blood mixed sample

with EDTA and soil-blood mixed sample without EDTA all from same blood collected from Jubilee Hospital were done. Also, clean blood and soil-blood mixed samples with EDTA were extracted using all the three different extraction methods (PrepFiler Forensic method, Promega DNA IQ method, Blood Miniprep method). Serological and DNA tests controls were done following protocols in **Appendices 1** and **2** after going through the same sample preparation method described in **3.4.1** above.

3.4.3 Serological tests

Twenty microliters (20 µl) of liquid supernatant from soil-blood mixed sample taken from each storage condition at each storage time was used for serological tests (Hemastix and Hexagon OBTI) after going through sample preparation method described in **3.4.1**. Serological tests were done following the kits' manufacturers' protocols in **Appendix 1**.

3.4.4 DNA extractions

Five hundred microliters (500 µl) of liquid supernatant from soil-blood mixed sample taken from each storage condition at each storage time was pippeted and used for each DNA extraction after going through sample preparation method described in **3.4.1**. DNA Extractions were done using the kits manufacturers' protocols in **Appendix 2**.

Sample identification numbers for the entire study is found in **Table 3.1** below. The first number(s) represent the storage duration in weeks, followed by the extraction method and the last number(s) or letter represent the storage condition. Samples without EDTA have **'n'** attached to the sample names

Table 3.1: Samples identification numbers

RT/25°C			4°C			-20°C		
P	**Q**	**B**	**P**	**Q**	**B**	**P**	**Q**	**B**
Sample names			**Sample names**			**Sample names**		
2PR	2QR	2BR	2P4	2Q4	2B4	2P-20	2Q-20	2B-20
4PR	4QR	4BR	4P4	4Q4	4B4	4P-20	4Q-20	4B-20
6PR	6QR	6BR	6P4	6Q4	6B4	6P-20	6Q-20	6B-20
8PR	8QR	8BR	8P4	8Q4	8B4	8P-20	8Q-20	8B-20
10PR	10QR	10BR	10P4	10Q4	10B4	10P-20	10Q-20	10B-20
12PR	12QR	12BR	12P4	12Q4	12B4	12P-20	12Q-20	12B-20
4PRn	4QRn	4BRn	4P4n	4Q4n	4B4n	4P-20n	4Q-20n	4B-20n
8PRn	8QRn	8BRn	8P4n	8Q4n	8B4n	8P-20n	8Q-20n	8B-20n
12PRn	12QRn	12BRn	12P4n	12Q4n	12B4n	12P-20n	12Q-20n	12B-20n
Controls								
Prep. Clean blood- PCB			Q. Clean blood- QCB			B. Clean blood- BCB		
Prep soil-blood mixed- PSB			Q. Soil-blood mixed - QSB			B. Soil-blood mixed- BSB		

P = PrepFiler Forensic kit; Q =Promega DNA IQ extraction kit; B = Blood Miniprep kit; RT=Room temperature/25°C; EDTA=Samples containing EDTA; No EDTA=Samples without EDTA

For the sample names, the first number(s) represent the storage duration in weeks, followed by the extraction method and the last number(s) or letter represent the storage condition; where R is for room temperature, 4 for 4°C and -20 for -20°C storage. Samples without EDTA have 'n' attached to the sample names.

3.4.5 Real-Time PCR Analysis

3.4.5.1 DNA Concentration determination

Total human DNA in the extracted samples was measured by Applied Biosystem's QuantiFiler trio kit using 7500 Real-time PCR and following the manufacturer's protocol in **Appendix 3**. The Small Autosomal target (SA) determined the actual DNA concentration.

3.4.5.2 DNA Quality Assessment

DNA quality in terms of degradation and presence of inhibitors was assessed by Applied Biosystem's QuantiFiler trio kit using 7500 Real-time PCR and following the manufacturer's protocol in **Appendix 3**. Degradation was assessed by comparing DNA concentration and Degradation index of a sample from a particular storage time to the previous storage time. Inhibitor presence was assessed by the Internal Positive control of each sample.

3.4.6 DNA Amplification (Multiplex PCR)

Amplification for 24 target sequences or markers (D3S1358, vWA, D16S539, CSF1PO, TPOX, D8S1179, D21S11, D18S51, D2S441, D19S433, TH01, FGA, D22S1045, D5S818, D13S317, D7S820, SE33, D10S1248, D1S1656, D12S391, D2S1338, DYS391, Y indel and Amelogenin) for the samples were done with GlobalFiler amplification kit using Applied Biosystem's GeneAmp 9700 PCR Machine. The manufacturer's protocol **(Appendix 4)** was followed for 29 cycle amplification.

3.4.7 Capillary Electrophoresis

Amplified DNA fragments were detected using Applied Biosystem's 3500 genetic analyzer following the manufacturer's protocol in **Appendix 5**.

3.4.8 Profile analysis

DNA profile analysis and electropherograms generation were done with GeneMapper *ID-X* 1.5 software.

3.5 Effect of Ethylenediaminetetraacetic acid (EDTA) on the study

Serological tests results from soil-blood mixed samples with EDTA and soil-blood mixed samples without EDTA were compared in terms of positive and negative results from each sample type, storage time and storage condition.

DNA concentrations and quality of soil-blood mixed samples with EDTA and soil-blood mixed samples without EDTA were also compared in terms of concentration, degradation indexes, internal positive controls cycle threshold values and number of detected alleles. Analysis was done with SPSS to know whether there was significant difference between the two sample types in terms of the parameters mentioned.

3.6 Statistical Analysis

DNA yields from the different storage conditions were subjected to One-way analysis of variance (ANOVA) using SPSS to ascertain whether or not they differ significantly from each other. T-tests were used for all other analysis using SPSS.

CHAPTER FOUR

4.0 RESULTS

4.1 Serological tests

Hemastix and Hexagon OBTI control tests for whole blood with EDTA, whole blood without EDTA, soil-blood mixed sample with EDTA and soil-blood mixed sample without EDTA all gave positive results. Throughout the study, the Hemastix and Hexagon OBTI kits tested positive for soil-blood mixed samples stored at all three conditions as seen in **Table 4.1**.

Table 4.1: Serological tests results for soil-blood mixed samples stored at various conditions overtime.

Time	**Hemastix**			**Hexagon OBTI**		
	RT	**4** ℃	**-20** ℃	**RT**	**4** ℃	**-20**℃
EDTA						
2 weeks	+	+	+	+	+	+
4 weeks	+	+	+	+	+	+
6 weeks	+	+	+	+	+	+
8 weeks	+	+	+	+	+	+
10 weeks	+	+	+	+	+	+
12 weeks	+	+	+	+	+	+
No EDTA						
4 weeks	+	+	+	+	+	+
8 weeks	+	+	+	+	+	+
12 weeks	+	+	+	+	+	+
Controls						
Clean blood with EDTA-	+		Soil-blood mixed with EDTA-	+		
Clean blood without EDTA-	+		Soil-blood mixed without EDTA-	+		

RT= Room temperature/25℃; 4℃= Fridge; -20℃= Freezer; += positive result; EDTA= Soil-blood mixed sample with EDTA; No EDTA= Soil-blood mixed sample without EDTA

4.2 Real-time PCR DNA quantification results

From the summary of the entire experiment as seen in **Table 4.2**, the internal positive control (IPC) of twenty samples (BSB, 2BR, 4BR, 6BR, 8BR, 10BR, 12BR, 2B4, 2B-20, 4B-20, 6B-20, 8B-20, 10B-20, 12B-20, 4BRn, 8BRn, 12BRn, 4B-20n, 8B-20n and 12B-20n) were flagged or not amplified. The Negative template control included in the quantification showed amplification for IPC but no amplification for other targets seen in **Figure 4.4**. This means all the RT-PCR assays worked well and that the sample preparation procedure was devoid of contamination. The positive control amplified and showed detectable DNA for all human and Y targets as observed in **Figure 4.5**. This indicates good amplification and good formulation of reagents. Slopes of -3.291, -3.364 and -3.231 were obtained from standard curve for the Y-target, Large autosomal and Small autosomal, respectively; an indication of 99.73% amplification efficiency. The C_T values for all amplified targets were less than 40 which suggest positive amplification in these samples.

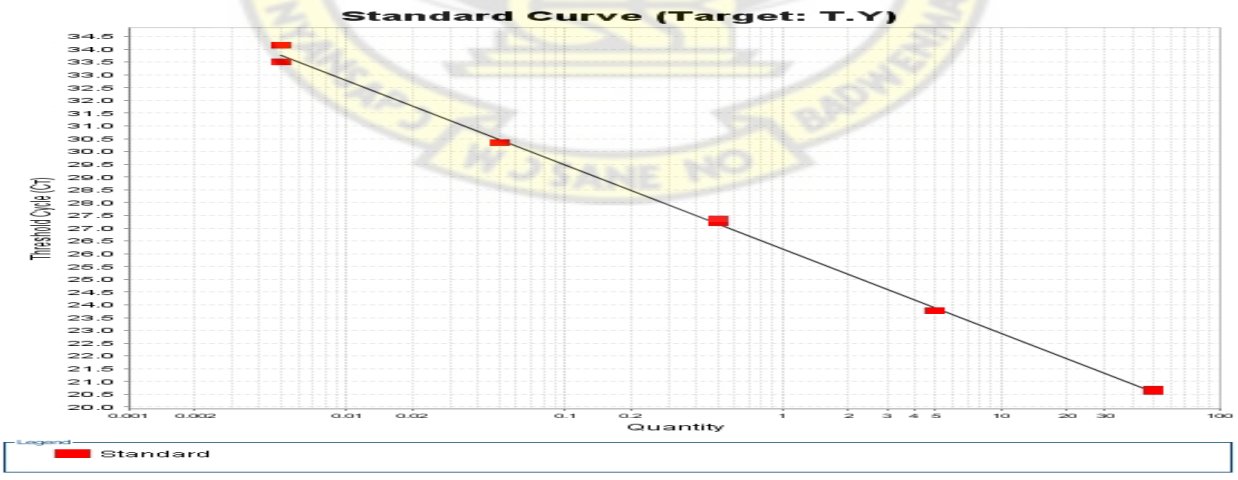


Figure 4.1: Standard curve for Y target

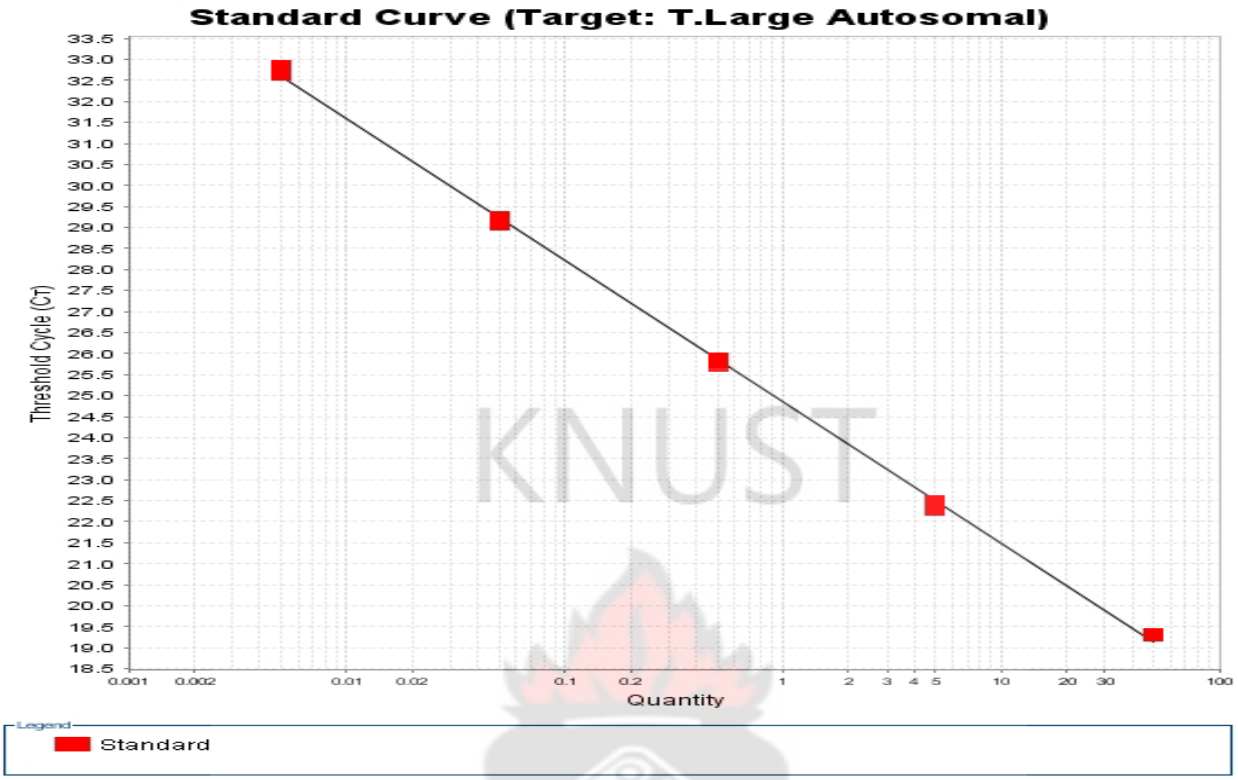


Figure 4.2: Standard curve for Large autosomal target

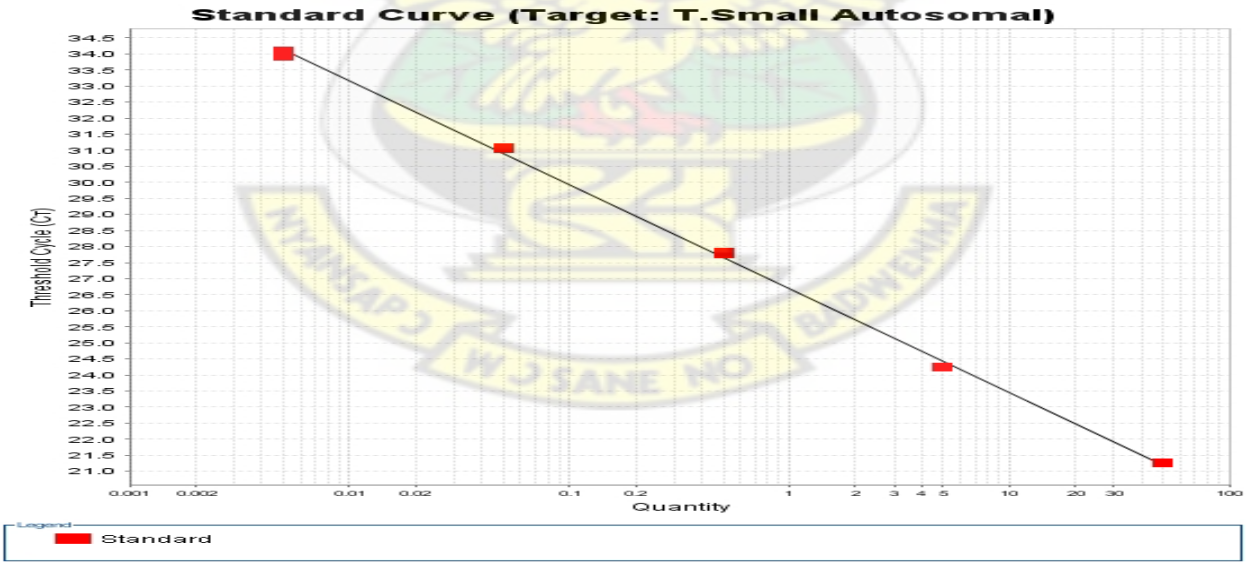


Figure 4.3: Standard curve for small autosomal target

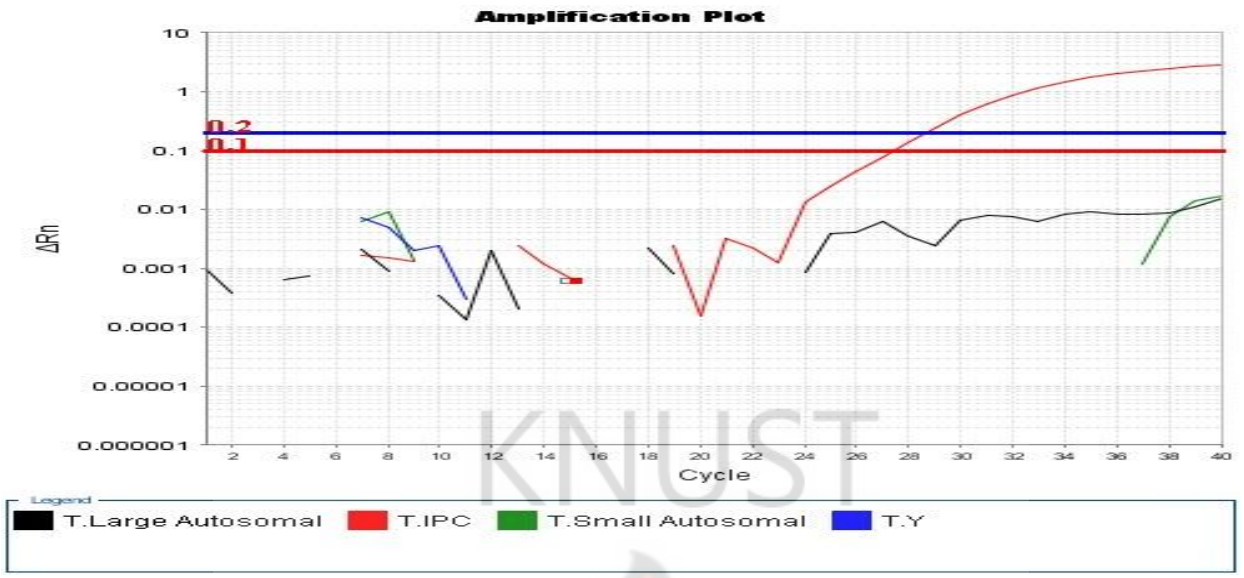


Figure 4.4: Amplification plot for the Negative template control (NTC)

ΔRn means Rn minus the baseline. Rn means the fluorescence of the reporter dye divided by the fluorescence of a passive reference dye; thus, Rn is the reporter signal normalized to the fluorescence signal of Applied Biosystems ROX dye.

