COMPARISON OF THE THREE FORENSIC DNA SAMPLING AND EXTRACTION TECHNIQUES ON VARIOUS MOCK CRIME SCENE SAMPLES FOR RELIABLE AND RAPID DNA ANALYSIS

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COMPARISON OF THE THREE FORENSIC DNA SAMPLING AND EXTRACTION TECHNIQUES ON VARIOUS MOCK CRIME SCENE SAMPLES FOR RELIABLE AND RAPID DNA ANALYSIS

by

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Thesis submitted in partial fulfilment of the requirements
for the degree of
Master of Science (Forensic Science)

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CERTIFICATE

This is to certify that the dissertation entitled "Comparison of the three Forensic DNA Sampling and Extraction Techniques on various Mock Crime Scene Samples for Reliable and Rapid DNA Analysis" is the bona fide record of research work done by Ms. Glenna Tan Jie Yee (P-SKM 0149/21) during the period from February 2022 to August 2022 under my supervision. I have read this dissertation and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation to be submitted in partial fulfilment for the degree of Master of Science (Forensic Science).

Supervisor,

……

(DR. NUR WALIYUDDIN HANIS BIN ZAINAL ABIDIN)

Date: 26th September 2022

DECLARATION

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

……..

(GLENNA TAN JIE YEE)

Date: 7th September 2022

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LIST OF SYMBOLS

®	Sum
µL	Microliter
µM	Micromolar
bp	Base pair
C	Celcius
dNTP	Deoxynucleoside triphosphate
kV	Kilovolt
min	Minute
mM	Millimolar
mm	Millimeter
n	Number of samples
ng	Nanogram
s	Second
™	Company trademark
U	Unit
v	Software version

LIST OF ABBREVIATIONS

A	Adenine
ABI	Applied Biosystems
AL	Allelic ladder
APH	Average Peak Height
AT	Analytical threshold
ATP	Adenosine Triphosphate
C	Cytosine
CE	Capillary electrophoresis
CW	Sample ID for Casework Direct System DNA extraction kit
CODIS	United States America Combined DNA Index System
ddH_2O	Deionized distilled water
DNA	Deoxyribonucleic Acid
DVI	Disaster Victim Identification
ENFSI	European Network of Forensic Institutes
EPG	Electropherogram
FDDM	Forensic DNA Databank of Malaysia
G	Guanine
GA	Genetic Analyzer
GFE	GlobalFiler™ Express PCR Amplification Kit
HgH	Human Growth Hormone
INTERPOL	The International Criminal Police Organization
KIMIA	Department of Chemistry Malaysia
MF	Sample ID for COPAN MicroFLOQ® Direct Swab
MX	Sample ID for Maxwell RSC 48 FSC DNA IQ Casework Kit

OL	Off ladder
PC	Positive Control
PCR	Polymerase Chain Reaction
RFU	Relative Fluorescence Unit
RSC	Rapid Sample Concentrator
RMP	Random Match Probability
STR	Short Tandem Repeat
SWGDAM	Scientific Working Group on DNA Analysis Methods
T	Thymine
USM	Universiti Sains Malaysia

PERBANDINGAN TIGA TEKNIK PERSAMPELAN DAN EXTRAKSI DNA PADA PELBAGAI SAMPEL PALSU KEJADIAN JENAYAH UNTUK PEMPROFILAN DNA YANG PANTAS DAN BOLEH DIPERCAYAI.

ABSTRAK

Keupayaan untuk menjana profil DNA yang boleh dipercayai (*reliable*) dan pantas melalui analisis tandem pendek berulang (STR) dapat membantu dalam penyiasatan jenayah melalui pengenalpastian identiti suspek. Kajian ini menilai prestasi kompilasi teknik persampelan dan ekstraksi yang terdiri daripada kit Maxwell RSC 48 FSC DNA IQ Casework, Casework Direct System dan COPAN MicroFLOQ® Direct Swab. Sebanyak 48 tindak balas menggunakan 16 sampel palsu kejadian jenayah untuk setiap teknik yang terdiri daripada sampel darah, air liur dan sampel DNA sentuhan yang biasa ditemui di tempat kejadian telah digunakan dalam kajian ini. Kuantiti sampel DNA yang diekstrak menggunakan kit Maxwell RSC 48 FSC DNA IQ Casework dikira menggunakan Spektrofotometer NanoDrop™ 2000. Selepas itu, sampel yang dikumpul menggunakan ketiga-tiga teknik diamplifikasi menggunakan kit GlobalFiler™ Express PCR Amplification. Produk tersebut kemudiannya dimuatkan ke ABI 3500xL Genetic Analyzer untuk elektroforesis kapilari sebelum dianalisis menggunakan perisian GeneMapper ID-X v1.4. Keputusan menunjukkan bahawa tujuh daripada 16 sampel berjaya dianalisis. Ketiga-tiga teknik tersebut menghasilkan panggilan alel STR autosomal lengkap sehingga 96% - 100%. Sementara itu, purata tinggi puncak alel (average allele peak height) menggunakan Maxwell RSC 48 FSC DNA IQ Casework Kit adalah yang tertinggi disebabkan proses penulenan automatik yang dijalankan berjaya mengestrak DNA dengan kepekatan yang tinggi. Dari segi masa dan kos analisis, COPAN MicroFLOQ® Direct Swab