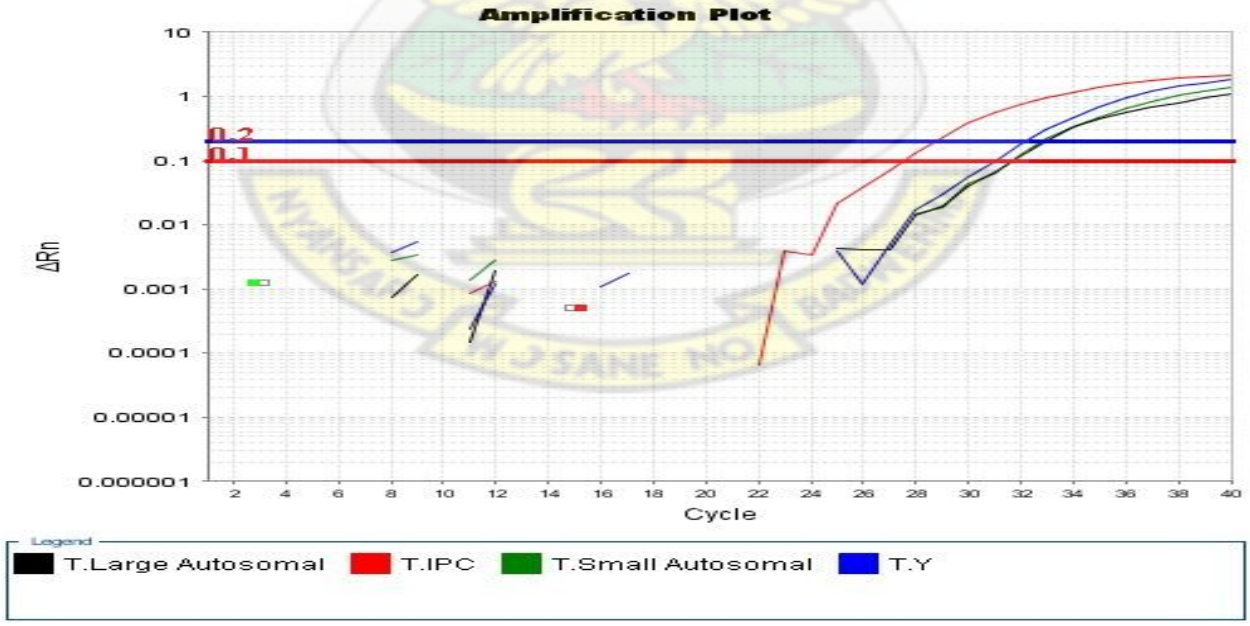


Figure 4.5: Amplification plot for Positive control showing amplification for all targets and IPC

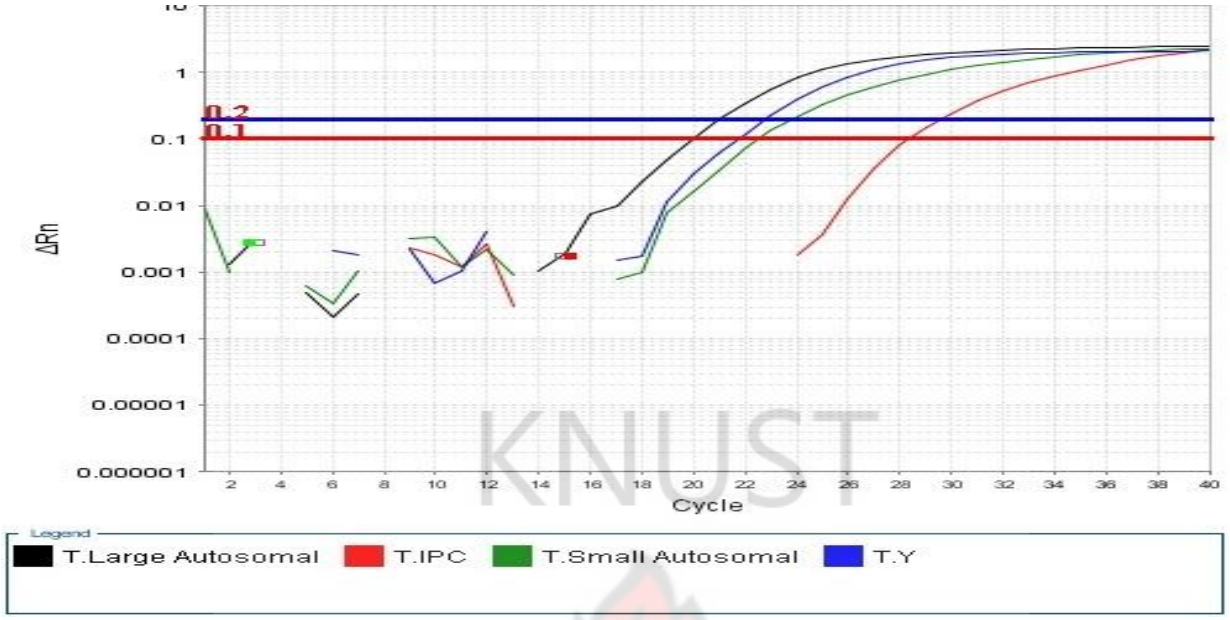


Figure 4.6: Amplification plot for sample PCB (Prepfiler clean blood) showing amplification for all targets and IPC

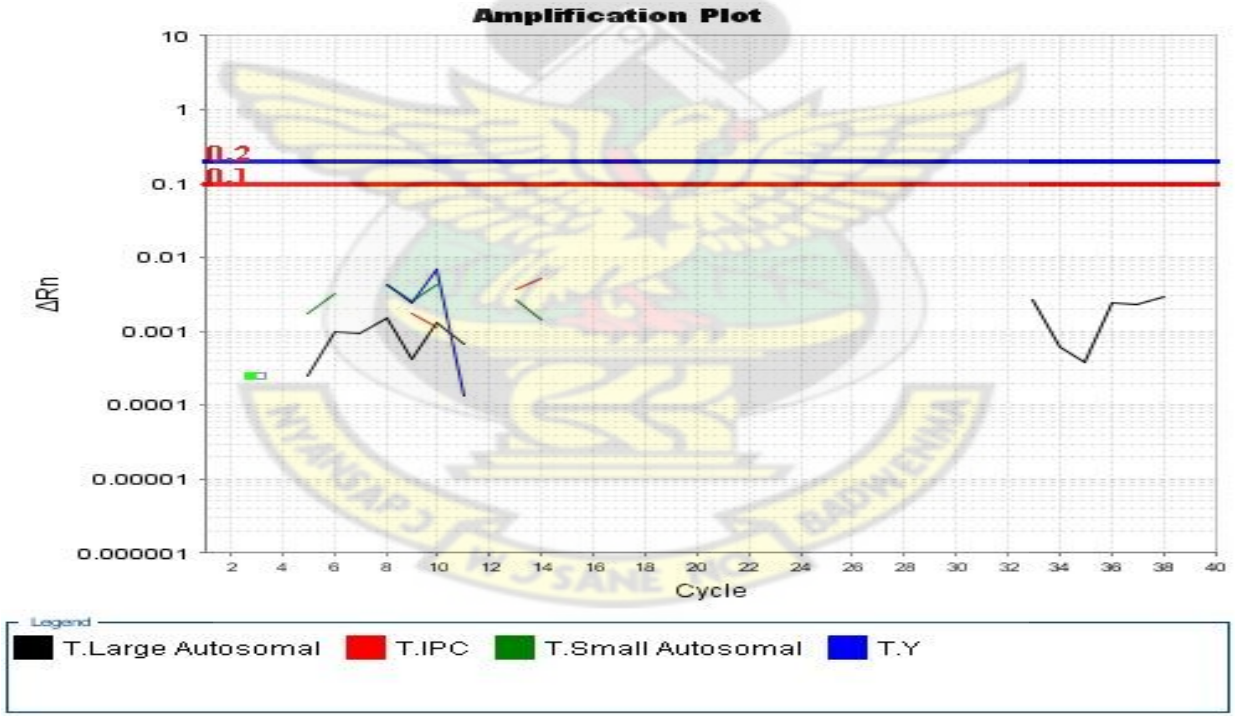


Figure 4.7: Amplification plot for sample 2BR showing no amplifications for targets and IPC

4.3 Assessing DNA degradation at the three storage conditions

This assessment was done to determine whetheher the three storage conditions were able to preserve the integrity of samples in terms of their DNA concentration and quality as storage time progressed. DNA concentrations and degradation indexes were compared among the various storage times and it was observed that DNA concentrations and quality decreased with increase in storage time for room temperature and 4 ℃ stored samples. -20 ℃ stored samples had increase and decrease in concentration for samples with increase in storage time but DNA quality remained intact.

Looking at the control samples in **Table 4.2**, the DNA from extracted soil-blood mixed sample (Sample BSB) using Blood Miniprep kit had its IPC and targets showing no amplifications. This means there was total amplification failure in this sample. The DNA extractions using PrepFiler and DNA IQ for both soil-blood mixed and clean/neat blood samples showed amplifications for all targets including IPCs and degradation indexes less than 1 (**Table 4.2**). This means DNA was of good quality without any degradation.

Table 4.2: DNA concentration, Degradation index and IPC status for the control samples

Sample	SA	LA	Y	DI	IPC
	Conc	Conc	Conc		
PSB	11.7224	14.917	10.5902	0.7858	A
PCB	28.6519	44.3452	36.8804	0.6461	A
BSB	-	-	-	-	N
BCB	9.2147	9.9929	7.8847	0.9221	A
QSB	6.8424	7.6631	5.3456	0.8929	A
QCB	24.0746	25.1912	20.0821	0.9557	A

SA=Small autosomal target; LA=Large autosomal target; Y=Y chromosome target; IPC=Internal positive control; Conc. =concentration (ng/µl); DI=Degradation index; A=Amplification; N=No amplification; - means no detectable DNA

PSB and PCB are PrepFiler kit extractions for soil-blood mixed and clean blood control samples, respectively. BSB and BCB are Blood Miniprep kit extractions for soil-blood mixed and clean blood control samples, respectively. QSB and QCB are DNA IQ kit extractions for soil-blood mixed and clean blood control samples, respectively.

4.3.1 Assessing DNA degradation at room temperature/25 °C storage

As seen in **Table 4.3**, samples 2PR and 2QR had degradation indexes less than 1 at 2 weeks storage. Their concentrations dropped compared to their controls (Figure 4.8). Sample 2BR had no amplification for IPC and all targets. At 4 weeks of storage at room temperature, samples 4PR and 4QR recorded degradation indexes less than 1 (**Table 4.3**) indicating DNA quality was still intact. Their concentrations dropped compared to 2 weeks stored samples (Figure 4.8). Sample 4BR had no amplificaion for IPC as well as other targets.

At 6 weeks of sample storage, samples 6PR and 6QR showed amplifications for all targets. Their degradation indexes were between 1-10 as seen in **Table 4.3**; an indication that DNA was

slightly to moderately degraded. Their concentrations were lower than 4 weeks stored samples as seen in **Figure 4.8**. Sample 6BR had no amplification for IPC and all other targets.

Table 4.3 shows that all Prepfiler and DNA IQ extracted samples had degradation indexes between 1-10 at weeks 8 to 12; an indication that DNA was slightly to moderately degraded. Their DNA concentrations kept on reducing week after week (**Figure 4.8**). All Blood Miniprep extracted samples at weeks 8 to 12 had no amplification for IPC as well as other targets.

There was significant difference in DNA concentrations of samples stored at room temperature compared to the control samples (PCB, PSB, BSB, BCB, QSB, QCB). A P value of 0.001 for Prepfiler extracted samples and a P value of 0.0055 for DNA IQ extracted samples confirms this. No statistics for Blood Miniprep extracted samples because they failed to amplify.

Table 4.3: DNA concentration, Degradation index and IPC status for Room temperature stored samples

Sample	SA	LA	Y	DI	IPC
	Conc.	Conc.	Conc.		
2PR	9.2058	12.6321	9.6974	0.7288	A
4PR	5.1765	6.5523	4.9149	0.7900	A
6PR	3.2528	3.0824	2.8496	1.0553	A
8PR	2.4214	1.6728	2.2871	1.4475	A
10PR	1.7201	1.2934	1.5628	1.3299	A
12PR	1.0423	1.0043	0.935	1.0378	A
2BR	-	-	-	-	N
4BR	-	-	-	-	N
6BR	-	-	-	-	N
8BR	-	-	-	-	N
10BR	-	-	-	-	N
12BR	-	-	-	-	N
2QR	7.0145	8.581	7.3859	0.8174	A
4QR	4.7136	5.6583	5.1061	0.8330	A
6QR	2.5688	0.6425	1.9197	3.9981	A
8QR	1.9318	1.4477	2.7993	1.3344	A
10QR	1.0700	0.7926	1.1056	1.3500	A
12QR	0.8386	0.4915	0.8807	1.7061	A

SA=Small autosomal target; LA=Large autosomal target; Y=Y chromosome target; IPC=Internal positive control; Conc. =concentration (ng/µl); A=Amplified; N=No amplification; DI=Degradation index; - means no detectable DNA

For the sample names, the first number(s) represent the storage duration in weeks; followed by the extraction method and the last number(s) or letter represents the storage condition. P stands for PrepFiler kit, B for Blood Miniprep kit and Q for DNA IQ kit. R stands for room temperature/25°C.

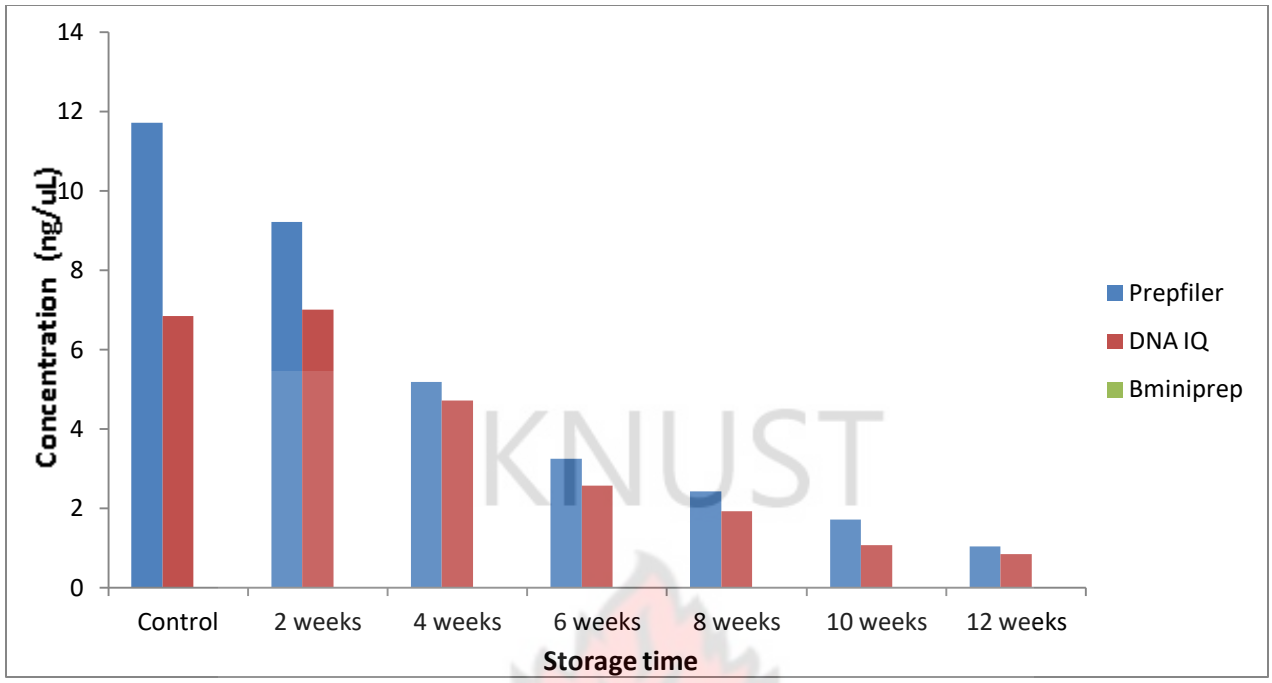


Figure 4.8: DNA concentrations of room temperature stored soil-blood mixed samples from the 3 extraction methods

4.3.2 Assessing DNA degradation at 4℃ storage

At 2 weeks of sample storage at 4 ℃, DNA concentrations of Samples 2P4 and 2Q4 reduced drastically (**Table 4.4**) compared to the control samples in **Table 4.2.** Their degradation indexes were between 1-10, meaning DNA was slightly to moderately degraded. Sample 2B4 showed no amplification for all targets and IPC.

At 4 weeks of storage, samples 4P4 and 4Q4 had their degradation indexes between 1-10 as seen in **Table 4.4** and their concentrations were lower than the 2 weeks extracted samples (**Figure 4.9**). Sample 4B4 amplified for only IPC but the IPC was flagged due to high C_T value of 34.16.

At 6 weeks, samples 6P4 and 6Q4 recorded degradation indexes between 1-10 and their concentrations were lower than the 4 weeks extractions (**Table 4.4**). Sample 6B4 had no detectable DNA.

At 8 weeks of storage at 4 °C, samples 8P4 and 8Q4 recorded degradation indeces between 1-10. Their concentrations dropped compared to the 6 weeks extractions. Sample 8B4 showed amplification for IPC only. At 10 and 12 weeks of storage at 4 °C, all samples from the 3 extraction methods showed amplifications for IPCs only. Reduction in DNA concentrations of 4 °C stored samples were very significant compared to their control samples with P value of 0.000 for both Prepfiler and DNA IQ extractions.

Table 4.4: DNA concentration, Degradation index and IPC status for 4°C stored samples

Sample	SA	LA	Y	DI	IPC
	Conc	Conc	Conc		
2P4	0.8615	0.8556	0.9733	1.0069	A
4P4	0.5096	0.437	0.7629	1.1661	A
6P4	0.1299	0.1003	0.0827	1.2951	A
8P4	-	-	-	-	A
10P4	-	-	-	-	A
12P4	-	-	-	-	A
2B4	-	-	-	-	N
4B4	-	-	-	-	A
6B4	-	-	-	-	A
8B4	-	-	-	-	A
10B4	-	-	-	-	A
12B4	-	-	-	-	A
2Q4	0.7048	0.6616	1.2143	1.0653	A
4Q4	0.2511	0.0454	0.2801	5.5308	A
6Q4	0.0388	0.0287	0.0357	1.3519	A
8Q4	-	-	-	-	A
10Q4	-	-	-	-	A
12Q4	-	-	-	-	A

SA=Small autosomal target; LA=Large autosomal target; Y=Y chromosome target; IPC=Internal positive control; Conc. =concentration (ng/µl); A=Amplified; N=No amplification; DI=Degradation index; - means no detectable DNA

For the sample names, the first number(s) represent the storage duration in weeks; followed by the extraction method and the last number(s) or letter represents the storage condition. P stands for PrepFiler kit, B for Blood Miniprep kit and Q for DNA IQ kit. 4 stand for 4 °C storage.

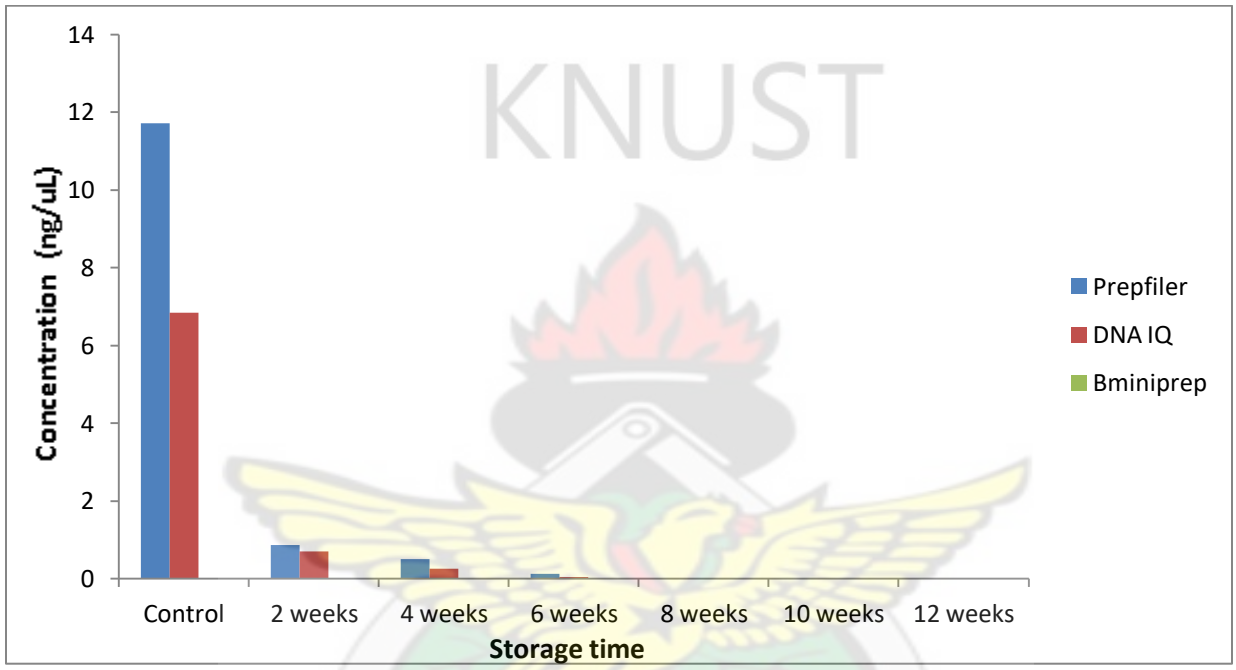


Figure 4.9: DNA concentrations of 4 °C stored soil-blood mixed samples from the 3 extraction methods

4.3.3 Assessing DNA degradation at -20 °C storage

Assessing the concentrations for PrepFiler and DNA IQ extracted samples, there was no particular pattern in terms of decrease or increase as storage time progressed (**Figure 4.10**). Degradation indexes of these samples were less than 1 meaning DNA quality was still intact even at 12 week storage period (**Table 4.5**). rom All Blood Miniprep extractions for -20 ^{o}C stored samples showed no amplifications for all targets and IPCs. There was no significant

difference in DNA Concentrations of samples stored at -20 ℃ compared to the concentrations of their control samples. P values of 0.324 and 0.161 for PrepFiler and DNA IQ extractions, respectively confirms this.

Table 4.5: DNA concentration, Degradation index and IPC status for -20 °C stored samples

Sample	SA	LA	Y	DI	IPC
	Conc	Conc	Conc		
2P-20	10.8187	16.2425	10.53	0.6661	A
4P-20	11.0896	16.4513	9.2983	0.6741	A
6P-20	13.0949	15.7872	10.5232	0.8295	A
8P-20	11.6075	14.8042	10.4406	0.7841	A
10P-20	11.9707	16.3692	12.2523	0.7313	A
12P-20	12.8158	13.8787	14.6747	0.9234	A
2B-20	-	-	-	-	N
4B-20	-	-	-	-	N
6B-20	-	-	-	-	N
8B-20	-	-	-	-	N
10B-20	-	-	-	-	N
12B-20	-	-	-	-	N
2Q-20	7.4269	7.4781	5.4733	0.9932	A
4Q-20	6.25	7.2257	5.0304	0.8650	A
6Q-20	7.2442	7.4683	5.3599	0.970	A
8Q-20	8.1467	8.5225	6.2267	0.9560	A
102-20	6.9633	7.6427	5.3417	0.9111	A
12Q-20	6.9745	7.4714	5.4336	0.9335	A

SA=Small autosomal target; LA=Large autosomal target; Y=Y chromosome target; IPC=Internal positive control; Conc. =concentration (ng/µl); DI=Degradation index; A=Amplified; N=No amplification; - means no detectable DNA

For the sample names, the first number(s) represent the storage duration in weeks; followed by the extraction method and the last number(s) or letter represents the storage condition. P stands for PrepFiler kit, B for Blood Miniprep kit and Q for DNA IQ kit. -20 stands for -20 ℃ storage.

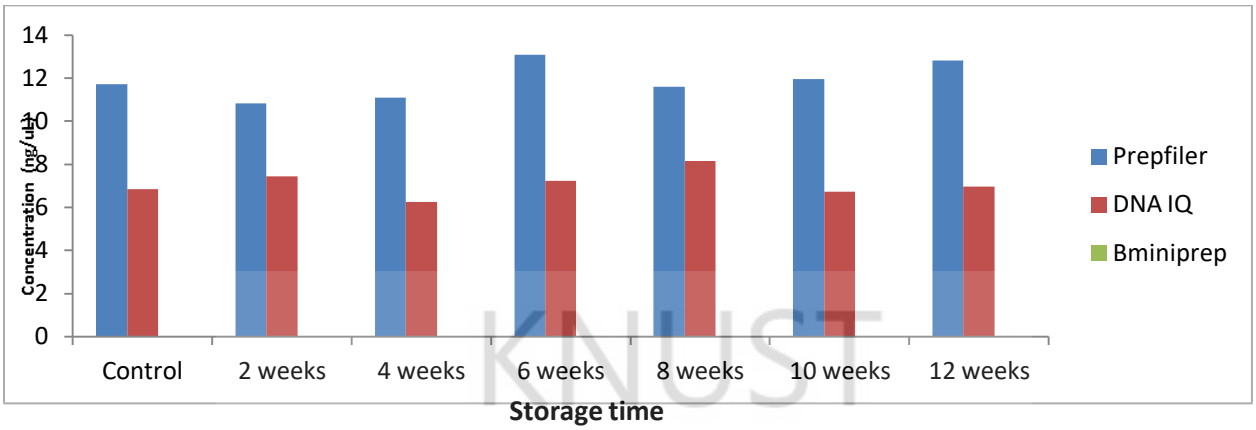


Figure 4.10: DNA concentrations of -20 °C stored soil-blood mixed samples from the 3 extraction methods

4.4 Comparing DNA degradation among the 3 storage conditions

Figures 4.11 and **4.12** show that samples stored at room temperature decreased in concentration as time of storage increased with concentration decrease being significant (P=0.001 for PrepFiler extractions and P=0.0055 for DNA IQ extractions). At 6 to 12 weeks of storage, degradation indexes of the PrepFiler and DNA IQ extracted samples rose to 1 or above. Blood Miniprep extracted samples showed no amplification for targets and IPC as seen in **Table 4.3**. **The** samples stored at -20 ^{o}C and extracted with PrepFiler and DNA IQ did not follow a particular pattern in terms of DNA concentration as time of storage increased with no significant difference in concentration (P=0.324 for PrepFiler extractions and P=0.161 for DNA IQ extractions) compared to the control samples. Their degradation indexes were less than 1 (**Table 4.5**). The soil-blood mixed samples stored at 4 °C decreased in concentration drastically (P=0.000 for both PrepFiler and DNA IQ extractions) and at 2 weeks of storage, their degradation indexes were between 1-10 as seen in **Table 4.4**. At 12 weeks, there were no amplifications for targets except IPC.

Significant difference was seen between the DNA concentrations of samples from room temperature and 4 ℃ storage (P=0.002). Again, significant difference was seen between the DNA concentrations of samples from room temperature and -20℃ storage (P=0.000). There was significant difference between the DNA concentrations of samples from 4 ℃ and -20 ℃ storage (P=0.000).

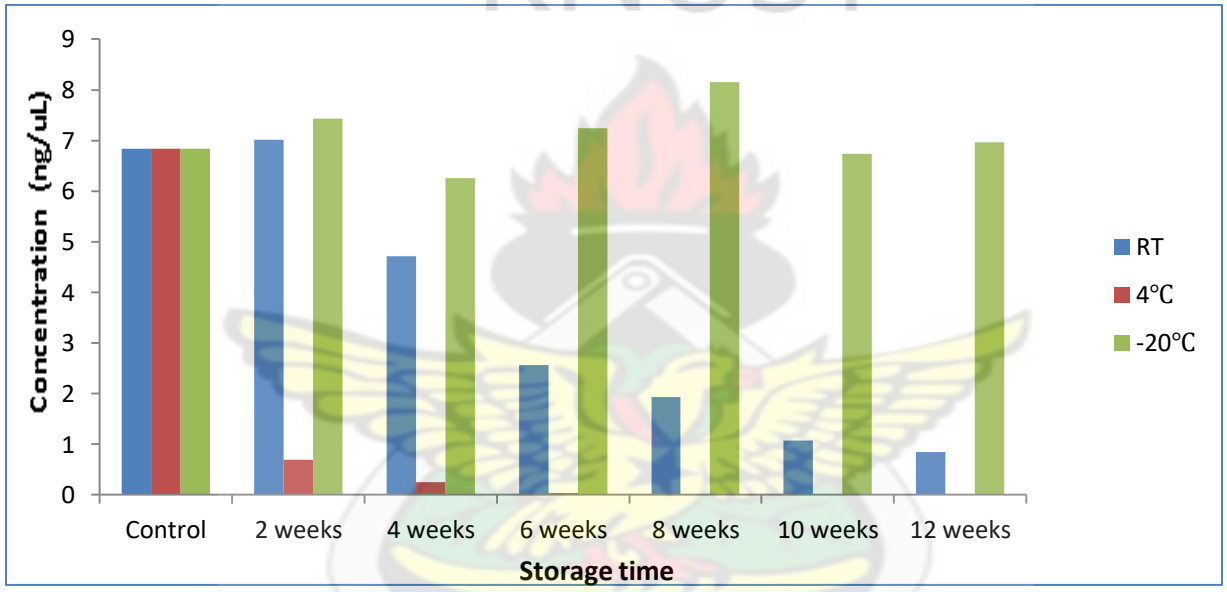


Figure 4.11: DNA concentrations at the 3 storage conditions from DNA IQ extracted samples; (RT=Room temperature/25 ℃)

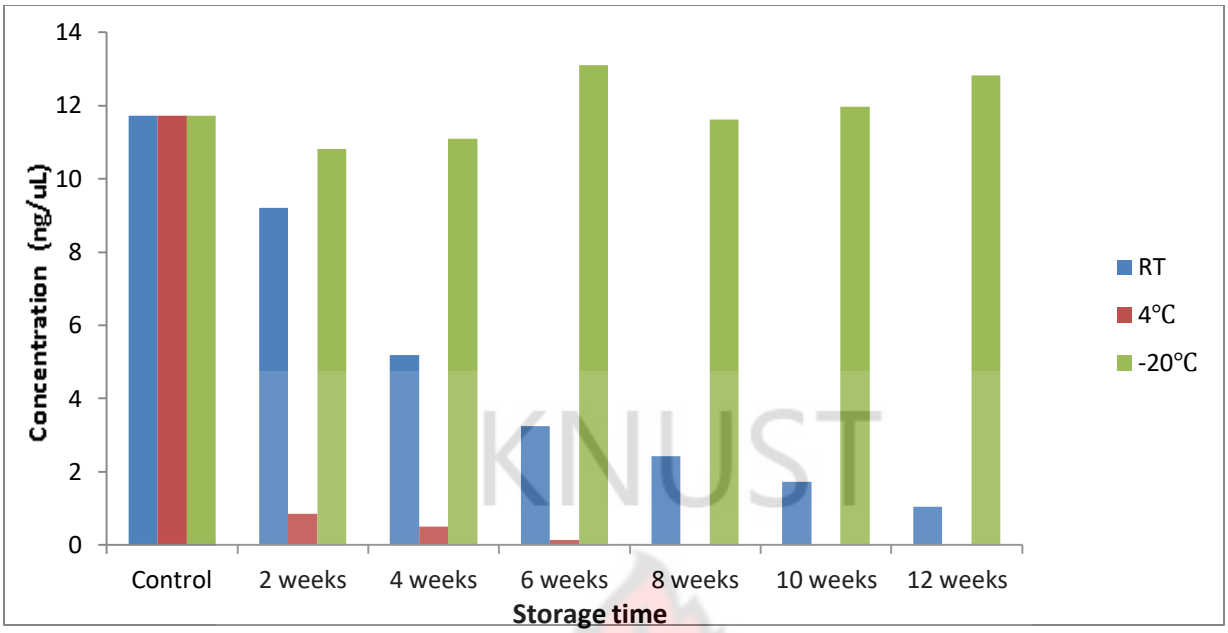


Figure 4.12: DNA concentrations at the 3 storage conditions from PrepFiler extracted samples; (RT=Room temperature/25 °C)

4.5 Comparing DNA extraction potency among the three methods

Assessment of the control samples from **Table 4.6** show PrepFiler Forensic kit yielding more DNA than DNA IQ kit for both clean blood and soil-blood mixed samples. DNA IQ also performed better than Blood Miniprep kit. PrepFiler Forensic kit yielded more DNA at the three storage conditions than DNA IQ with no significant difference (P=0.603) in concentrations. There was no significant difference (P=0.469) in DNA concentrations of PrepFiler and DNA IQ extractions of room temperature stored samples. There was also no significant difference (P=0.693) in DNA concentrations of PrepFiler and DNA IQ extractions of 4°C stored samples. However, difference in DNA concentrations of PrepFiler and DNA IQ extractions from -20°C stored samples was significant (P=0.000).

Table 4.6: DNA concentrations (ng/µl) from soil-blood mixed samples at 3 storage conditions

Storage time	RT			4°C			-20°C		
	Prep.	**IQ.**	**BM.**	**Prep.**	**IQ.**	**BM.**	**Prep.**	**IQ.**	**BM.**
EDTA									
2 weeks	9.21	7.01	N	0.86	0.70	N	10.82	7.43	N
4 weeks	5.18	4.71	N	0.51	0.25	-	11.09	6.25	N
6 weeks	3.25	2.57	N	0.13	0.04	-	13.10	7.24	N
8 weeks	2.42	1.93	N	-	-	-	11.61	8.15	N
10 weeks	1.72	1.07	N	-	-	-	11.97	6.74	N
12 weeks	1.04	0.84	N	-	-	-	12.82	6.97	N
No EDTA									
4 weeks	5.19	4.30	N	0.48	0.21	-	11.16	7.78	N
6 weeks	3.37	2.52	N	0.18	0.05	-	11.14	6.84	N
12 weeks	1.13	0.81	N	-	-	-	11.40	6.38	N
Controls									

PrepFiler clean blood (PCB)- 28.65ng/µl DNA IQ clean blood (QCB)- 24.07ng/µl
PrepFiler soil-blood mixed (PSB)- 11.72ng/µl DNA IQ soil-blood mixed (QSB)- 6.84ng/µl
Blood Miniprep clean blood (BCB)- 9.21ng/µl Blood Miniprep soil-blood mixed (BSB)- No amplification

Prep. =PrepFiler Forensic kit; IQ. =Promega DNA IQ extraction kit; BM. =Blood Miniprep kit; RT=Room temperature/25°C; N=No amplification; - means no detectable DNA

4.6 Assessing PCR Inhibition among the three extraction methods

Blood Miniprep extraction for clean blood control sample BCB showed amplification for IPC. All Blood Miniprep extractions for control soil-blood mixed sample, soil-blood mixed samples stored at room temperature and soil-blood mixed samples stored at -20 °C showed no amplification for IPCs. Blood Miniprep extractions for sample stored at 4 °C for 2 weeks showed no amplification for IPC. At 4 weeks of sample storage at 4 °C, there was amplification for IPC of Blood Miniprep extracted sample with the IPC being flagged because of high IPC C_T value of 34.16. At 6 weeks onwards, there were amplifications for IPCs of Blood Miniprep extractions for 4 °C stored samples (**Table 4.7**). All PrepFiler and DNA IQ extractions for control clean blood samples PCB and QCB as well as soil-blood mixed samples PSB and QSB showed amplifications for IPCs. Also, all PrepFiler and DNA IQ extractions for soil-blood mixed samples stored at the 3 conditions showed amplifications for IPCs. There was no significant difference (P=0.887) in IPC C_T values of PrepFiler and DNA IQ extracted samples.

Table 4.7: IPC C_T values from soil-blood mixed samples at the 3 storage conditions

Storage time	RT			4°C			-20°C		
	Prep.	**IQ.**	**BM.**	**Prep.**	**IQ.**	**BM.**	**Prep.**	**IQ.**	**BM.**
EDTA									
2 weeks	26.09	25.27	N	25.63	25.76	N	28.91	28.63	N
4 weeks	28.11	25.76	N	25.50	27.55	34.16	28.52	28.47	N
6 weeks	25.65	25.82	N	25.50	27.45	27.49	28.30	28.01	N
8 weeks	25.69	25.49	N	27.09	27.33	27.37	29.14	27.60	N
10 weeks	25.58	27.25	N	27.28	27.50	27.35	28.36	28.10	N
12 weeks	27.43	27.63	N	27.39	27.24	27.40	28.50	28.41	N
No EDTA									
4 weeks	27.59	28.17	N	27.89	27.71	35.05	27.52	28.15	N
6 weeks	27.73	27.39	N	27.48	27.28	27.37	28.54	27.77	N
12 weeks	27.26	27.72	N	27.39	27.51	27.47	28.54	27.74	N

Controls

Prep. Clean blood (PCB)- 27.26 IQ. Clean blood (QCB)- 26.30 BM. Clean blood (BCB)- 25.35
Prep soil-blood mixed (PSB)- 28.91 IQ. Soil-blood mixed (QSB)- 27.18 BM. Soil-blood mixed (BSB)- no amplification

Prep. =PrepFiler Forensic kit; IQ. = Promega DNA IQ extraction kit; BM. =Blood Miniprep kit; RT=Room temperature/25°C; N=No amplification; EDTA=Samples with EDTA; No EDTA=Samples without EDTA

4.7 Electrophoresis

Allelic ladders that were run alongside the samples in the capillary electrophoresis passed as seen in **Figure 4.13**; the negative control showed a null profile as seen in **Figure 4.14**; and the positive control gave a full STR profile as seen in **Figure 4.15**. This means the capillary electrophoresis run was good with all assays performing well. There was no contamination of the samples or reagents.