mengatasi dua teknik lain diikuti dengan pengekstrakan Casework Direct System yang mengambil masa satu jam. Sementara itu, kit Maxwell FSC DNA IQ Casework mengambil masa pengekstrakan yang lebih lama dan kos yang lebih tinggi (1 jam 30 minit dan purata RM 76.15 setiap tindak balas). Tambahan pula, penggunaan kit tersebut perlu digunakan bersama instrumen Maxwell RSC 48 automatik yang bernilai RM 450,000 setiap instrumen adalah jauh lebih mahal berbanding dua teknik yang lain. Secara keseluruhan, ketiga-tiga teknik yang berbeza mempunyai kelebihan dan kelemahan tersendiri tetapi COPAN MicroFLOQ® Direct Swab mempunyai kelebihan berbanding dua teknik lain dari segi masa, keberkesanan kos dan kemudahan untuk digunakan. Teknik tersebut menawarkan banyak kelebihan kerana sifat amplifikasi PCR langsungnya, keupayaan pengesanan yang agak tinggi dan profil DNA berkualiti yang dihasilkan. Hal ini menyebabkan COPAN MicroFLOQ® Direct Swab mampu menjana profil DNA dalam masa yang singkat dan sekali gus berpotensi menjadi teknik persampelan yang dipilih oleh pegawai penguatkuasa undang-undang.

COMPARISON OF THE THREE FORENSIC DNA SAMPLING AND EXTRACTION TECHNIQUES ON VARIOUS MOCK CRIME SCENE SAMPLES FOR RELIABLE AND RAPID DNA ANALYSIS.

ABSTRACT

The capability to generate reliable DNA profiles rapidly via short tandem repeat (STR) analysis to identify the suspect could greatly assist in crime investigations. This study evaluated the performance of a compilation of sample collection and extraction techniques consisting of the Maxwell RSC 48 FSC DNA IQ Casework Kit, Casework Direct System, and the COPAN MicroFLOQ® Direct Swab. A total of 48 reactions consisting of 16 mock casework samples for each technique ranging from bloodstain, saliva stained and touch DNA samples that were commonly encountered in crime scene were used in this study. The samples extracted using the Maxwell RSC 48 FSC DNA IQ Casework Kit were quantified using the NanoDrop™ 2000 Spectrophotometer. Subsequently, the collected samples for the three techniques were amplified using the GlobalFiler™ Express PCR Amplification Kit. The amplified products were then loaded for capillary electrophoresis via the ABI 3500xL Genetic Analyzer before analyzed using the GeneMapper ID-X v1.4 software. Results demonstrated that the three techniques generated relatively high percentage of autosomal STR allele call in total (100%, 100% and 96% respectively). Particularly, the COPAN MicroFLOQ™ with 96% was possible to analyze wide range of DNA samples where seven out of 16 samples were typed successfully. Meanwhile, the same number of sample types (n=6) with 100% autosomal STR allele call percentage and full consistent profiles were generated via the extraction using the two extraction kits. Notably, the average peak height across the samples using the former extraction kit

was the highest which attributed to the automated extraction and purification employed that corresponded to the high DNA concentration yielded. This were followed by Casework Direct System and COPAN MicroFLOQ . In terms of turnaround time and cost associated, the MicroFLOQ® Direct Swab outperfomed the other two techniques followed by the Casework Direct System extraction which took one hour for complete extraction. Meanwhile, the Maxwell FSC DNA IQ Casework Kit which took a longer time and higher cost for the entire extraction (1 hour 30 minutes and average RM 76.15 per reaction respectively). Not to mention, the use of the kit coupled with the automated Maxwell RSC 48 instrument (RM 450, 000 per instrument) were on the more expensive end as compared to the other two techniques. Overall, the three different techniques had their respective merits and pitfalls but the COPAN MicroFLOQ® Direct Swab had an edge over the other two methods in terms of time, cost-effectiveness and ease to use. It also offered numerous advantages due to its direct PCR amplification properties, relatively high detection ability and quality DNA profiles produced. This resulted in the COPAN MicroFLOQ® Direct Swab capable to generate DNA profiles reliably in a short time thus potentially become a novel preferable collection technique employed by law enforcement officers.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Deoxyribonucleic acid or also known as DNA is undeniably a powerful crime solving tools nowadays. Ever since the discovery of DNA profiling technique by Sir Alec Jeffreys in 1985 and the collective efforts by pioneer researchers, it has greatly assisted in the forensic investigations based on the core principle that no two individuals have the same DNA, except for identical twins (Gill *et al.*, 1985). This makes this unique biological molecule highly distinguishable in terms of identifying the source of evidence collected and thus the identity of the contributor. In Malaysia, the prominent role of DNA had been seen in cases that shocked the nation like the Canny Ong's murder (Zabdi *et al.*, 2008) and Noritta Samsudin's case (Ahmad *et al.*, 2013). It had resulted either in the conviction of the actual perpetrator or the acquittal of the innocent individual from the crime that they did not commit.