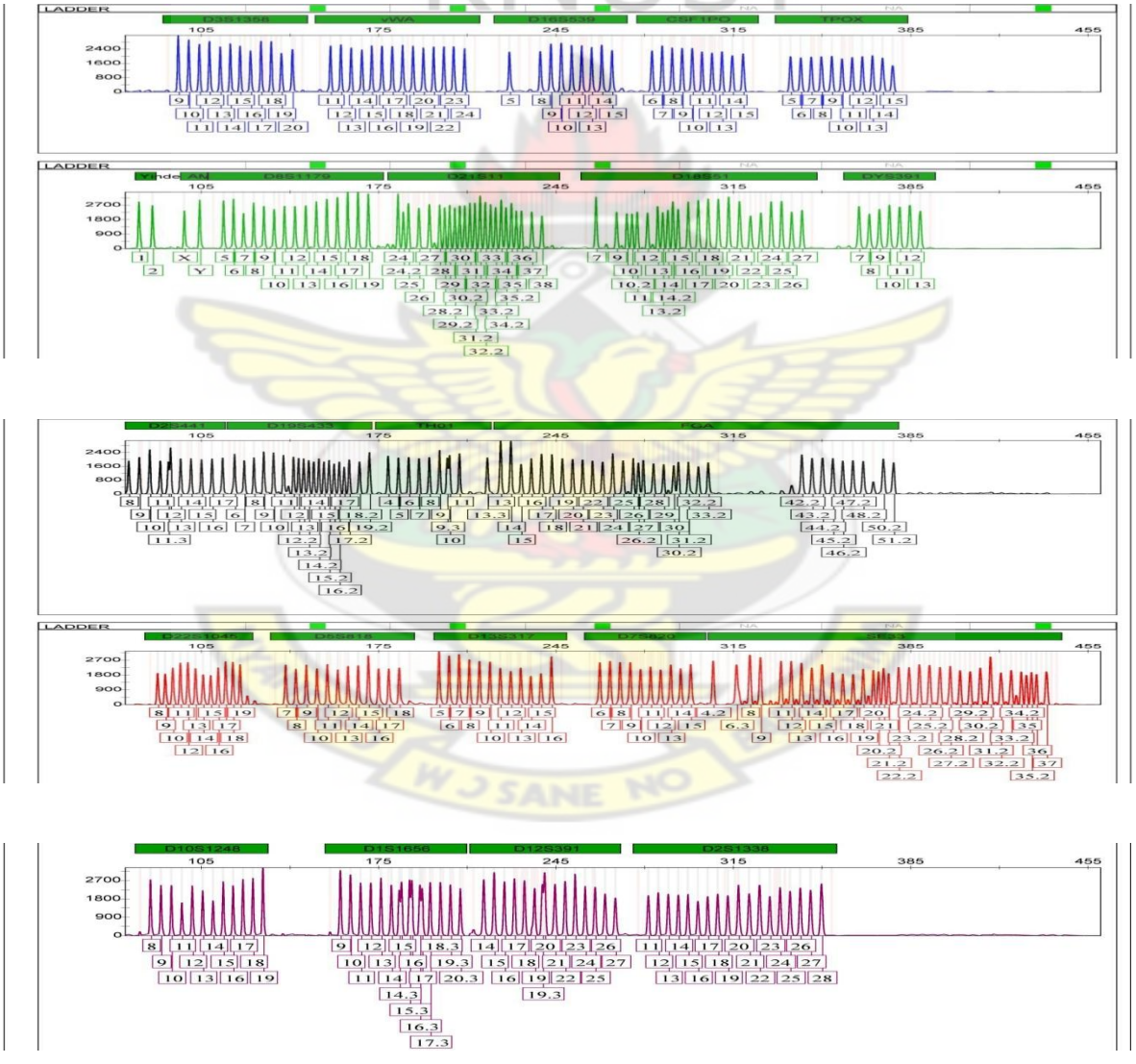


Figure 4.13: Electropherogram of Allelic Ladder from the GlobalFiler PCR Amplification Kit

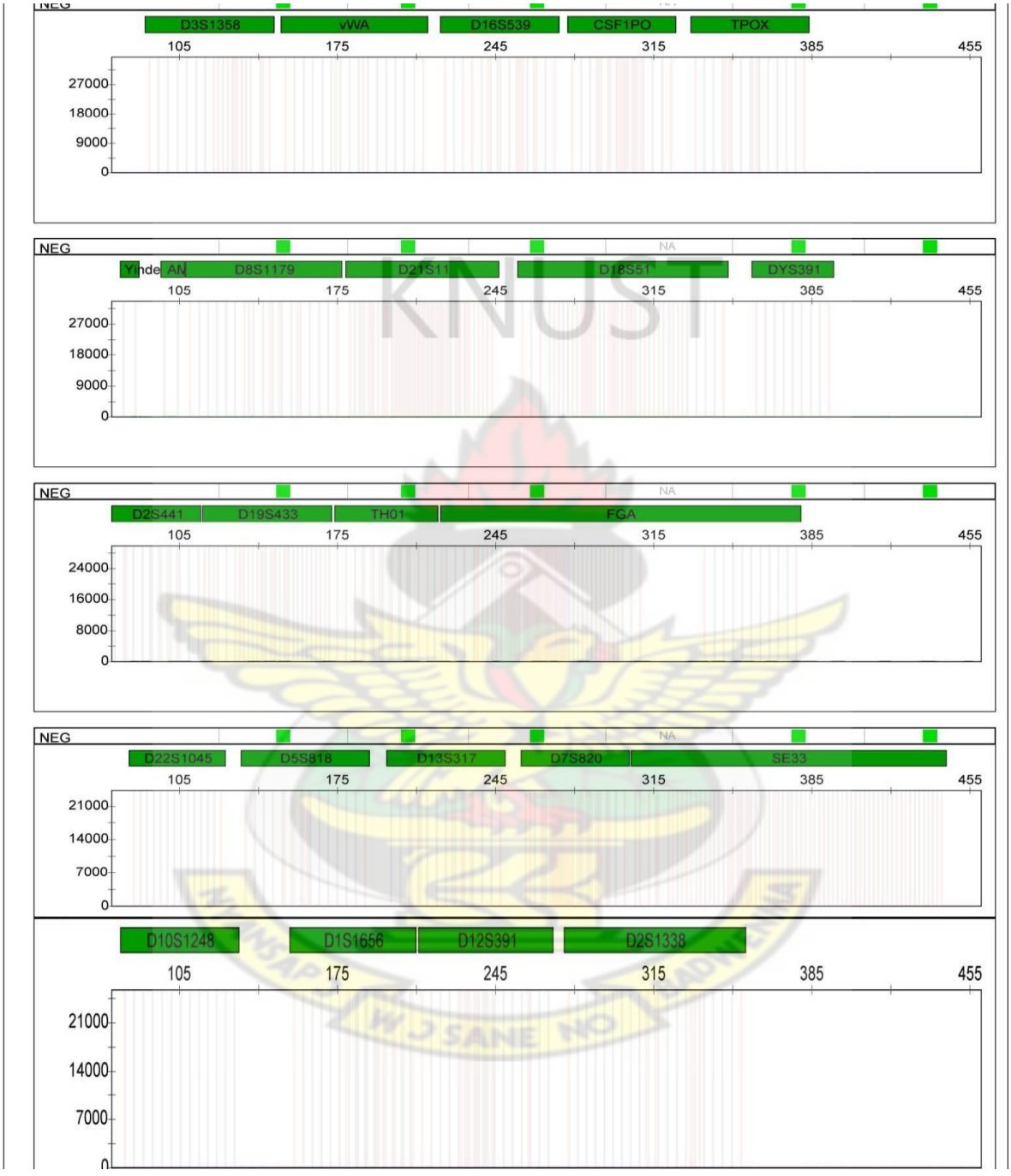


Figure 4.14: Electropherogram of Negative control run alongside the samples

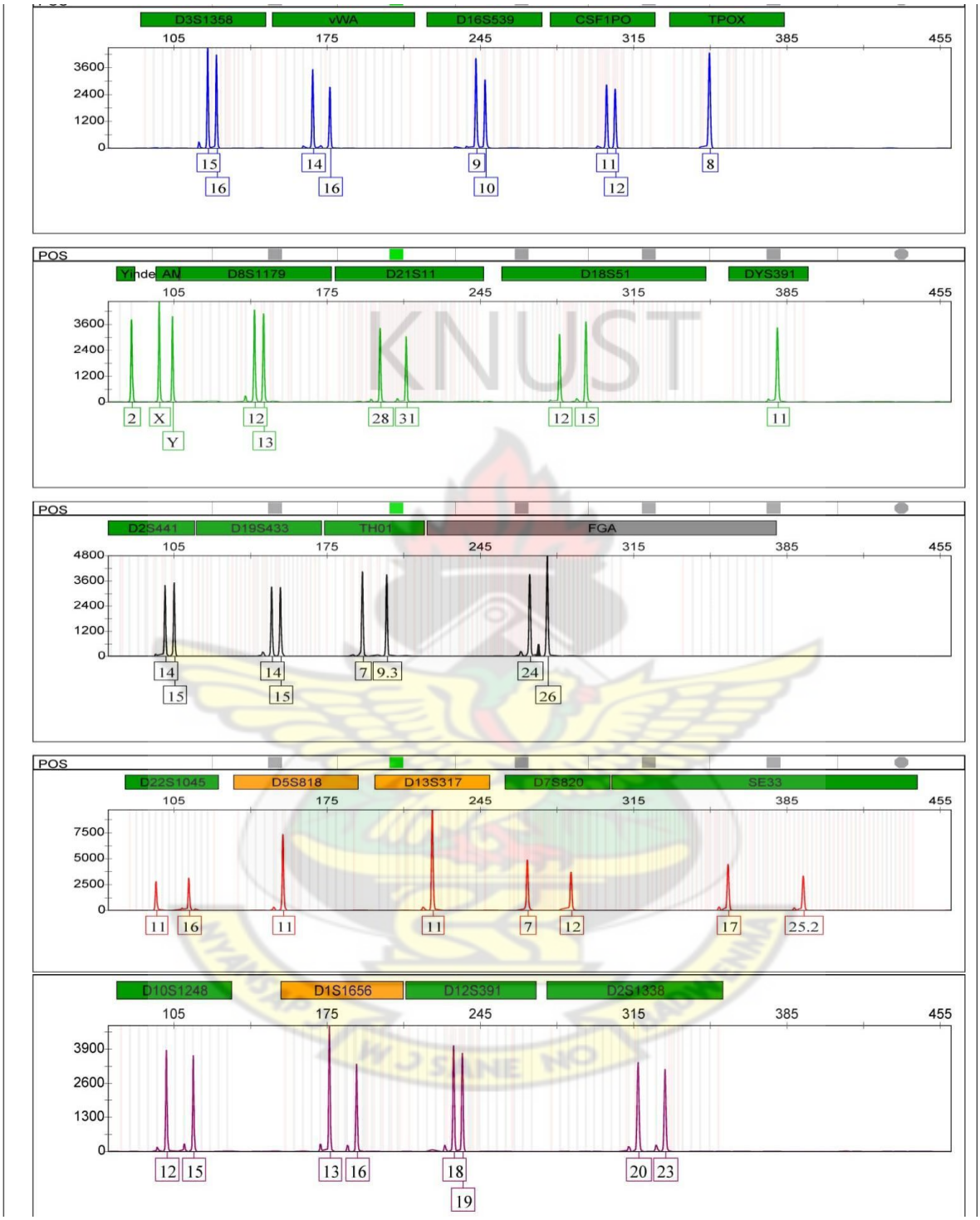


Figure 4.15: Electropherogram of Postive Control run alongside the samples

4.7.1 Detected alleles for Control samples

All control samples gave full alleles at all autosomal markers and Amelogenin except Blood Miniprep extracted soil-mixed sample (sample BSB) (**Table 4.8**).

Table 4.8: Amplified and detected alleles at each loci/marker for control samples

Marker	Sample					
	PCB	**PSB**	**BCB**	**QSB**	**QCB**	**BSB**
D8S1179	12,13	12,13	12,13	12,13	12,13	-
D21S11	30,31.2	30,31.2	30,31.2	30,31.2	30,31.2	-
D7S820	10,11	10,11	10,11	10,11	10,11	-
CSF1PO	11	11	11	11	11	-
D3S1358	14,17	14,17	14,17	14,17	14,17	-
TH01	6,7	6,7	6,7	6,7	6,7	-
D13S317	11,13	11,13	11,13	11,13	11,13	-
D16S539	10	10	10	10	10	-
D2S1338	25,26	25,26	25,26	25,26	25,26	-
D19S433	13,13.2	13,13.2	13,13.2	13,13.2	13,13.2	-
vWA	15,17	15,17	15,17	15,17	15,17	-
TPOX	11,12	11,12	11,12	11,12	11,12	-
D18S51	13,16	13,16	13,16	13,16	13,16	-
D5S818	11,13	11,13	11,13	11,13	11,13	-
FGA	22,24	22,24	22,24	22,24	22,24	-
Y INDEL	2	2	2	2	2	-
DYS391	10	10	10	10	10	-
D2S441	11	11	11	11	11	-
D22S1045	10,16	10,16	10,16	10,16	10,16	-
SE33	13.2,22	13.2,22	13.2,22	13.2,22	13.2,22	-
D10S1248	12,14	12,14	12,14	12,14	12,14	-
D1S1656	17.3	17.3	17.3	17.3	17.3	-
D12S391	15,19	15,19	15,19	15,19	15,19	-
AMEL	X,Y	X,Y	X,Y	X,Y	X,Y	-
Total alleles	42	42	42	42	42	0

PCB=PrepFiler clean blood extraction; PSB=PrepFiler soil-blood mixed extraction; QCB=DNA IQ clean blood extraction; QSB=DNA IQ soil-blood mixed extraction; BCB=Blood Miniprep clean blood extraction; BSB=Blood Miniprep soil-blood mixed extraction

4.7.2 Detected alleles for samples stored at the three conditions

PrepFiler and DNA IQ extractions for samples stored at room temperature and -20 °C all gave full STR profiles as seen in **Table 4.9**. At 4 weeks of sample storage at 4 °C, there were allelic drop outs for PrepFiler and DNA IQ extracted samples. At 6 weeks of storage onwards, there were no alleles observed for PrepFiler and DNA extracted samples stored at 4 °C. It can be observed that, throughout this study, there were no alleles detected for Blood Miniprep extracted soil-blood mixed samples.

Table 4.9: Number of amplified and detected alleles for soil-blood mixed samples stored at the three conditions

Storage time	RT/25 °C			4 °C			-20 °C		
	Number of alleles			Number of alleles			Number of alleles		
	P	Q	B	P	Q	B	P	Q	B
EDTA									
2 weeks	42	42	0	42	42	0	42	42	0
4 weeks	42	42	0	40	34	0	42	42	0
6 weeks	42	42	0	0	0	0	42	42	0
8 weeks	42	42	0	0	0	0	42	42	0
10 weeks	42	42	0	0	0	0	42	42	0
12 weeks	42	42	0	0	0	0	42	42	0
No EDTA									
4 weeks	42	42	0	38	36	0	42	42	0
8 weeks	42	42	0	0	0	0	42	42	0
12 weeks	42	42	0	0	0	0	42	42	0

RT=Room temperature; P=PrepFiler Extraction; Q=DNA IQ Extraction; B=Blood Miniprep Extraction; EDTA=Samples with EDTA; No EDTA= samples without EDTA

4.8 Assessing effect of EDTA on samples at the three storage conditions

From **Tables 4.6, 4.7, 4.9** and **4.10**, there was no significant difference between samples containing EDTA and those without EDTA stored at room temperature in terms of DNA concentration (P=0.966), degradation indexes (P=0.989), number of detected alleles, and IPC C_T values (P=0.103). There was also no significant difference between samples containing EDTA and those without EDTA stored at 4 °C in terms of DNA concentration (P=0.988), degradation indexes (P=0.973), number of detected alleles (P=1.000) and IPC C_T values (P=0.618). There was no significant difference between samples containing EDTA and those without EDTA stored at -20 ^{o}C in terms of DNA concentration (P=0.778), degradation indexes (P=0.716) , number of amplified targets and IPC C_T values (P=0.139).

Table 4.10: Degradation indexes of soil-blood mixed samples stored at the 3 storage conditions

Storage time	RT			4°C			-20°C		
	Prep.	**BM.**	**IQ.**	**Prep.**	**BM.**	**IQ.**	**Prep.**	**BM.**	**IQ**
EDTA									
2 weeks	0.73	-	0.82	1.01	-	1.07	0.67	-	0.99
4 weeks	0.79	-	0.83	1.17	-	5.53	0.67	-	0.87
6 weeks	1.06	-	3.99	1.30	-	1.35	0.83	-	0.97
8 weeks	1.45	-	1.33	-	-	-	0.78	-	0.96
10 weeks	1.33	-	1.35	-	-	-	0.73	-	0.91
12 weeks	1.04	-	1.71	-	-	-	0.92	-	0.93
No EDTA									
4 weeks	0.81	-	0.76	1.78	-	3.50	0.74	-	0.98
6 weeks	1.57	-	1.85	2.00	-	1.86	0.75	-	0.96
12 weeks	2.93	-	1.45	-	-	-	0.76	-	0.86

Controls

Prep. Clean blood (PCB)- 0.6461 IQ. Clean blood (QCB)-0.9557 BM. Clean blood (BCB)- 0.9221

Prep soil-blood mixed (PSB)- 0.7858 IQ. Soil-blood mixed (QSB)- 0.8929 BM. Soil-blood mixed (BSB)-no amplification

Prep. =PrepFiler Forensic kit; IQ. = Promega DNA IQ extraction kit; BM. =Blood Miniprep kit; RT=Room temperature/25°C; N=No amplification; EDTA=Samples with EDTA; No EDTA=Samples without EDTA; - means no detectable DNA

CHAPTER FIVE

5.0 DISCUSSION

Throughout the study, both Hemastix and Hexagon OBTI tests were positive for soil-blood mixed samples stored at all three storage conditions. This suggests that hemoglobin was present in the samples throughout the study and its concentration was enough for detection by the kits. This implies that all the three storage conditions were able to preserve blood for serological tests.

All samples containing EDTA anticoagulant and those without EDTA tested positive for both Hemastix and Hexagon OBTI tests. This means EDTA did not have any effect on the hemoglobin in the blood so far as Hemastix and Hexagon OBTI serological tests were concerned. This agrees with work by Matheson and Veall in 2014 where EDTA didn't had effect on blood in Hemastix testing.

Blood Miniprep extracted control soil-blood mixed sample had no amplification for IPC and all targets , suggesting that there was total amplification failure in this sample as a result of possible presence of high concentration of PCR inhibitors. These inhibitors possibly came from the soil as observed in work by Braid *et al*., in 2003, because the Blood Miniprep extraction for clean blood showed amplification for all targets and IPC.

PrepFiler Forensic kit extractions for clean blood and soil-blood mixed control samples had amplifications for all targets and IPCs with degradation indexes of less than 1. This indicates that DNA was not degraded or inhibited; which agrees that the kit was able to extract undegraded DNA devoid of inhibitors. This result supports finding by Brevnov *et al.* in 2009 on developmental validation of the PrepFiler kit in which the kit extracted undegraded and inhibitor free DNA from environmentally challenged samples with PCR inhibitors but disagrees with

similar work by Lasota in 2014 where the PrepFiler forensic kit failed to extract quality DNA from soil contaminated body fluid.

DNA IQ extractions for the control samples showed amplifications for all targets and IPCs; an indication that the kit removed inhibitors totally or at least brought them to a level that could not prevent amplification. Degradation indexes were also less than 1 suggesting that the DNA wasn't degraded. This is in agreement with similar work by Kasu and Shires in 2015 in which DNA IQ kit was able to extract high quality DNA from blood contaminated with humic acid rich soil.

PrepFiler and DNA IQ extractions of 2 weeks room temperature stored samples had amplifications for all targets, IPCs and degradation indexes less than 1. Concentration of PrepFiler extracted sample decreased compared to its control but that of DNA IQ didn't. This means there was decrease in DNA quantity but not quality. The decrease in concentration could be as a result of microorganisms from the soil (Waksman, 1931) degrading the DNA (Ogata *et al.,* 1963) but degradation wasn't prominent enough to affect the degradation index. On the part of the DNA IQ extracted sample, an experimental error could have caused a non reduction in its concentration.

At 4 weeks of storage at room temperature, PrepFiler and DNA IQ extractions showed amplifications for all targets and IPCs. Their concentrations dropped compared to 2 week extractions but their degradation indexes were less than 1. Microbial activity could have caused this as explained earlier. Once again, DNA quantity decreased but quality wasn't affected.

At 6 to 12 weeks of storage at room temperature, all PrepFiler and DNA IQ extracted samples amplified at all targets and IPCs. Their degradation indexes were between 1-10 and this means the large autosomal targets had degraded more than the small autosomal targets because

degradation index is a ratio of small autosomal concentration to large autosomal concentration. This show that the samples were slightly to moderately degraded. Also their concentrations kept on decreasing week after week. This means DNA quantity as well as quality decreased. Effective degradation as a result of microbial activity defragmenting the DNA into smaller fragments can account for the rise in degradation index as well as decrease in concentration. All

Blood Miniprep extracted samples from room temperature storage failed to amplify and severe PCR inhibition can account for this. This means Blood Miniprep kit could not remove potential PCR inhibitors during extraction.

PrepFiler and DNA IQ extractions from 4℃ stored samples at 2 weeks reduced in concentration drastically with degradation indexes between 1-10. This means the samples were slightly to moderately degraded as a result of microbial activity from the soil. The drastic decrease in concentration shows that the temperature and other storage conditions were very favourable for microbes and as such they degraded the DNA within the 2 week period. This confirms study by Rohatgi and Kapoor in 2014 on the effect of soil types and time on blood DNA quantity and quality over a period of 4 weeks in which there was decrease in DNA quantity and quality from week one to week four. Blood Miniprep extracted sample at 2 week storage failed to amplify with flagged IPC indicating possible severe inhibitors in the sample as a result of the soil.

Concentrations of PrepFiler and DNA IQ samples continued to decrease at varying rates from 2 weeks to 6 weeks at 4 ℃ storage. Their degradation indexes (DIs) were between 1-10. At 8 weeks to 12 weeks of storage, their concentrations were zero (0) with no DIs indicating the samples were significantly degraded. Microbial activity defragmenting the DNA to an extent that

they probably became either unrecoverable or microbial activity degrading all white cells could account for this.

Blood Miniprep extracted samples from 4 °C storage failed to amplify at 2 weeks storage. High concentrations of possible PCR inhibitors in the sample could account for this. At 4 weeks storage, there was amplification for IPC but with high IPC C_T value; an indication of degradation of the inhibitors to a concentration that couldn't prevent IPC amplification. This agrees with study by Seo *et al*., (2012) which showed that incorrect DNA quantification due to the presence of PCR inhibitors may affect experiment results and that Inhibitors can affect the IPC C_T value of a sample and raise it above the avarage IPC C_T of the quantification standards. At 6 to 12 weeks, the IPCs were amplified with normal IPC C_T values. These amplifications might have arisen as a result of degradation of the possible inhibitors in the samples stored at 4 °C from 6 weeks onwards such that their quantity could not affect amplification in any way. Work by Filip and Tesařová in 2004 confirmed that microbial activity degrades humic acid with the humic acid serving as a source of Carbon or Nitrogen for the microbes.

All PrepFiler and DNA IQ extractions from samples stored at -20 °C amplified with degradation indexes less than 1. Their concentrations didn't follow a particular pattern in terms of decrease or increase compared to the control samples. This means the samples were not degraded and inhibitors were removed during extraction. Blood Miniprep extracted samples failed to amplify; an indication of severe presence of possible inhibitors which prevented amplification. The inhibitors could be attributed to the presence of soil.

The IPCs for all samples extracted with PrepFiler and DNA IQ kits were unflagged. This means PrepFiler and DNA IQ kits were able to remove potential inhibitors or bring their concentration

to the bearest minimum during the DNA extraction process such that they could not affect amplification.

Samples stored at -20 °C did not follow a particular pattern in terms of decrease or increase in DNA concentrations throughout the study and their DIs were less than 1, whiles samples stored at room temperature recorded decrease in concentrations as storage time increased. Samples stored at 4 °C also recorded decrease in concentrations such that no DNA was detected at 8 weeks of storage and beyond. This means storage at -20 °C and room temperature/25 °C were better than 4°C. This disagrees with similar work by Cushwa and Medrano (1993) on whole blood in which storage at 4 °C and -20 °C were the best in terms of DNA yield and quality compared to room temperature (25 °C) storage for a 4 week period, but their blood was stored in tube at room temperature whiles room temeperature storage in this study was done in dry state. Microbial activity could thus be said to be the major influence of DNA degradation at 4 °C storage in this study due to presence of a lot of moisture. Heat could be said to have caused the decrease in DNA concentrations of room temperature stored samples overtime. It could be argued that conditions at -20 °C storage (temperature and moisture) did not favour microbial activity, and hence this condition can be used to store soil-mixed blood crime scene samples when brought to the laboratory.

PrepFiler extracted samples recorded the highest DNA concentration at all three storage conditions compared to DNA IQ and Blood miniprep kits. This means PrepFiler is the most potent kit in this study in terms of DNA yield.

All control samples except Blood Miniprep extracted soil-blood mixed sample gave full alleles at all autosomal, Y and Amelogenin markers. This means all samples except Blood Miniprep

extracted soil-blood mixed were undegraded and uninhibited. Results of no profile from control Blood Miniprep soil-blood mixed extracted sample could be attributed to presence of possible PCR inhibitors from the soil which prevented amplification at all target markers.

PrepFiler and DNA IQ extractions for room temperature and -20 °C stored samples all gave full STR Profiles because DNA degradation wasn't prominent enough to affect STR typing of samples at these conditions after 12 weeks of storage.

Blood Miniprep extracted samples from room temperature and -20 °C all gave null profiles because there were possible PCR inhibitors present which prevented amplification of target sequences of these samples. This disagrees with work by Ludeman *et al*. in 2018 where presence of inhibitors like hematin and humic acid in DNA samples was overcome by GlobalFiler kit. Extraction for 2 week sample from 4 °C storage gave null STR Profile because there was severe inhibition which prevented amplification of the sample. At week 4, there was no profile because inhibition was still present in moderate amount and DNA was also in low quantity such that GlobalFiler kit couldn't amplify the sample. At 6 week storage and onwards, no profile was detected though IPC values from quantification were good; an indication that the samples were totally degraded.

PrepFiler and DNA IQ extractions for 2 week 4 °C stored samples gave full profiles meaning DNA was still intact to the detection of the assays of the GlobalFiler PCR amplification kit. At week 4, there were allele drop-outs at some markers for both PrepFiler and DNA IQ extractions meaning there was significant degradation at of markers. This was seen in work by Ludeman *et al*. in 2018 where there were allele drop outs for degraded samples profiled using GlobalFiler kit.

At week 6 and beyond, no profile was obtained indicating total degradation which reduced amplicon sizes such that GlobalFiler kit couldn't amplify these samples.

Samples containing EDTA and those without EDTA stored at the various storage conditions and extracted with the three methods didn't vary in terms of concentration, degradation indexes, IPC C_T values and number of alleles detected. This means EDTA didn't have any effect on the samples and their storage conditions and hence results from this study actually mimics what happens in real crime scenes. This agrees with work by Khosravinia and Ramesha in 2007 in which EDTA added to collected blood samples had no impact on quantity and quality of DNA as well as PCR.

CHAPTER SIX

6.0 CONCLUSIONS

The three Storage conditions (Room temperature/25 ℃, 4 ℃ and -20 ℃) had no effect on the samples in terms of performing Hemastix and Hexagon OBTI serological tests.

Soil-blood mixed samples with and without EDTA all tested positive for Hemastix and Hexagon OBTI serological tests. Thus, EDTA had no effect on serological tests in this study.

Storage time had effect on DNA quantity and quality of samples stored at room temperature and 4 °C; hence storing soil-blood mixed samples at -20 °C was the best in this study whiles storage at 4 ℃ was the worst.

PrepFiler forensic kit was the most potent in terms of ability to extract more DNA followed by DNA IQ Kit. Though Blood Miniprep kit extractions were mostly inhibited, the controls and few samples that amplified confirms that this kit was the worst in terms of DNA extraction potency. Promega DNA IQ and PrepFiler forensic extraction kits or methods were able to remove potential PCR Inhibitors whiles the Blood Miniprep kit performed poor in removing PCR inhibitors from the soil-blood mixed samples.

There was no significant difference in DNA yield from samples with and without EDTA. Moreover, DNA quality did not differ between samples with and without EDTA. The electropherograms from samples with and without EDTA also confirms this. Hence, EDTA did not have any effect on the outcome of this study and that findings from this study can be applied to real life scenarios.

6.1 RECOMMENDATIONS

The study time should be extended to know whether storage condition and storage time will have effect on the results of the Hemastix and OBTI serological tests. The study time should also be extended to see if there will be allele dropouts in room temperature/25 °C stored samples as time of storage progresses. Other storage and DNA extraction methods could also be utilized on soil-blood mixed samples.

REFERENCES

Akane, A., Matsubara, K., Nakamura, H., Takahashi, S., and Kimura, K. (1994). Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. Journal of Forensic Science, 39(2), 362-372.

Alcamo, E.I. (1996). DNA Technology the awesome skill, WCB Publishers, Times mirror higher education group Inc. ISBN 0-697-21248-3.

Anand, U., Agarwal, R., and Anand, C. V. (1994). Test for occult blood. The benzidine reaction: a text book error. Biochemical Education, 22(4), 212.

Applied Biosystems. (2008). PrepFiler Forensic DNA Extraction Kit user guide. P/N 4390932 Rev. B. Available from http://www.appliedbiosystems.com.

Barni, F., Lewis, S. W., Berti, A., Miskelly, G. M., and Lago, G. (2007). Forensic application of the luminol reaction as a presumptive test for latent blood detection. Talanta, 72(3), 896-913.

Bessetti, J. (2007). An introduction to PCR inhibitors. Profiles in DNA, 28, 159-67.

Braid, M. D., Daniels, L. M. and Kitts, C. L. (2003). Removal of PCR inhibitors from soil DNA by chemical flocculation. Journal of Microbiological Methods, 52(3), 389-393.

Brevnov, M. G., Pawar, H. S., Mundt, J., Calandro, L. M., Furtado, M. R., and Shewale, J. G. (2009). Developmental validation of the PrepFiler™ forensic DNA extraction kit for extraction of genomic DNA from biological samples. Journal of forensic sciences, 54(3), 599-607.

Brown, R., and Davenport, J. (2015). Forensic Science: Advanced Investigations, Copyright Update. Nelson Education.

Buckwalter S.P., Sloan L.M., Cunningham S.A., Espy M.J., Uhl J.R., Jones M.F., Vetter E.A., Mandreka J., Cockerill F.R., Pritt B.S., Patel R., Wengenack N.L. (2014). Inhibition controls for qualitative real-time PCR assays: are they necessary for all specimen matrices?. Journal of clinical microbiology, 52(6), 2139-2143.

Budowle, B., Baechtel, F.S. and Adams, D.E. (1990). Validation with regard to environmental insults on the RFLP procedure for forensic purposes, American Chemical Society Series.

Butler, J. M. (2011). Advanced topics in forensic DNA typing: methodology. Academic Press.

Carvalho, S. P. M., Sales-Peres, A., Ribeiro-Bicudo, L. A., and Silva, R. H. A. D. (2010). Quality evaluation of DNA obtained from stored human saliva and its applicability to identification in Forensic Dentistry. Revista Odonto Ciência, 25(1), 48-53.

Claridge, J. (2016). Serology: Bodily fluids, http://www.exploreforensics.co.uk/serology.html accessed 10th June 2016.

Colotelo, A.H. (2009). Evaluation and Application of Presumptive Tests for Blood for Fish Epithelial Injury Detection, The Faculty of Graduate Studies and Research, Department of Biology Carleton University, pp 11-15.

Conti, T. and Buel, E. (2011). Forensic Stain Identification by RT-PCR Analysis, https://www.ncjrs.gov/pdffiles1/nij/grants/226809.pdf.

Cushwa, W. T. and Medrano, J. F. (1993). Effects of blood storage time and temperature on DNA yield and quality. Biotechniques, 14(2), 204-207.

Dawson, L. A., and Hillier, S. (2010). Measurement of soil characteristics for forensic applications. Surface and Interface Analysis, 42(5), 363-377.

Dutelle, A. W. (2016). An introduction to crime scene investigation. Jones and Bartlett Publishers.

Eckert, W. G. (1996). Introduction to Forensic Sciences, Second Edition, CRC Press.

Elkins, K. M. (2012). Forensic DNA biology: a laboratory manual. Academic Press.

Evans, C. (2009). Criminal investigations: crime scene investigation, InfoBase publishing, New York, ISBN-13: 978-0-7910-9405-1.

Filip, Z., and Tesařová, M. (2004). Microbial degradation and transformation of humic acids from permanent meadow and forest soils. International biodeterioration & biodegradation, 54(2), 225-231.

Fisher, B. A., and Fisher, D. R. (2012). Techniques of crime scene investigation. CRC Press.

Fortin, N., Beaumier, D., Lee, K., and Greer, C. W. (2004). Soil washing improves the recovery of total community DNA from polluted and high organic content sediments. Journal of Microbiological Methods, 56(2), 181-191.

Gefrides, L., and Welch, K. (2011). Forensic biology: serology and DNA. In The forensic laboratory handbook procedures and practice (pp. 15-50). Humana Press.

Goodwin, W., Linacre A., Hadi S. (2011). An Introduction to Forensic Genetics, Wiley.

Gryson, N. (2010). Effect of food processing on plant DNA degradation and PCR-based GMO analysis: a review. Analytical and bioanalytical chemistry, 396(6), 2003-2022.

Gunn, A. (2011). Essential forensic biology. 2nd edition John Wiley & Sons, ISBN 9780470758045 (HB).

Harry, M., Gambier, B., Bourezgui, Y., and Garnier-Sillam, E. (1999). Evaluation of purification procedures for DNA extracted from rich organic samples: interference with humic substances. Analusis, 27(5), 439-441.

Hermon, D., Shpitzen, M., Oz, C., and Glattstein, B. (2003). The use of the Hexagon OBTI test for detection of human blood at crime scenes and on items of evidence: Part I: Validation studies and implementation. Journal of Forensic Identification, 53(5), 566.

Hillyer, C. D. (2007). Blood Banking and Transfusion Medicine: Basic Principles and Practice, Churchill Livingstone/Elsevier.

Houck, M. M., and Siegel, J. A. (2009). Fundamentals of forensic science. Academic Press.

Ingham, J. (1932). An improved and simplified benzidine test for blood in urine and other clinical material. Biochemical Journal, 26(4), 1124.

International Agency for Research on Cancer (IARC). (2000). Some antiviral and antineoplastic drugs, and other pharmaceutical agents: views and expert opinion of an IARC Working Group on the Evaluation of Carcinogenic Risks to Humans, Lyon 12-19 October 1999 (Vol. 76). Geneva: World Health Organization.

James, N. (2012). DNA Testing in Criminal Justice: Background, Current Law, Grants, and Issues, Congressional Research Service.

James, S. H., Nordby, J. J., and Bell, S. (Eds.). (2005). Forensic Science: An Introduction to Scientific and Investigative Techniques, Second Edition, Taylor & Francis.

James, S. H., Nordby, J. J., and Bell, S. (Eds.). (2002). Forensic science: an introduction to scientific and investigative techniques. CRC Press.

Jenny, H. (1994). Factors of soil formation: a system of quantitative pedology. Courier Corporation. ISBN 0-486-68128-9.

Karmakar, R. N. (2003). Forensic medicine and toxicology. Academic Publishers.

Kasu, M., and Shires, K. (2015). The validation of forensic DNA extraction systems to utilize soil contaminated biological evidence. Legal Medicine, 17(4), 232-238.

Khosravinia, H. and Ramesha, K. P. (2007). Influence of EDTA and magnesium on DNA extraction from blood samples and specificity of polymerase chain reaction, African Journal of Biotechnology Vol. 6 (3), ISSN 1684–5315, pp. 184-187.

Kirby, L.T. (1992). DNA Fingerprinting An Introduction, Oxford University Press Inc, New York, ISBN 0-19-511867-7.

Knietsch, A., Waschkowitz, T., Bowien, S., Henne, A., and Daniel, R. (2003). Metagenomes of complex microbial consortia derived from different soils as sources for novel genes conferring formation of carbonyls from short-chain polyols on Escherichia coli. Journal of molecular microbiology and biotechnology, 5(1), 46-56.

Kobilinsky, L. F., Levine, L., and Margolis-Nunno, H. (2007). Forensic DNA analysis. InfoBase Publishing.

Lakay, F. M., Botha, A., and Prior, B. A. (2007). Comparative analysis of environmental DNA extraction and purification methods from different humic acid-rich soils. Journal of applied microbiology, 102(1), 265-273.

Lasota, T. (2014). Purification of human DNA for DNA profiling from body fluid contaminated soil using superparamagnetic iron oxide nanoparticles (spions) (Doctoral dissertation, University of Central Lancashire).

Legg, K.M. (2013). Development and Testing of a Rapid Multiplex Assay for the Identification of Biological Stains, Doctoral thesis, Faculty of Natural Science and Mathematics, University of Denver.

Lorente, J. A., Entrala, C., Alvarez, J. C., Arce, B., Heinrichs, B., Lorente, M. and Villanueva, E. (2001). Identification of missing persons: The spanish" phoenix" program. Croatian medical journal, 42(3), 267-270.

Ludeman, M. J., Zhong, C., Mulero, J. J., Lagacé, R. E., Hennessy, L. K., Short, M. L., and Wang, D. Y. (2018). Developmental validation of GlobalFiler™ PCR amplification kit: a 6-dye multiplex assay designed for amplification of casework samples. International journal of legal medicine, 1-19.

Luftig, M. A., and Richey, S. (2000). DNA and Forensic science. New Eng. L. Rev., 35.

Matheson, C. D., and Veall, M. A. (2014). Presumptive blood test using Hemastix® with EDTA in archaeology. Journal of Archaeological Science, 41, 230-241.