Related to that, the short tandem repeat (STR) which is a highly variable genetic marker is being used extensively in Forensic DNA profiling. It offers great discrimination power and is considered as the gold standard in human identification which greatly aid in criminal justice through the identification of the perpetrator's identity (Kayser, 2015; Udogadi *et al.*, 2020). Based on the standard DNA typing protocol by the Scientific Working Group of DNA Analysis (SWGDAM), the primary step in Forensic DNA profiling is the DNA extraction process before proceeding with quantification, separation and STR analysis. This particular process involves the isolation of DNA from the biological material which facilitates the subsequent downstream analysis for generating quality DNA profiles. The current DNA extraction process include either the conventional manual method or in the form of

commercialized kits, where both have their respective merits and pitfalls during analysis.

Starting from the conventional method, the organic (phenol-chloroform) extraction method is the gold standard for DNA extraction and has been widely used to extract DNA for the longest period of time (Butler, 2014; Dairawan & Shetty, 2020; Bukyya *et al.*, 2021). This is mainly attributed to the method's high sensitivity and the capability of extracting DNA from a wide variety of biological samples. However, the main shortcoming of this manual method is the use of toxic reagents (phenol and chloroform) during the extraction process. Plus, the process is rather time-consuming and laborious where it might take up to three days merely for the extraction process of each samples. This undeniably poses a challenge to the contemporary problems faced by the workforce involved in forensic DNA profiling such as the high laboratory workload due to the great amount of casework samples received daily, lack of trained analysts and budgetary constraints.

All of this has led to the increase use of the commercial DNA extraction kits following the demand for a rapid and less complex DNA extraction method to handle the high workload capacity, and reduce the turnaround time and backlog in the forensic laboratories. Consequently, in this current research project, two DNA extraction techniques from Promega Corporation, (1) Casework Direct System and (2) Maxwell® Forensic Sample Concentrator (FSC) DNA IQ™ Casework kit were used to study the quality of DNA profiles when dealing with simulated casework samples particularly.

The Casework Direct System (Promega Corporation, USA) adopts a no-wash protocol where DNA could be extracted rapidly and produce amplification-ready lysate in a

relatively shorter time (Graham, 2018). On the contrary, the Maxwell® FSC DNA IQ™ Casework kit which employed the automated nucleic acid purification method using the Maxwell® Rapid Sample Concentrator (RSC) 48 machine can produce clean DNA samples for the downstream analysis following multiple washing process and the use of paramagnetic particles that facilitate DNA binding. Additionally, the automated Maxwell® RSC 48 System is very convenient and offers high throughput where it can process the extraction of 48 samples simultaneously within 30 minutes.

On top of that, following the advancement of technology in the forensic DNA field it has led to the emergence of the microFLOQ™ Direct Swab capable of direct amplification after collection. This has greatly speed up the forensic DNA profiling process as it is possible to bypass the DNA extraction and quantification step using this new technique. The capability of direct amplification for this particular technique is mainly attributed to the lysing agent present on the nylon-flocked swab head where DNA could be lysed directly. In addition, the relative small size of the swab head (approximately 1 mm in diameter) also resulted in more convenient sampling from difficult areas like narrow seams. Plus, it involves less-sample consumption thus allowing the preservation of greater portion of valuable forensic samples for further testing if desired in the future (Ambers *et al.*, 2018; Chong *et al.*, 2019).

Therefore, in this research, the three different Forensic DNA technique, namely the Casework Direct System, Maxwell® FSC DNA IQ™ Casework Kit on the Maxwell® RSC 48 System, and the COPAN MicroFLOQ™ Direct Swab were used to analyse the DNA profiles of the mock crime scene samples. The extracted DNA samples were amplified using the GlobalFiler™ Express PCR Amplification Kit. To the best of our knowledge, there is no study that evaluated the performance of the aforementioned

techniques, particularly in terms of the quality of DNA profiles generated which able to provide reliable DNA profile from a variety of mock crime scene samples.

1.2 Problem Statement

Any novel method proposed need to be well validated before adopting as the protocol to be used for law enforcement agencies. DNA analysis techniques that are capable to generate a reliable DNA profile of crime scene related samples in a short time would be deemed useful for Forensic DNA analysts and beneficial in the crime investigation process as well. The profiles generated could be quickly matched with the reference samples analysed by the Malaysia's Forensic DNA Databank (FDDM) which would help to save significant time by reducing the need of passing information through different departments and thus identify the perpetrator's identity rapidly.

Therefore, in this research, mock crime scene samples were used and processed using the three different Forensic DNA techniques, namely the Casework Direct System (Promega), Maxwell® FSC DNA IQ™ Casework Kit on the Maxwell® RSC 48 System, and the COPAN MicroFLOQ™ Direct Swab. To the author's knowledge, there is still no study that compared the performance of all these three techniques simultaneously and amplified using the GlobalFiler Express Kit which is the amplification kit often used with reference or single-sourced samples. Hence, this research evaluated the performance of the aformentioned techniques, particularly in terms of the quality of DNA profiles generated , turnaround time and cost associated.

1.3 Objectives

1.3.1 General Objective

This research aims to evaluate the performance between different DNA sample extraction and collection methods namely the Casework Direct System (Promega), Maxwell® FSC DNA IQ™ Casework Kit using the Maxwell® RSC 48 system, and COPAN MicroFLOQ Direct Swab in generating reliable DNA profiles from various mock crime scene samples.