McCord, B., Opel, K., Funes, M., Zoppis, S., and Meadows Jantz, L. (2011). An investigation of the effect of DNA degradation and inhibition on PCR amplification of single source and mixed forensic samples. US Department of Justice, 1-66.

Mitchell, J.K, and Soga K. (1976). Fundamentals of soil behavior: Wiley New York.

National Academy of Sciences .(2015). Support for Forensic Science research: Improving the Scientific Role of the National Institute of Justice, National Academies Press, Washington DC, ISBN 978-0-309-37645-7 | DOI: 10.17226/21772.

National Academy of Sciences. (2009). Strengthening Forensic Science in the United States: A Path Forward, The national academies press, Washington DC, ISBN 978-0-309-13135-3 | DOI 10.17226/12589.

National Research Council. (1992). Committee on DNA Technology in Forensic Science, DNA technology in forensic science. Washington DC, ISBN: 0-309-58369-1.

Newton, D. E. (2007). Forensic chemistry. InfoBase Publishing.

Ogata, K., Nakao, Y., Igarasi, S., Omura, E., Sugino, Y., Yoneda, M., and Suhara, I. (1963). Degradation of nucleic acids and their related compounds by microbial enzymes. Agricultural and Biological Chemistry, 27(2), 110-115.

Petricevic S., DNA Profiling in Forensic Science, Institute of Environmental Science and Research Ltd. (ESR), New Zealand Institute of Chemistry, http://nzic.org.nz/ChemProcesses/biotech/12D.pdf, accessed 7th June 2016.

Prahlow, J. A. (2010). Forensic pathology for police, death investigators, attorneys, and forensic scientists. Springer Science & Business Media. Chapter 2, ISBN: 978-1-58829-975-8.

Promega. (2016). DNA IQ™ System—Database Protocol Technical Bulletin, https://www.promega.com/-/media/files/resources/protocols/technical-bulletins/101/dna-iq-system-database-protocol.pdf accessed 15th March 2017.

Rådström, P., Knutsson, R., Wolffs, P., Lövenklev, M., and Löfström, C. (2004). Pre-PCR processing. Molecular biotechnology, 26(2), 133-146.

Robe, P., Nalin, R., Capellano, C., Vogel, T. M., and Simonet, P. (2003). Extraction of DNA from soil. European Journal of Soil Biology, 39(4), 183-190.

Rohatgi, R., and Kapoor, A. K. (2014). Effect of Different Types of Soil and Time Intervals on Isolation and Quantification of DNA: A Forensic Management Technology Perspective., International Journal of Emerging Research in Management &Technology ISSN: 2278-9359 Volume-3 Issue-7.

Rudin, N. and K. Inman (2001). An Introduction to Forensic DNA Analysis, Second Edition, CRC Press.

Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989). Molecular cloning: a laboratory manual (No. Ed. 2). Cold spring harbor laboratory Press.

Schrader, C., Schielke, A., Ellerbroek, L. and Johne, R. (2012). PCR inhibitors – occurrence, properties and removal, Journal of Applied Microbiology 113, 1014-1026 ,ISSN 1364-5072.

Seo, S. B., Lee, H. Y., Zhang, A. H., Kim, H. Y., Shin, D. H., and Lee, S. D. (2012). Effects of humic acid on DNA quantification with QuantiFiler® Human DNA Quantification kit and short tandem repeat amplification efficiency. International journal of legal medicine, 126(6), 961-968.

Shahzad, M. S., Bulbul, O., Filoglu, G., Cengiz, M., and Cengiz, S. (2009). Effect of blood stained soils and time period on DNA and allele drop out using Promega 16 Powerplex® kit. Forensic Science International: Genetics Supplement Series, 2(1), 161-162.

Shaler, R. C. (2002). Modern forensic biology. Forensic science handbook, 1, 525-613.

Sharma, M., Khajja B.S., Godara, D.R., and Mathur, G.K. (2011). Case Study: Theft and Murder Unraveled by Forensic Investigation http://www.forensicmag.com/article/2011/06/case-study-theft-and-murder-unraveled-forensic-investigation, accessed 10th June, 2016.

Spalding, R. P. (2003). Identification and characterization of blood and bloodstains. Forensic Science, An introduction to Scientific and Investigative Techniques, 244-245.

Stevens, C. D. (2009). Clinical Immunology and Serology: A Laboratory Perspective, F.A. Davis.

Sutlović, D., Definis Gojanović, M., and Anđelinović, Š. (2007). Rapid extraction of human DNA containing humic acid. Croatica chemica acta, 80(1), 117-120.

Taupin, J. M. (2013). Introduction to Forensic DNA Evidence for Criminal Justice Professionals. CRC Press.

ThermoFisher Scientific. (2016). GlobalFiler PCR Amplification kit user guide, Pub no. 4477604E.

ThermoFisher Scientific. (2016). QuantiFiler™ HP and Trio DNA Quantification Kits User Guide, https://tools.thermofisher.com/content/sfs/manuals/4485354.pdf accessed 17th March 2017.

Tobe, S. S., Watson, N., and Daeid, N. N. (2007). Evaluation of six presumptive tests for blood, their specificity, sensitivity, and effect on high molecular weight DNA. Journal of forensic sciences, 52(1), 102-109.

Triggs, C. M., Buckleton J. S. and Walsh S. J. (2004). Forensic DNA Evidence Interpretation, CRC Press.

UNODC (United Nation's office on drugs and crime). (2009). Crime scene and physical evidence awareness for non-forensic personnel, United Nations publication Sales No. E.09.IV.5, ISBN 978-92-1-130273-8.

Vandewoestyne, M., Lepez, T., Van Hoofstat, D., and Deforce, D. (2015). Evaluation of a visualization assay for blood on forensic evidence. Journal of forensic sciences, 60(3), 707-711.

Virkler, K., and Lednev, I. K. (2009). Analysis of body fluids for forensic purposes: from laboratory testing to non-destructive rapid confirmatory identification at a crime scene. Forensic Science International, 188(1), 1-17.

Waksman, S. A., and Starkey, R. L. (1931). Soil and the Microbe. New York: Wiley.

Walsh, P. S., Erlich, H. A., and Higuchi, R. (1992). Preferential PCR amplification of alleles: mechanisms and solutions. Genome Research, 1(4), 241-250.

Williams, G. A. (2012). Identification and Resolution of Capability Gaps in Forensic Science, Doctoral Thesis, University of Huddersfield.

Wilson, I. G. (1997). Inhibition and facilitation of nucleic acid amplification. Applied and environmental microbiology, 63(10), 3741–3751.

Zhou, J., Bruns, M. A., and Tiedje, J. M. (1996). DNA recovery from soils of diverse composition. Applied and environmental microbiology, 62(2), 316-322.

Zipper, H., Buta, C., Lämmle, K., Brunner, H., Bernhagen, J., and Vitzthum, F. (2003). Mechanisms underlying the impact of humic acids on DNA quantification by SYBR Green I and consequences for the analysis of soils and aquatic sediments. Nucleic acids research, 31(7), e39-e39.

https://en.wikipedia.org/wiki/DNA accessed 7th September 2016.

http://www.biopioneerinc.com/2016/molecular-biology/genomic-dna-isolation-kits/blood-genomic-dna-miniprep-kit-100-preps/ accessed 8th April, 2018.

http://www.bluestar-forensic.com/pdf/en/instructions_hexagon_obti.pdf, accessed 21st February 2017.

http://www.epigentek.com/catalog/fitamp-general-tissue-section-dna-isolation-kit-p-31.html accessed 7th September 2016.

https://www.tes.com/lessons/cfjSt8FlQVcD1w/agglutination-and-precipitation accessed 17th March 2017.

APPENDICES

Appendix 1

Hexagon OBTI test protocol

1. Unscrew the red cap of the tube holding the tube upwards

2. Collect suspected blood sample with an appropriate collection tool

3. Put the presumed blood into the tube with transport medium and shake gently.

4. Screw the cap tightly.

5. Remove test cassette from the pouch and place it on a level surface.

6. Holding the tube vertically, break the tip.

7. Dispense exactly two full drops into the sample well (S) at the lower end of the cassette.

8. Wait for about 5 minutes to read the results.

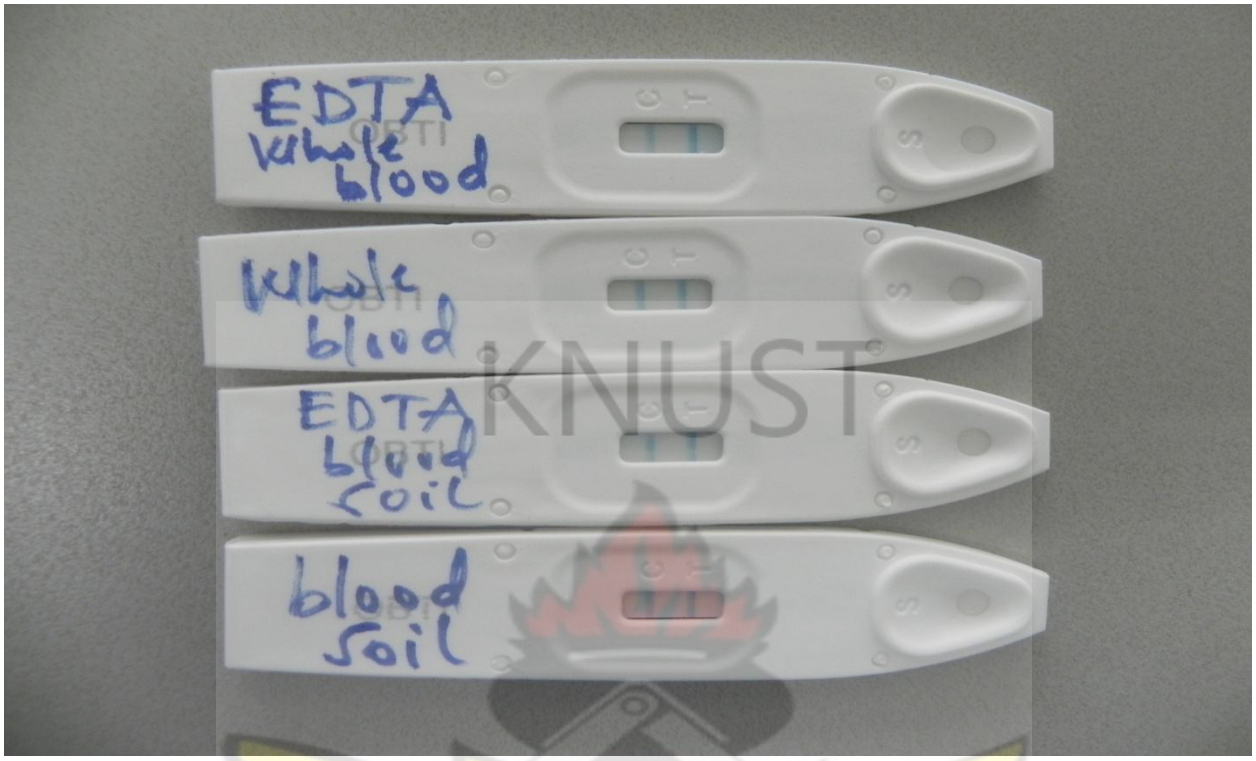
9. Negative results should be confirmed after 10 minutes.

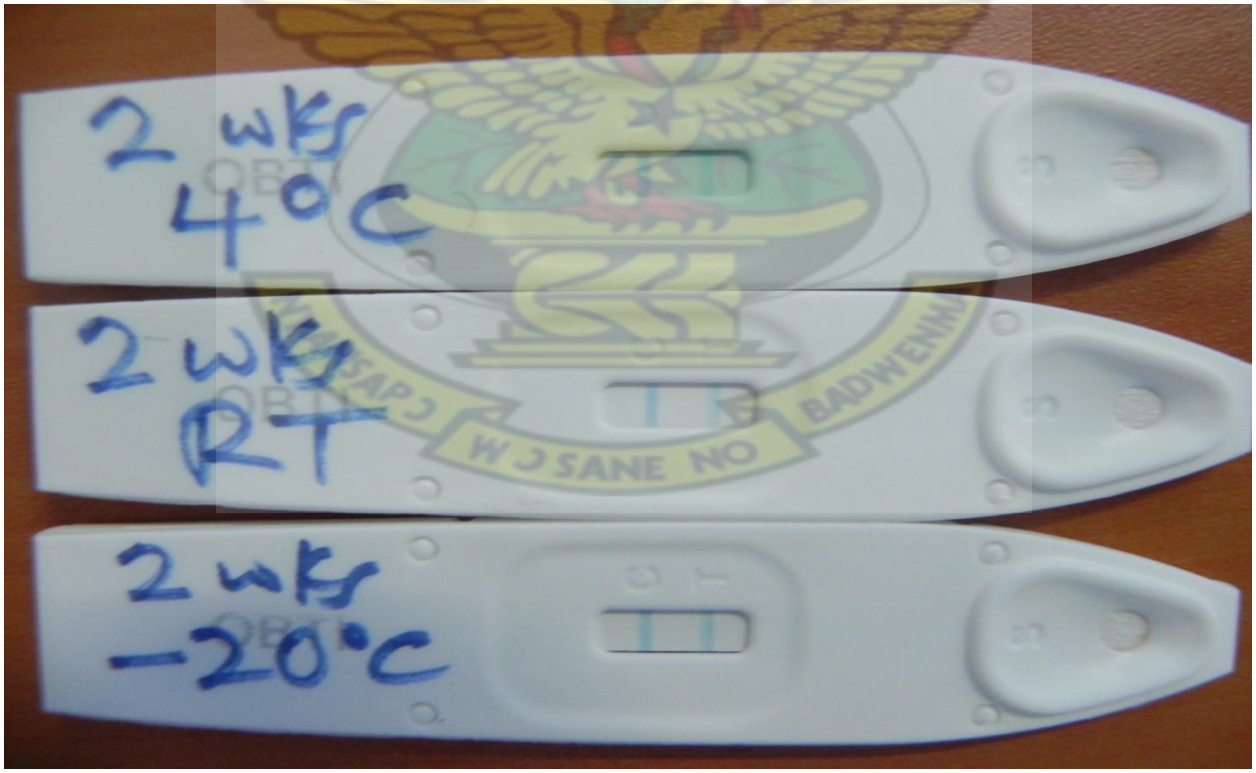
10. Only one blue control line (C) appearing in the cassette indicates a negative result.

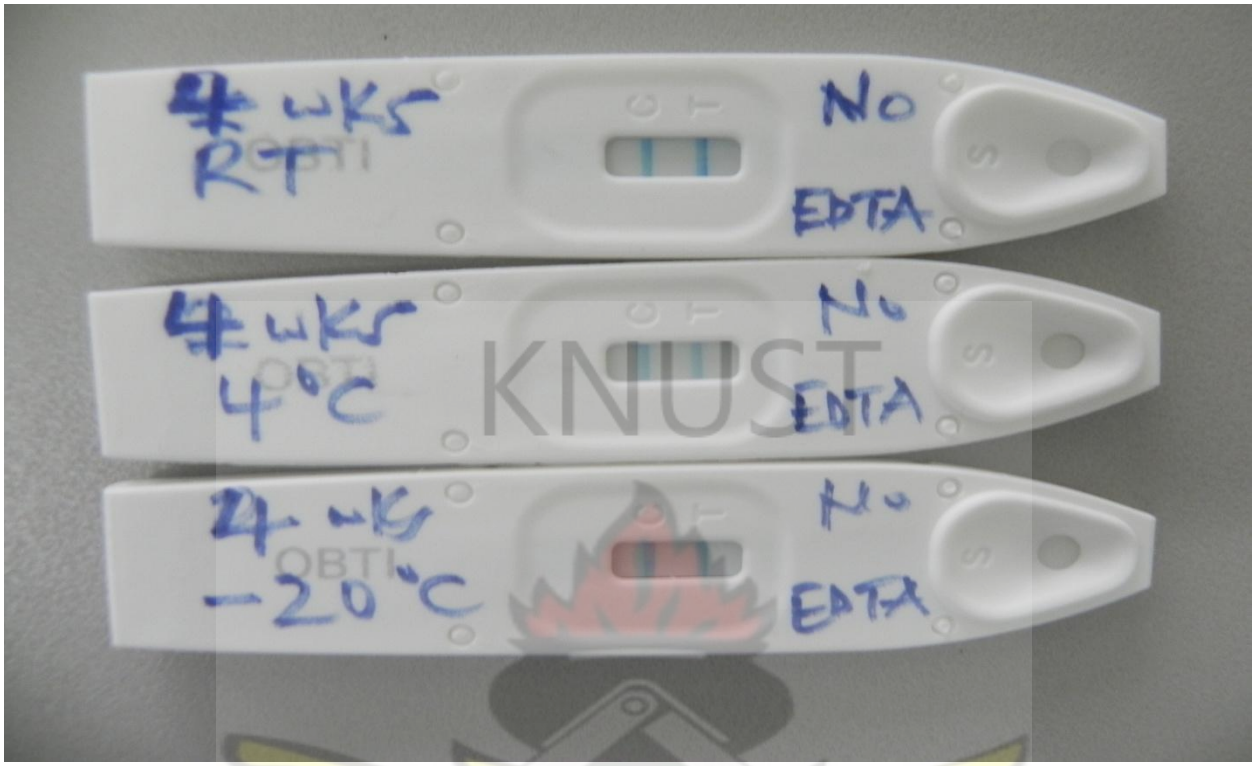
11. Two blue lines one at the C region and the other at the T region appearing in the cassette indicates positive results.

12. No control line or only the test line appearing indicates invalid result. Repeat the test with a new kit.

Hexagon OBTI test results for the study







Hemastix test protocol

1. Apply 1 drop of deionized water to the pad end of the test strip

2. Rub the damp pad onto the stain in question

3. Note color change within 60 seconds.

4. A color change to orange through green or blue indicates a positive result. No color change indicates a negative result. A negative result means there is no blood present or is below the limit of detection of the test.

Appendix 2

Prepfiler Forensic DNA extraction protocol

1. Put amount of blood free from soil into Eppendorf tube.

2. Bring the thermal shaker temperature to 70 °C.

3. To the tube that contains the sample, add 500 µL of PrepFiler™ Lysis Buffer.

4. Cap the tube, vortex it for 5 seconds, then centrifuge it briefly.

5. Place the tube in a thermal shaker, and then incubate at 70 °C and 900 rpm for 30 minutes.

6. Centrifuge the tube at maximum speed (≈16,110 x g) for 5 minutes.

7. Transfer the clear (free of residual soil) supernatant to a new 1.5-mL

8. Allow the sample lysate to come to room temperature (approximately 5 minutes).

9. Vortex the PrepFiler™ Magnetic Particles tube approximately 5 seconds, invert the tube to confirm that no visible pellet remains in the bottom of the tube, then centrifuge briefly.

10. Pipette 20 µL of magnetic particles into the tube containing the sample lysate.

11. Cap the sample lysate tube, vortex it at low speed (approximately 500 to 1,200 rpm) for 10 seconds, then centrifuge it briefly.

12. Add 300 µL of isopropanol to the sample lysate tube.

13. Cap the sample lysate tube, vortex it at low speed (approximately 500 to 1,200 rpm) for 5 seconds, then centrifuge it briefly.

14. Place the sample lysate tube in a shaker or on a vortexer (with adaptor), then mix at room temperature at 1,000 rpm for 10 minutes.

15. Vortex the sample DNA tube:

a. If magnetic particles are present on the sides of the sample DNA tube above the meniscus, invert the tube to resuspend the particles.

b. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) for 10 seconds, then centrifuge briefly.

16. Confirm that the magnet in the magnetic stand is properly aligned.

17. Place the sample DNA tube in the magnetic stand and observe that the magnetic particles form a pellet against the back of the tube. Wait until the size of the pellet stops increasing (approximately 1 to 2 minutes).

18. With the sample DNA tube remaining in the magnetic stand, use a pipette to carefully remove and discard all visible liquid phase.

19. Perform steps a through e three times:

a. Add 300 µL of prepared PrepFiler™ Wash Buffer to the sample DNA tube.

b. Cap the sample DNA tube and remove the tube from the magnetic stand.

c. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) until there is no visible magnetic particle pellet on the side of the tube (approximately 5 seconds), then centrifuge briefly.

d. Place the sample DNA tube in the magnetic stand for 30 to 60 seconds.

e. With the sample DNA tube remaining in the magnetic stand, use a pipette to carefully remove and discard all visible liquid phase.

20. With the sample DNA tube remaining in the magnetic stand, open the tube, then allow the magnetic particles-bound DNA to air-dry for 7 to 10 minutes

21. Bring the thermal shaker temperature to 70 °C.

22. Add 50 µL of PrepFiler Elution Buffer to the sample DNA tube.

23. Cap the sample DNA tube, vortex it at maximum speed (approximately 10,000 rpm) until there is no visible magnetic particle pellet on the side of the tube (approximately 5 seconds), then centrifuge it briefly.

24. Place the sample DNA tube in a thermal shaker, then incubate at 70 °C and 900 rpm for 5 minutes.

25. Vortex the sample DNA tube at maximum speed (approximately 10,000 rpm) until there is no visible magnetic particle pellet on the side of the tube (approximately 2 seconds), then centrifuge briefly.

26. Place the sample DNA tube in the magnetic stand, then wait until the size of the pellet at the side of the tube stops increasing (at least 1 minute).

27. Pipette the liquid in the sample DNA tube (which contains the isolated genomic DNA) to a new spin tube or 1.5-mL microcentrifuge tube for storage.

Promega DNA IQ extraction protocol

Vortex the stock resin bottle for 10 seconds at high speed or until resin is thoroughly mixed. For each sample,

1. Prepare the resin/Lysis Buffer mixture using 7µl of resin and the volume of prepared Lysis Buffer indicated

2. Place an amount of blood in an Eppendorf tube

3. Add 80µl of the Incubation Buffer/Proteinase K solution to the liquid blood sample. Incubate at 56°C for 1 hour.

4. Vortex the resin/Lysis Buffer mixture for 2 seconds at high speed to ensure suspension of resin. Add the volume of resin/Lysis Buffer mixture indicated below to the sample. The resin/Lysis Buffer mixture should be mixed again if the resin begins to settle while dispensing aliquots.

5. Vortex the sample/resin/Lysis Buffer mixture for 3 seconds at high speed. Incubate for 5 minutes at room temperature. Vortex mixture for 3 seconds once every minute during this 5-minute incubation.

6. Vortex tube for 2 seconds at high speed. Place the tube in the magnetic stand. Separation will occur instantly.

Note: If resin does not form a distinct pellet on the side of the tube, vortex the tube and quickly place back in the stand.

7. Carefully remove and discard all of the solution without disturbing the resin pellet on the side of the tube.

8. Add 100µl of prepared Lysis Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.

9. Return tube to the magnetic stand, and remove and discard all Lysis Buffer.

10. Add 100µl of prepared 1X Wash Buffer. Remove tube from the magnetic stand, and vortex for 2 seconds at high speed.

11. Return tube to the magnetic stand. Dispose of all Wash Buffer.

12. Repeat Steps 10 and 11 two more times for a total of three washes. Be sure that all of the solution has been removed after the last wash.

13. With the tube in the magnetic stand and the lid open, air-dry the resin for 5 minutes.

! Do not dry for more than 20 minutes, as this may inhibit removal of DNA.

14. Add 50 µL of Elution Buffer.

15 Close the lid and vortex tube for 2 seconds at high speed. Incubate at 65°C for 5 minutes.

16. Remove the tube from the heat source, and vortex for 2 seconds at high speed. Immediately place on the magnetic stand. Tubes must remain hot until placed in the magnetic stand, or yield will decrease.

17. Carefully transfer the DNA-containing solution to a container of choice.

Blood Miniprep kit extraction protocol

1. Place amount of blood free from soil into an Eppendorf tube.

2. Centrifuge at 3000rpm for 3 minutes. Discard supernatant.

3. Add 0.8ml TBP buffer to the collection tube, vortex gently, then 3000rpm for 3 minutes. Discard supernatant.

4. Repeat step 2. If the blood pellet looks mauve, then continue next step.

5. Add 0.5ml TBM buffer to the collection tube, intensify vortex, then add 3ul Proteinase K, then incubate at 55^{O}C for 30 minutes.

6. If insoluble material is visible, centrifuge for 2 minutes at 5000rpm. Save the supernatant to an Eppendorf tube, and then add 260ul absolute ethanol.

7. Apply the mixture to BP-10 column that is in a 2ml collection tube. Spin at 8000rpm for 1 minute. Discard the flow-through in the collection tube.

8. Add 500ul of wash solution, and spin at 8000rpm for 1 minute.

9. Repeat washing step 8.

10. Discard flow-through. Spin at 8000rpm for an additional minute to remove residual amount of wash solution.

11. Place the column into a clean 1.5ml Eppendorf tube. Add 30-50ul elution buffer into the center part of the membrane in the column. Incubate the tube at 37 or 50^{O}C for 2 minutes.

12. Spin at 10000rpm for 1 minute to elute DNA from the column.

Appendix 3

QuantiFiler trio kit protocol

Prepare the DNA quantification standards·

Standard	Concentration ng/uL	Example volumes	Dilution factor
1	50.000	10 µL [100 ng/µL stock] + 10 µL QuantiFiler DNA dilution buffer	2X
2	5.000	10 µL [Std. 1] + 90 µL QuantiFiler DNA dilution buffer	10X
3	0.500	10 µL [Std. 2] + 90 µL QuantiFiler DNA dilution buffer	10X
4	0.050	10 µL [Std. 3] + 90 µL QuantiFiler DNA dilution buffer	10X
5	0.005	10 µL [Std. 4] + 90 µL QuantiFiler DNA dilution buffer	10X

To prepare the DNA quantification standards dilution series:

1. Label five microcentrifuge tubes: Std. 1, Std. 2, Std. 3, and so on.

2. Dispense the required amount of diluent (QuantiFiler DNA Dilution Buffer) to each tube as shown on the table above.

3. Prepare Std. 1:

a. Vortex the QuantiFiler Trio DNA Standard 3 to 5 seconds.

b. Using a new pipette tip, add the appropriate volume of QuantiFiler Trio DNA Standard for your dilution series to the tube for Std. 1.

c. Mix the dilution thoroughly.

4. Prepare Std. 2 through 5:

a. Using a new pipette tip, add the appropriate volume of the prepared standard to the tube for the next standard as shown in table.

b. Mix the standard thoroughly.

c. Repeat steps a and b for each subsequent standard until you complete the dilution series.

To prepare the reactions:

1. Calculate the volume of each component needed to prepare the reactions, using the table below.

Component	Volume per reaction uL)
QuantiFiler Trio Primer Mix	8
QuantiFiler Trio PCR Reaction Mix	10

2. Prepare the reagents:

- Thaw the QuantiFiler Trio Primer Mix completely, then vortex 3 to 5 seconds and centrifuge briefly before opening the tube.
- Gently vortex the QuantiFiler Trio PCR Reaction Mix before using.

3. Pipette the required volumes of components into an appropriately sized polypropylene tube.

4. Vortex the PCR mix 3 to 5 seconds, then centrifuge briefly.

5. Dispense 18 µL of the PCR mix into each reaction well or tube.

6. Add 2 µL of sample, standard, or control to the applicable wells or tubes.

7. Seal the reaction plate with the Optical Adhesive Cover, or the strip tube with the optical 8-cap strip.

8. Remove bubbles: While the plate is inside the base, tap the base on the benchtop to bring the bubbles to the liquid surface. Lift the plate, and then inspect each well for bubbles; tap each well with a marker, pen, or gloved fingertip.

9. Centrifuge the plate at 3000 rpm for about 20 seconds in a tabletop centrifuge with plate holders to remove any bubbles.

10. Run plate on machine

Appendix 4

DNA Amplification protocol

1. Vortex the master mix and primer set from the GlobalFiler kit for 3 seconds.

2. Pipette the required volumes of components into an appropriate tube.

Reaction component	Volume per reaction
Master Mix	7.5ul
Primer Set	2.5ul

3. Vortex the reaction mixture for 3 seconds and centrifuge briefly.

4. Pipette 10ul of reaction mixture into each PCR tube

5. Add 15ul of prepared DNA sample to the PCR tube.

6. Program the thermal cycler and start run.

Initial incubation step	Cycle (29 cycles)		Final extension	Final hold
	Denature	Anneal/extend		
Hold	Cycle		hold	hold
95^{O}C 1 minute	94^{O}C 10 seconds	59^{O}C 90 seconds	60^{O}C 10 mins	4^{O}C up to 24 hrs

Appendix 5

STR Typing protocol

1. Pipette required volumes of components into an appropriate tube.

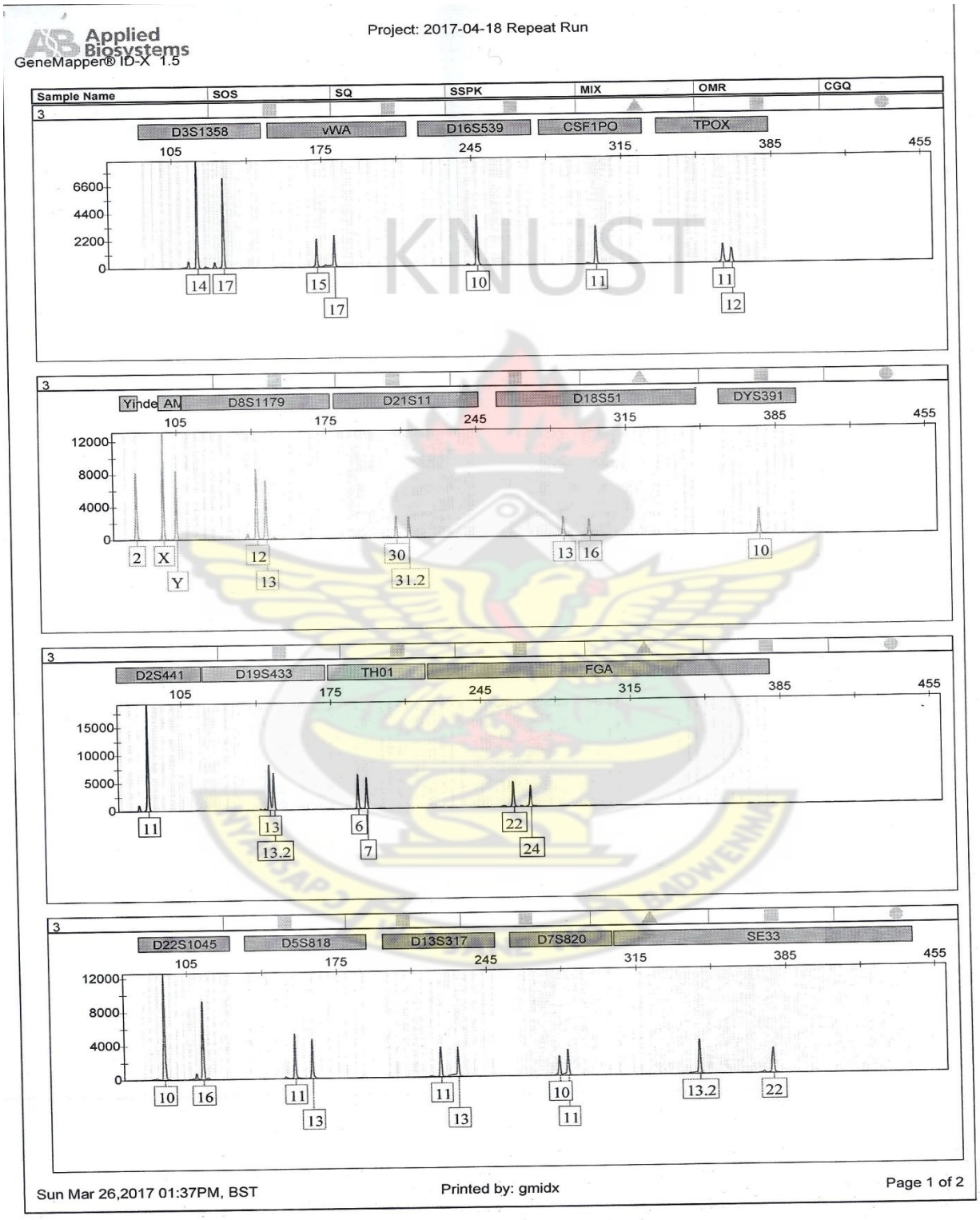
Reagent	Volume per reaction
GeneScan 600 LIZ Size standard v2.0	0.4ul
HiDi Formamide	9.6ul

2. Vortex the tube and centrifuge briefly.

3. Into each well of a 96-well plate, put 10ul of GeneScan/Formamide mixture and 1ul of amplified DNA (PCR product) or Allelic ladder.