1.3.2 Specific objectives

i. To study the quality of DNA profile generated by different DNA extraction and collection methods.
ii. To determine the autosomal STR allele call percentage based on the profiles yielded by different DNA extraction and collection methods.
iii. To compare the turnaround time and cost associated using the different DNA extraction and collection methods.

1.4 Significance of Study

This study would help to determine which DNA analysis technique is capable to generate a reliable DNA profile in shorter time and reduced cost. This is achieved through the evaluation of a compilation of Forensic DNA techniques ranging from various sampling, extraction techniques and then amplified using GlobalFiler™ Express PCR Amplification kit. To be particular, this is done through comparison of the performance between the three Forensic DNA techniques, namely the Casework Direct System (Promega), Maxwell® FSC DNA IQ Casework Kit on the Maxwell®

RSC 48 System, and the COPAN MicroFLOQ Direct Swab on a wide range of forensic-related samples. This is because evaluation and validation are required to be carried out prior applying on the actual samples collected by the law enforcement agencies. Therefore, this research would be beneficial to the law enforcement agencies by providing reference and guidelines regarding which aformentioned technique is capable to provide DNA profile in terms of the quality of DNA profiles, turnaround time and cost associated of the selected technique used.

CHAPTER 2

LITERATURE REVIEW

2.1 Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid was first discovered in 1869 but it's importance in forensic investigations was first realized by Alec Jeffrey and its double-helix structure described using the Watson and Crick model (Bukyya *et al.*, 2021). Cell is the basic unit of life in human and DNA which is mostly located in the cell's nucleus stores vital genetic information that is capable to pass down from one generation to the other. It is made up of nucleotides which consist of sugar, phosphate group and nitrogen bases. The nitrogen bases include Adenine (A), Guanine (G), Thymine (T) and Cytosine (C). The discovery of DNA particularly on its significance in assisting forensic investigations had revolutionized the criminal justice system (Panneerchelvam & Norazmi, 2003; and Butler, 2010).

The first use of DNA fingerprinting dated back to 1987 in the Colin Pitchfork case. The case revolved around the rape-murder of two girls happened during 1983 and 1986 respectively. This was a good example of the use of DNA fingerprinting technology, because it first exonerated the innocent individual, Richard Buckland then convicted the actual perpetrator, Colin Pitchfork. Initially, Richard Buckland was one of the suspect for the case and had decided to turn in due to public pressure.

However, DNA had excluded him as the perpetrator as his DNA samples did not match with the semen samples present in the crime scene. Instead, it matched with an individual called Colin Pitchfork after a witness heard that he asked his friend to provide blood sample on his behalf during the sample collection when no suspect was found. At

last he finally confessed to the crime he committed after presented with the DNA evidence that matches with his DNA thus making him the first person being convicted using DNA profiling.

2.2 Forensic DNA Profiling in Malaysia

Forensic DNA profiling can help to link a perpetrator to a crime involve or exonerate innocent individuals from a crime that they did not commit by using autosomal short tandem repeat (STR). It involves side-by-side comparison thus reference samples are usually collected from known individuals before comparing with that from the crime scene in order to proclaim a match and know the source of identity for the crime scene samples.

In Malaysia, the law enforcement agencies namely the Royal Malaysia Police (RMP) DNA Databank Laboratory and the Chemistry Department of Malaysia (KIMIA) have the jurisdiction to analyse and store DNA profiles legally in accordance to the DNA Identification Act 2009 (Act 699) and the DNA Identification and Regulation Act 2012. In this regard, there were seven indices stated in Section 3(3) of the aforementioned act which include crime scene, suspected person, convicted offenders, detainees, drug dependants, missing persons and voluntary index. Crime scene and paternity kinship samples were usually sent to KIMIA for analysis whereas the remaining indices which considered the reference samples such as that from suspected person, detainees, drug dependant and missing persons were often analyzed by the RMP DNA Databank Laboratory (Hakim *et al.*, 2020).

Nevertheless, both the agencies collaborated together and mutually shared the information through the DNA profiles uploaded from those samples into the DNA database from time to time. Since the official establishment of the national DNA database or also well-known with the name of Forensic DNA Databank Malaysia (FDDM) at the year of 2015, the number of successful matches had increased significantly following the large number of DNA profiles stored in the database. This greatly improves the matching rates as compared to the time before, where matching was performed merely by KIMIA on a case-to-case basis, which often faced challenges in scenarios especially when there is no suspect (Abdul Rahman *et al.*, 2021). Based on the statistics from Royal Malaysia Police DNA D13, Bukit Aman, there is up to 186 hits (DNA matches) out of the 207,581 total DNA profiles stored in FDDM as of 15th May 2022.

Additionally, the great success matching rate could also attributed to the extensive use of the autosomal STR profiling in the forensic DNA analysis of our country.The amplification of STR loci which offers high discriminating power had resulted in it preferably used by the law enforcement agencies in Malaysia during genotyping for human identification testing. This highly polymorphic genetic marker which comes in small repetitive units (2-6 base pairs) greatly varies between different individuals thus offer unique identification.

Currently, the GlobalFiler™ Express PCR Amplification Kit which contain additional 8 loci were employed by the law enforcement agencies as compared to the previously used AmpFiSTR Identifiler® PCR Amplification kit that amplifies 16 loci during their standard analysis protocol since the year of 2017. The increase in the STR loci used

also indirectly leads to the increase in the discrimination power of the profiles generated (Hakim *et al.*, 2020).

The four main steps of the DNA profiling is inclusive of extraction, quantitation, amplification and capillary electrophoresis. DNA extraction is the initial step in DNA analysis where it involves the process of releasing the DNA from the cell. The extracted DNA will then proceed with the quantitation steps in which the quantity of the DNA will be determined. However, according to the paper by Francisco *et al.* (2020) the quantification of DNA does not necessary equates to the success in generating STR profiles especially when dealing with 'Touch' DNA that consist of low quantity of DNA.

Therefore, the amplification step is crucial during DNA analysis particularly with casework samples that are mostly degraded. There will be multiple copies produced from the extracted DNA. Then, the amplified DNA will be separated through capillary electrophoresis to allow for subsequent identification. Last but not least, the analysis and interpretation step will be carried out where the DNA profiles from the crime scene samples will be compared to the known DNA profiles stored in the database.

2.2.1 Short tandem repeat (STR) markers

Short tandem repeats (STR) or also known as microsatellites had been widely used in the current forensic DNA technology due to their polymorphic nature. It is a popular DNA markers that can be amplified easily by polymerase chain reaction (PCR) because of its small size. It consist of short repeated sequences of up to 2 – 6 base pairs (bp) and is mostly located in the noncoding region.

Furthermore, STR is greatly variable among individuals, this leads to STR capable to offer high power of discrimination during DNA analysis. Research studies have shown that the more STR loci is being used, the greater the probability in discriminating between the profiles of two individuals (Udogadi *et al.*, 2020).

2.3 GlobalFiler™ Express PCR Amplification Kit

The Applied Biosystems™ GlobalFiler™ Express PCR Amplification Kit (GFE, Thermo Fisher Scientific) is a mutiplex PCR assay with 6 dyes and capable to amplify up to 24 loci, including 21 autosomal STR loci, 1 Y-indel, 1 DYS391 and 1 Amelogenin sex-determining marker. This PCR amplification kit is optimized to amplify human genomic DNA from reference DNA samples that often contain high content, single-source DNA such as that of buccal swab, blood on FTA card and etc.

Nevertheless, previous studies had showed the feasibility and quality DNA profiles generated through the usage of GFE coupled with other techniques on various mock and casework samples. The study by Ambers *et al.* (2018) had evaluated the feasibility of the microFLOQ® Direct Swab to amplify bloodstain, saliva stained and touch samples directly using the GFE. Their results demonstrated that it is possible to yield DNA profiles from samples that contain limited amount of DNA using sample collection via microFLOQ® swab and direct amplification with GFE.

In addition, Hakim et al. (2019) had employed GFE in the STR amplification stage of their study to assess the performance of autosomal STR profiling between the two DNA extraction kits namely, the Casework Direct System and the Maxwell 16 System DNA IQ Casework Pro Kit. Their samples involved actual casework samples as well such as

swab from knife handle, cigarette butt, tissue, and plastic straw. They managed to obtain informative DNA profiles through the usage of GFE for amplification in order to compare the performance between the two aforementioned extraction kits.

2.4 Maxwell ® Rapid Sample Concentrator (RSC) 48 System

According to the technical information by Promega (2022), the Maxwell® Rapid Sample Concentrator (RSC) system is an automated nucleic acid purification platform that uses the DNA IQ™ chemistry. It is compact and the instrument occupies minimal bench space. For the Maxwell® RSC 16 instrument it is capable to perform extraction of 16 samples simultaneously whereas the Maxwell RSC 48 instrument can extract up to 48 samples at one time.

The Maxwell® RSC system is mainly used for research purposes whereas the Forensic Sample Concentrator (FSC) system is usually meant for forensic purposes. Unlike the Maxwell® FSC instrument that can only store one system, the Maxwell® RSC instrument can store both systems including the RSC and FSC system. Hence, in our project, the Maxwell® RSC 48 instrument that is equipped with the Maxwell® FSC system is used for the study.

Despite the Maxwell® RSC 48 system is a relatively new model as compared to the Maxwell® 16 system, where the new model now comes along with a surface tablet and modern intuitive interface. It is still capable to provide the same great performance in extracting clean DNA samples that will facilitate the subsequent STR analysis. The instrument which uses the technology of magnetic particle mover can help to reduce the risk of cross-contamination.

Previous studies by Dumache *et al.* (2019) had also recorded the use of the Maxwell® RSC 16 instrument in their research where the DNA extracted using the Maxwell® RSC Whole Blood DNA Kit and Maxwell® RSC Buccal Swab DNA Kit were performed by the automated Maxwell® RSC 16 instrument.

In addition, another study by Dumache *et al.* (2021) had performed genetic DNA identification from bone remains in kinship analysis by using automated extraction system. They extracted the reference DNA samples from the deceased's relative using the Maxwell® FSC DNA IQ™ Casework Kit on the automated Maxwell® RSC 48 instrument. This shows the capability of Maxwell® RSC instrument with the FSC system in various forensic-related applications.