Appendix 6

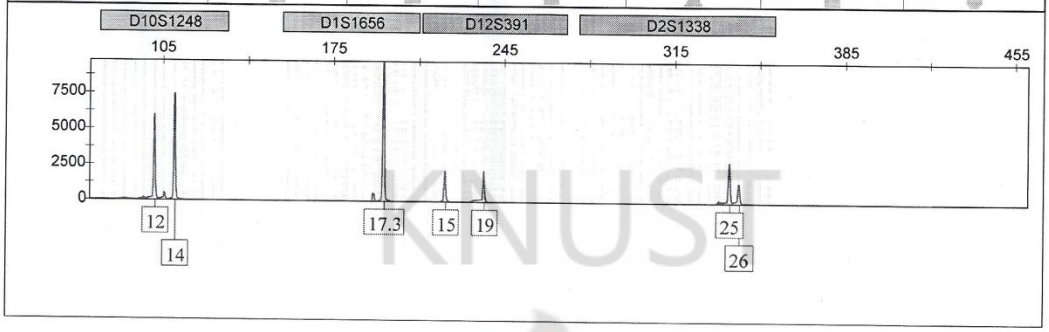
Some STR Profiles



Applied Biosystems
GeneMapper® ID-X 1.5

Project: 2017-04-18 Repeat Run

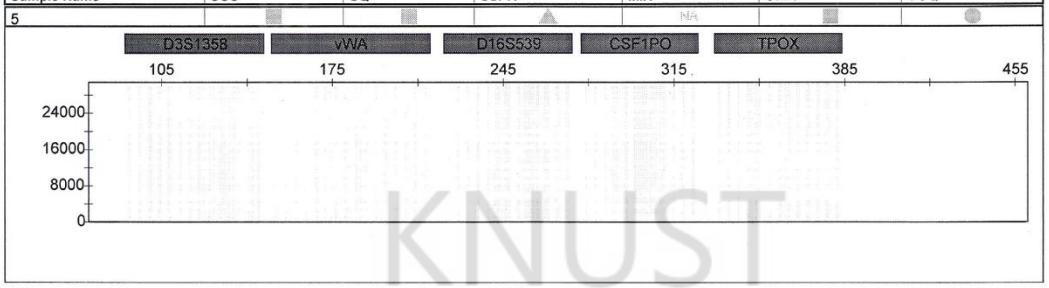
Sample Name	SOS	SQ	SSPK	MIX	OMR	CGQ
3						

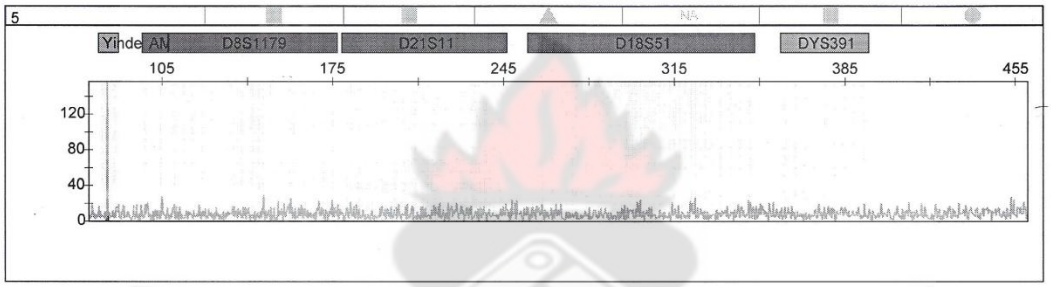


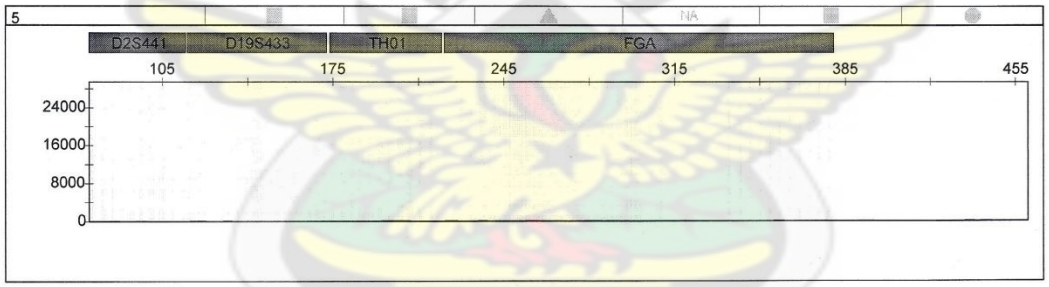
Applied Biosystems
GeneMapper® ID-X 1.5

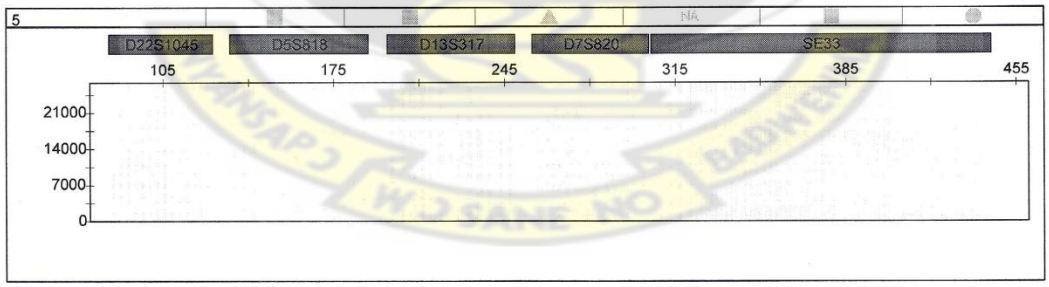
Project: 2017-04-18 Repeat Run

Sample Name	SOS	SQ	SSPK	MIX	OMR	CGQ







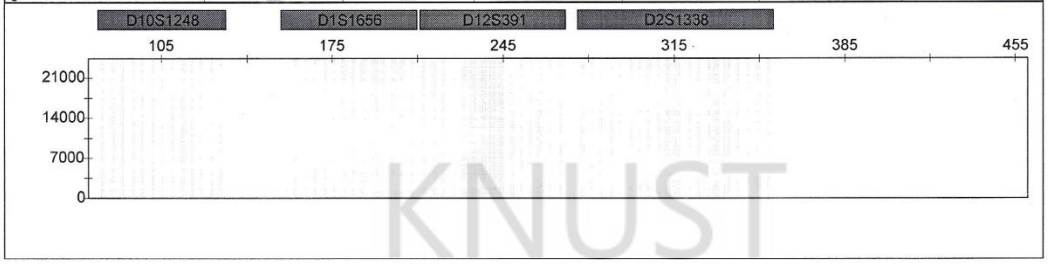


Sun Mar 26,2017 01:45PM, BST Printed by: gmidx Page 1 of 2

Applied Biosystems
GeneMapper® ID-X 1.5

Project: 2017-04-18 Repeat Run

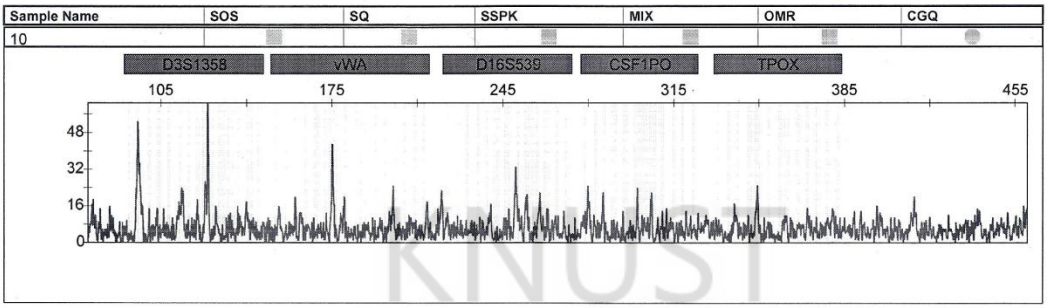
Sample Name	SOS	SQ	SSPK	MIX	OMR	CGQ
5				NA		

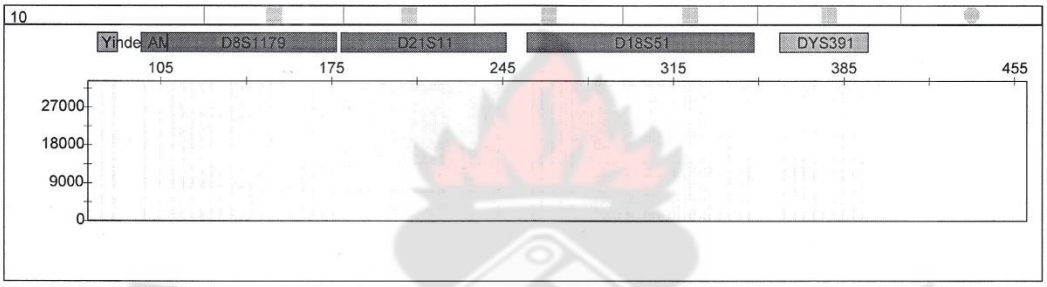


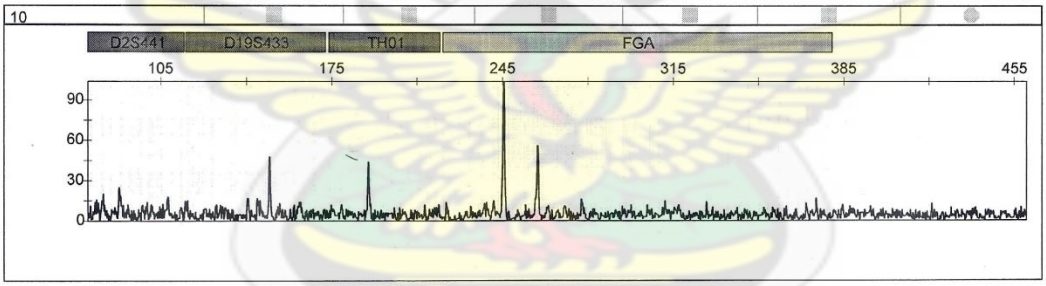
Applied Biosystems
GeneMapper® ID-X 1.5

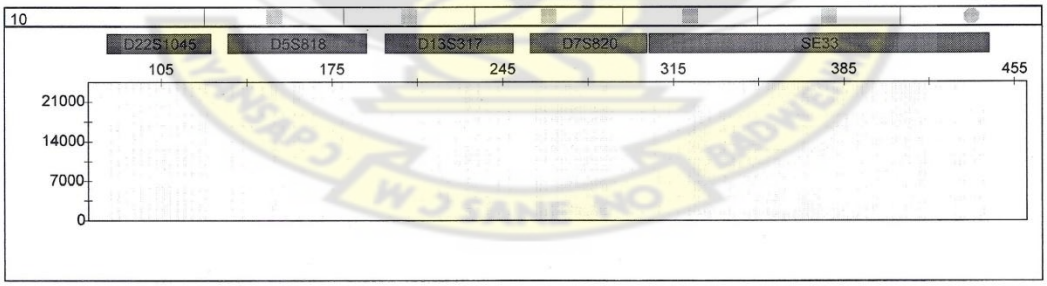
Project: 2017-04-18 Repeat Run

Sample Name	SOS	SQ	SSPK	MIX	OMR	CGQ









Sun Mar 26,2017 01:47PM, BST

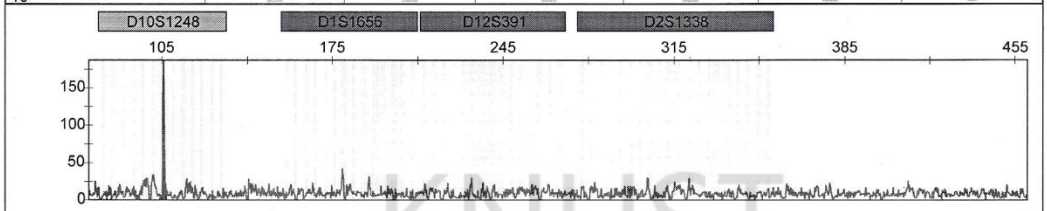
Printed by: gmidx

Page 1 of 2

Applied Biosystems
GeneMapper® ID-X 1.5

Project: 2017-04-18 Repeat Run

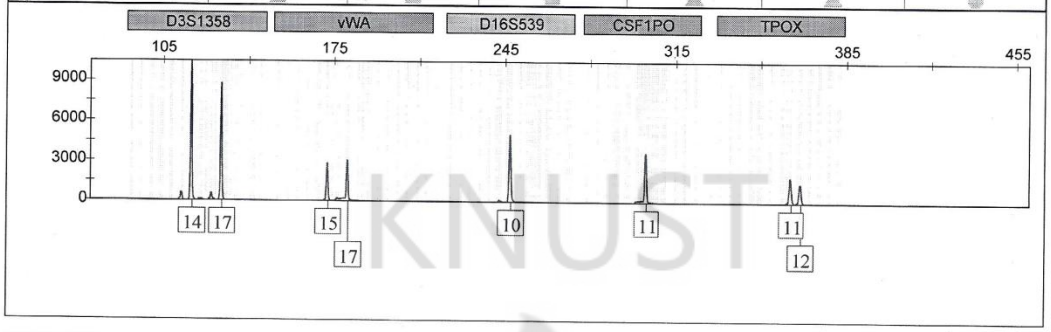
Sample Name	SOS	SQ	SSPK	MIX	OMR	CGQ
10						

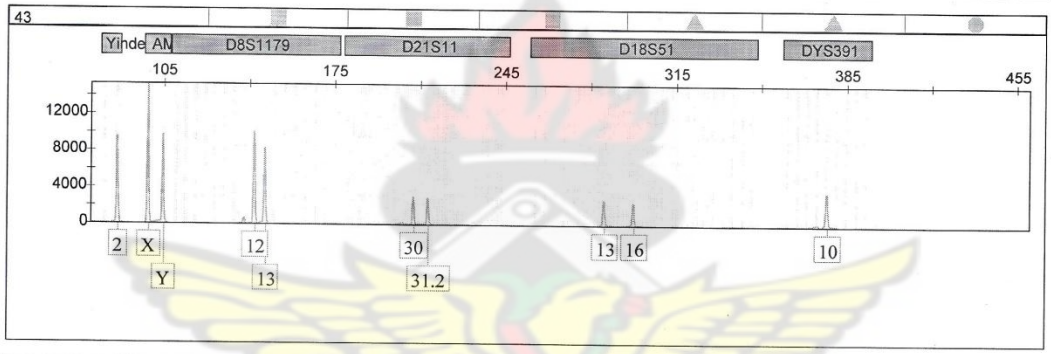


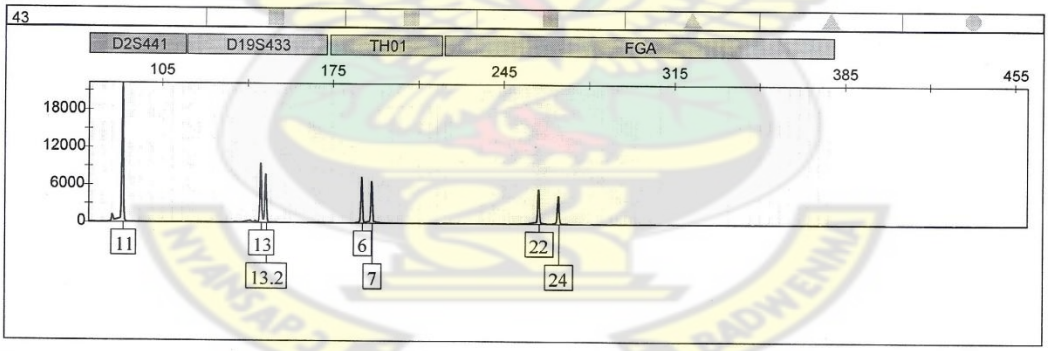
Applied Biosystems
GeneMapper® ID-X 1.5

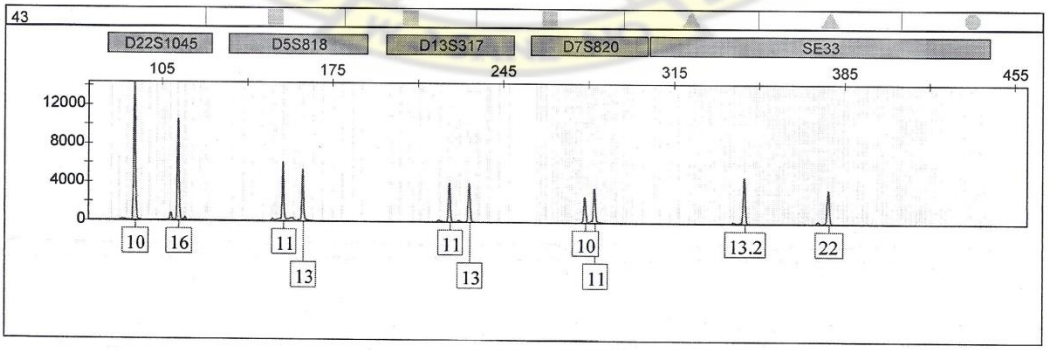
Project: 2017-04-18 Repeat Run

Sample Name	SOS	SQ	SSPK	MIX	OMR	CGQ
43						









Sun Mar 26,2017 02:05PM, BST

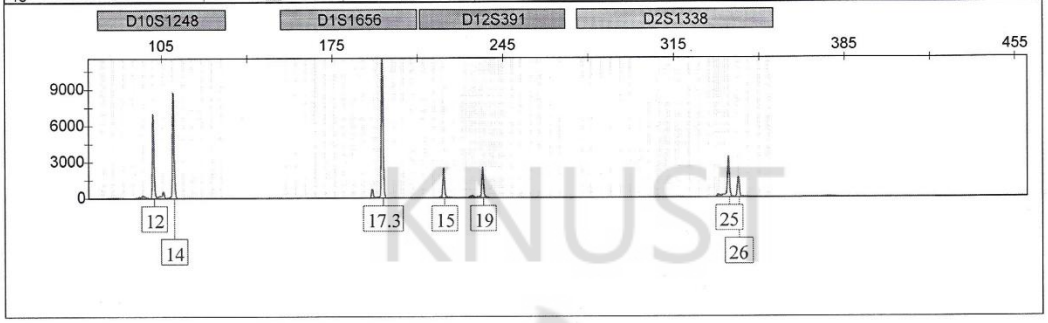
Printed by: gmidx

Page 1 of 2

Applied Biosystems
GeneMapper® ID-X 1.5

Project: 2017-04-18 Repeat Run

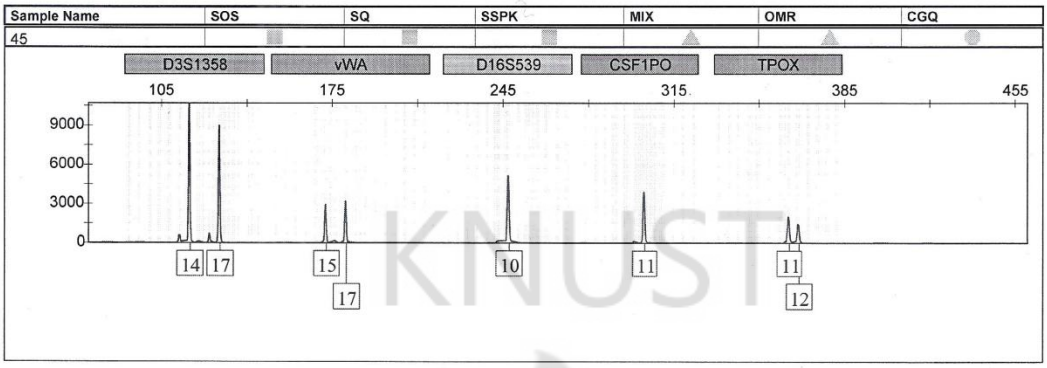
Sample Name	SOS	SQ	SSPK	MIX	OMR	CGQ
43						

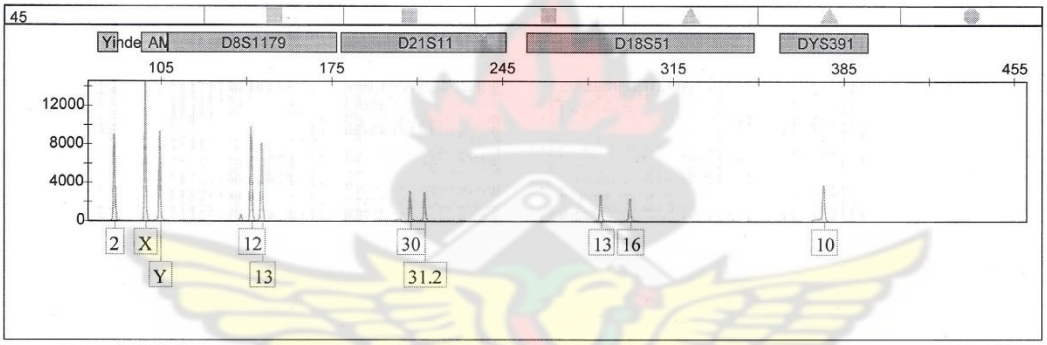


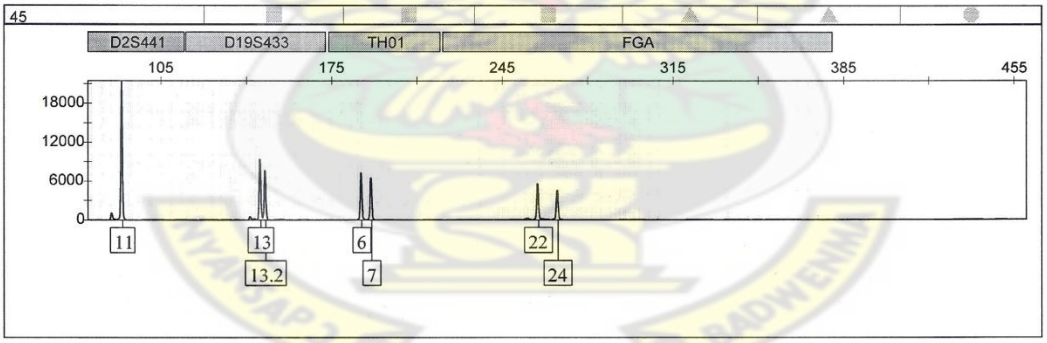
Applied Biosystems
GeneMapper® ID-X 1.5

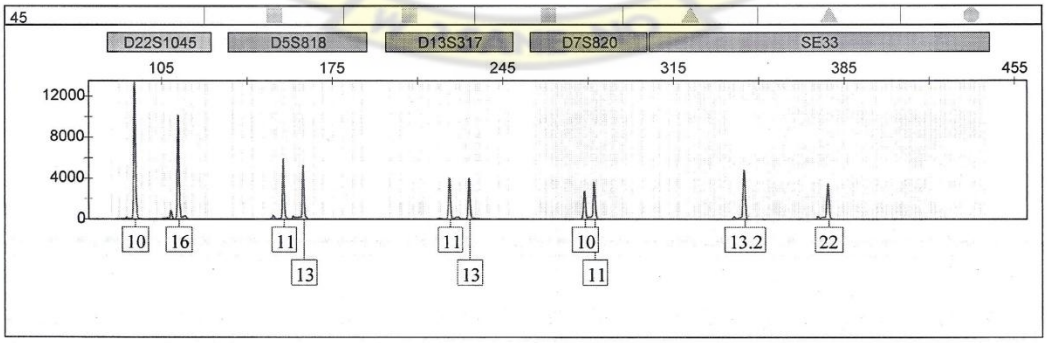
Project: 2017-04-18 Repeat Run

Sample Name	SOS	SQ	SSPK	MIX	OMR	CGQ









Sun Mar 26,2017 02:07PM, BST

Printed by: gmidx

Page 1 of 2

Applied Biosystems
GeneMapper® ID-X 1.5

Project: 2017-04-18 Repeat Run

Sample Name	SOS	SQ	SSPK	MIX	OMR	CGQ
45