2.4.1 Maxwell® Forensic Sample Concentrator (FSC) DNA IQ™ Casework Kit

The Maxwell® Forensic Sample Concentrator (FSC) DNA IQ™ Casework Kit is capable to provide extraction and purification of nucleic acids automatically for a wide range of casework samples. Similar to the Maxwell® RSC system mentioned previously, it also uses the DNA IQ™ chemistry to purify crime scene samples that often comes with low abundance of DNA.

The Maxwell® FSC DNA IQ™ Casework Kit comes along with the lysis buffer, resin and wash buffer contained in the prefilled cartridges. The DNA IQ ™ Resin is used to purify the DNA thus maximized the yield of DNA and purity for the STR analysis later.

Spin baskets and microfuge tubes that were easy to use and can mimimize the risk of cross-contamination were also equipped with the kit. However it is to bear in mind that, the use of the Casework Extraction kit for sample preprocessing is required before carrying out the DNA extraction step in order to increase the efficiency of DNA extraction later (Graham *et al.*, 2020).

Plus, the Maxwell® FSC DNA IQ™ Casework Kit is also compatible with the Maxwell® FSC or Maxwell® RSC instruments thus making it very convenient to use for automated extraction methods. When use with the Maxwell® instruments , the magnetic particle handling instrument will efficiently mixed the DNA IQ™ Resin with the purification reagents in the prefilled cartridges which reduces hands-on time and greatly reduces the risk of potential contamination.

Loten *et al.* (2018) had performed a validation study by using the Maxwell® FSC DNA IQ™ Casework Kit on the Maxwell® FSC Instrument to extract genomic DNA from forensic biological samples. To be particular, the samples used in their study include blood, saliva, buccal swabs and semen samples, which were often encountered in crime scenes. The result of their study displayed that the aforementioned kit could produce reproducible, high purity and quality of DNA from a variety of casework samples after evaluated with a set of experiments and conducted under the recommended validation standards and guidelines.

2.4.2 DNA IQ™ System

The DNA IQ™ System under Promega Corporation (Madison, WI) which stands for isolation and quantitation, is designed specifically for forensic and paternity purposes.

This system is also one of the solid-phase extraction methods where it employs novel paramagnetic particles for an easy and efficient DNA extraction, The silica-coated paramagnetic resin is used for DNA binding and elution in the DNA IQ™ system which will result in the nucleic acids selectively absorb to the silica support (Promega, 2016).

Firstly, the DNA molecules are bound to the magnetic particles reversibly in an acidic pH solution (< pH 7.5). Next, the silica-coated magnetic beads is drawed by the magnet to the bottom of the tube and the impurities left in the solution can be removed. Multiple washing is then performed to clean the DNA that bind to the magnetic particles. Finally, the tube is heated for a few minutes in order to release a definite amount of DNA into the solution and the isolation process is completed (Butler, 2010).

Hence, the quantity of DNA isolated for this approach is dependent on the number and capacity of the magnetic particles used. However, considering centrifugation steps are unrequired for this approach, the magnetic bead procedures of this system enables simple, rapid DNA isolation which is also compatible to use with other automated methods, such as the Maxwell RSC or FSC system.

Numerous studies had showed that DNA IQ™ system which employed the paramagnetic resin technology is capable to perform a better DNA extraction as compared to the conventional extraction methods. This is showed in the study by Frégeau *et al.* (2010) where it shows that the DNA IQ™ can provide better DNA profiles than the organic phenol/chloroform extraction method. In addition, the paper by Ip *et al.* (2015) also demonstrated that DNA extracted using IQ has higher successs rate than the Chelex® 100 for the subsequent DNA analysis. This leads to DNA IQ™ system is widely used in forensic laboratories around the world including that in Brazil

(Francisco *et al.*, 2020). However, in Malaysia, the DNA IQ™ system is yet to be adopted in any of the law enforcement agencies in analysing casework samples.

2.5 Casework Direct System

The Casework Direct System (formerly known as Caswork Direct Kit, Custom) which is also under Promega Corporation (Madison, WI) is a relatively new DNA extraction kit developed for forensic purposes. It comes along with a buffer and reducing agent just as other relatively new extraction methods. However, the unique feature of this extraction kit which makes it stand out from the former extraction methods is that, it adopts a no-wash protocol which minimizes the risk of DNA loss during the washing process. This resulted in this extraction kit to potentially emerged as a powerful extraction tool for the retrieval of touch DNA samples.

Based on the Promega's developmental validation paper by Graham et al.(2020), the Casework Direct System is capable to produce DNA lysates from a wide variety of forensic casework samples, ranging from semen, blood stain to touch DNA that usually contain minute amount of DNA. Furthermore, the author also stated that their study proved that this extraction kit is a suitable, accurate and reproducible method for the rapid isolation of DNA.

Plus, the aforementioned extraction kit can generate the lysates ready for amplification rapidly within 35 minutes. This can helps to save the time required for analysis and also makes it an ideal kit to process low abundance DNA samples, particularly on casework 'touch DNA' samples or on sexual assault evidence due to the chance of recovering minute amount of DNA will be improved tremendously with the usage of this extraction

kit. This is greatly attributed to no multiple washing procedures were adopt which could potentially maximized and retain the original amount of nucleic acid present to samples that initially contain limited quantity of DNA, such as that of touch DNA.

This is supported by Francisco *et al.* (2020) where they compared the efficiency of the two extraction kits, namely the DNA IQ™ system and the Casework Direct System, on touch DNA samples by simulating 104 crime scene experiments. Their study concluded that the Casework Direct System is much more efficient than the DNA IQ™ system when it comes to process the touch DNA samples. To be particular, the percentage of Casework Direct System in obtaining useful STR profiles is 98.1% as compared to that extracted using DNA IQ™ system (61.5%) in their research.

Nevertheless in the paper by Hakim *et al.* (2019), they mentioned that the Maxwell 16 System DNA IQ Casework Pro Kit can produce better results than the Casework Direct Kit after comparison. However, the authors suggested that Casework Direct System is still a better option in terms of the extraction of reference samples which is usually clean and contain relatively high amount of DNA as compared to the crime scene samples. On top of that, Francisco *et al.* (2020) also recommended in their paper that more studies have to be carried out in order to verify the ability and performance of the relatively new Casework Direct System in recovering touch DNA under different scenarios.

2.6 COPAN MicroFLOQ® Direct Swab (MF)

COPAN MicroFLOQ® Direct is a DNA collection tool that enables direct amplification of DNA collected from crime scene samples. This innovative development as a result of the collaboration between the French Gendamerie Forensic Research Institute

(IRCGN ™) and COPAN contains fibers that comes with lysis treatment which allows it to the skip the DNA extraction step of the standard typing protocol (COPAN 2022). Related to that, it is capable to perform rapid DNA processing of forensic-related samples and obtain DNA profiles with 24 markers within 2 hours.

Furthermore, the relative small diameter of the microFLOQ® also offers several advantage during on-site collection as compared to that of the traditional swabbing method. The study by Ambers *et al.* (2018) mentioned that microFLOQ® is the miniaturised version of the 4N6FLOQSwabs™ which consist of short nylon fibers arranged perpendicularly at the tip of the shaft and with the addition of the lysing agent on the swab head. The small-sized swab head which is around 1 mm in diameter is capable to retrieve DNA from areas that are difficult to access or from narrow seams such as floor crackings. Plus, the small sampling area involved also will result in less sample consumption thus preserving more samples available for further testing if required in the future.

All of these features had resulted in microFLOQ® Direct emerged as a potential rapid and efficient on-site DNA collection tool on a variety of biological samples. The same paper by Ambers *et al.* (2018) had also evaluated the efficacy of direct amplication of the microFLOQ® Direct swabs with the GlobalFiler™ Express system on human bloodstains, saliva and touch samples. The results were then compared it with the traditional method which is via manual extraction. The study demonstrated that the human DNA samples collected using the microFLOQ® swabs are capable to produce better results than the conventional extraction workflow.

In addition, the study by Chong *et al.* (2019) compared the microFLOQ™ workflow with that of the standard workflow, which used the DNA IQ™ chemistry on the Mawell® 16 for DNA extraction, followed by the quantification, amplification and capillary electrophoresis steps. The results displayed that the average peak height generated using the direct amplification of the microFLOQ™ Direct swab produce comparable or better results with that of the standard workflow. Therefore, it is indicated that the microFLOQ™ workflow has great potential to be used to obtain DNA profiles rapidly and reliably from casework samples.

Moreover, Sherier *et al.* (2019) had examined the capability of the microFLOQ® Direct swab to collect minute amout of DNA from challenging substrate such as on porous materials. The DNA collection from porous surface like cotton cloth usually posses additional difficulties due to the biological fluid will be absorbed into the porous surface and trapped within the fibrous matrix. Despite the challenges present, the result of their study demonstrated that microFLOQ® Direct swab is capable to retrive DNA collected from bloodstains, saliva and semen on cotton cloth.

Consequently, Hazirah *et al.* (2020) had conducted a preliminary evaluation regarding the optimum PCR cycle number and volume of GlobalFiler™ Express Kit (GFE) required for the microFLOQ™ workflow on buccal swab and blood samples. They found out that a minimum of 8µl GFE and 25 cycles and 27 cycles were required to generate a full DNA profile for buccal and blood samples respectively using the direct amplification of the microFLOQ™ workflow. This optimization performed would be beneficial particularly for the DNA laboratories that are planning to incorporate the microFLOQ™ workflow on the aformentioned biological samples collected.

Another comparative study were also carried out by Loockerman *et al.* (2021) where the microFLOQ™ Direct swab was coupled with the QIAGEN Investigator QS GO! Kit and compared with the traditional methods using 4N6FLOQSwabs. Their samples focused on the DNA swabbed from decomposing human remains which is very common during disaster victim identification (DVI) and often required rapid identification. Their study concluded that the use of microFLOQ ™ swabs with Investigator 24Plex GO! Kit could facilitate DVI process by providing rapid DNA profiling from samples collected from decomposing human remains.

This is also supported by Castriciano *et al.* (2022) which concluded that rapid DNA profiling of crime scene samples is facilitated using microFLOQ™ which greatly help in identifying the victim or perpetrator's identity in a shorter time. In a nutshell, the microFLOQ™ Direct swab that is capable for direct amplification enabled the elimination of purification and extraction steps thus reduces the time required and cost associated. This indirectly will also improve the laboratory performance that usually have to handle a high laboratory workload capacity.

2.7 Touch DNA

One of the famous quote in Forensic Science is that by Sir Edmund Locard which stated that 'every contact leaves a trace', Related to that, touch DNA which can also be referred as contact DNA is defined as the trace evidence left at the crime scene through handling or in contact with an item. This is attributed to the constant shedding of epithelial cells from human skin through daily activities (Templeton *et al*., 2015; Sessa *et al.*, 2019). The challenges in recovering touch DNA often lies in the insufficient

amount of DNA available to yield DNA profiles. Despite there are often present in low amount, they could played a significant role particularly in cases where no other biological fluid is available at the scene such as that during property crime (Bonsu *et al.*, 2020).

Furthermore, since the generation of the first DNA profile from touch DNA in 1997 (Templeton *et al.*, 2017). There were numerous research that had studied the recovery rate of touch DNA from various substrates using multiple methods. Cavanaugh and Bathrick (2018) had reviewed the success rates on the direct PCR amplification of forensic touch and challeging DNA samples. They stated that some items might be easier to recover touch DNA as compared to the others.

This is also supported by Dziak *et al.* (2018) which stated that samples such as socks, fabric gloves, eyeglasses and headwear had a higher success rate during the analysis of trace DNA samples. They also mentioned that the STR profiling from trace DNA samples that originate from a single source which means samples that often used by single user such as that of socks or headwear will be easier to yield quality profiles as compared to items that frequently reachable by other people which would result in mixtures.

Related to that, the authors categorized the recommended touch DNA items that would have higher success rates after direct PCR amplification. The first category include porous clothing item that directly contact with the skin, such as headwear, shirt collars, socks and etc. Next, is the non-porous worn items such as eyeglasses, gloves. Thirdly is items that had contact with the mouth but often present in low quantity, such as that of drinking bottle, cigarette butts or even vape pod that has increased users nowadays.

In this regard, after reviewing the possible sample types from different categories that had higher possibilities to yield DNA profiles based on previous study conducted by other researchers. The suitable touch DNA samples such as drinking bottle, face mask, socks, vape pod, touch DNA on glass, base of screwdriver and fingerprint enhanced with black carbon fingerprint powder were selected as part of the mock casework samples to be evaluated using the three techniques employed in this study.

CHAPTER 3

MATERIALS AND METHODS

3.1 Introduction

This chapter includes the methodology and materials used during the laboratory experiment of this particular research. Most of the laboratory work was carried out at Royal Malaysia Police's (RMP) Forensic DNA Databank Laboratory in Cheras, Selangor, Malaysia. Meanwhile, the quantitation part was performed at the Human Identification Unit/DNA (HID) Laboratory , School of Health Sciences (PPSK), in Universiti Sains Malaysia (USM), Kubang Kerian, Kelantan. A total of 48 reactions including 16 mock casework samples were processed for each of the three techniques, which were using the Casework Direct System (Promega), Maxwell® FSC DNA IQ™ Casework Kit on the Maxwell® RSC 48 System and COPAN MicroFLOQ™ Direct Swab respectively. The mock crime scene samples covered a variety of biological samples which include swab from drinking bottle, face mask, chewing gum, touch DNA on glass, phone, vape pod, toothbrush to bloodstain on jeans.

3.1.1 General Laboratory Practice

The laboratory work for this research were carried out in compliance with the DNA Identification Act 2009 (Act 699) and the DNA Identification Regulations Act 2012. The experimental work were performed following the laboratory protocols in the RMP Forensic DNA Databank Laboratory which was accredited under the MS ISO/IEC 17025: 2017 by the Department of Standards Malaysia.

All instruments used were calibrated regularly and the equipments used were sterilised. Lab coat was worn all times and a new set of disposable nitrile gloves and surgical face

masks were used during different stage of experimental procedures. The DNA extraction, polymerase chain reaction (PCR) amplification and capillary electrophoresis procedures were carried out at different working areas to avoid cross-contamination (pre-and post-PCR rooms).

3.2 Materials

All the materials and instruments used in this research were listed in Table 3.1 according to the DNA analysis stages

Table 3.1 The list of materials and apparatus used in this study were listed below.

Materials/ Instrument	Brand
1. Sampling (General)	
MicroFLOQ® Direct Swab	COPAN, Brescia, Italy
Forceps	Merck, USA
Iso-propanol (70%)	Merck, USA
Kimwipes	Kimberly- Clark Worldwide, USA
Micropipette (10 µl, 200 µl, 1000 µl)	Rainin ™, California
Normal Saline Solution (Sterile Isotonic Sodium Chloride Solution)	Klean & Kare, MY
Sterile cotton swab	Labchem, MY
2. DNA Extraction	
Casework Direct System	Promega, Madison, WA, USA
Casework Extraction Kit	Promega, Madison, WA, USA
ClickFit Microtube, 1.5 ml	Promega, Madison, WA, USA
CW Microfuge Tubes, 1.5 ml	Promega, Madison, WA, USA
CW Spin Baskets	Promega, Madison, WA, USA