DNA ANALYSIS BY POLYMERASE CHAIN REACTION OF SALIVA TRACES ON CIGARETTE BUTTS EXPOSED TO INDOOR AND OUTDOOR ENVIRONMENTAL CONDITIONS

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To my beloved mother and father
ACKNOWLEDGEMENT

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Thank You.
ABSTRACT

Cigarette butts are one of the most common carriers of saliva traces in forensic practice. There is a growing need to perform trace analyses such as DNA salivary analyses on cigarette butts found at a crime scene for identification purpose. However, examination of saliva traces left on cigarette butts as evidences are complicated due to the availability of the biological material in trace amounts for analysis and its rapid degradation due to extreme effects of environmental factors. The aim of this study was to compare the DNA quality and quantify the amount of DNA preserved in saliva found on cigarette butts subjected to various temperatures and humidity. Several cigarette butt samples were smoked, collected and were exposed to outdoors and indoors for 1 day, 3 days and 7 days. The samples were subjected to DNA extraction, quantification, DNA amplification using polymerase chain reaction (PCR) for the locus YNZ-22 and DNA typing. The results from this study showed that the purity of the DNA in the indoor experiment were higher (A_{260/280} 1.76 – 1.91) than the purities of the outdoor experiment (A_{260/280} 1.26 – 1.65) of cigarette butts. The concentration of the DNA found on the saliva traces on cigarette butts can be very variable in the outdoor experimental set-up (377.99 – 585.83 ng/µL) compared to the indoor (266.38 – 290.18 ng/µL); attributable to the differences in the humidity as well as the temperature. In conclusion, the purity obtained in this study ranges from low to high, and samples with intermediate to high purity was proven to enable successful DNA profiling. Since the concentration of DNA reported in this study may constitute human as well as non-human DNA, the interpretation of the DNA purity is a better mean for elucidating its potential value in forensic aspect compared to the DNA concentration.
ABSTRAK

Puntung rokok merupakan salah satu pembawa kesan air liur paling biasa dalam amalan forensik. Keperluan adalah semakin meningkat untuk melaksanakan analisis DNA sampel air liur pada puntung rokok yang ditemui di tempat kejadian untuk tujuan pengenalpastian. Walau bagaimanapun, analisis kesan air liur pada bahan bukti adalah rumit kerana terdapat bahan biologi dalam jumlah surih untuk analisis serta degradasi cepat kesan faktor persekitaran yang melampau. Tujuan kajian ini adalah untuk membandingkan kualiti DNA dan mengukur kuantiti DNA yang terdapat dalam air liur pada puntung rokok yang terdedah kepada pelbagai suhu dan kelembapan. Beberapa sampel puntung rokok dihisap, dikumpul dan didedahkan kepada persekitaran luaran dan dalaman untuk 1 hari, 3 hari dan 7 hari. Sampel melalui proses pengekstrakan DNA, kuantifikasi, amplifikasi DNA menggunakan tandakbalas berantai polimerase untuk lokus YNZ-22 dan pemprofilan DNA. Hasil daripada kajian ini menunjukkan bahawa ketulenan DNA dalam eksperimen dalaman adalah lebih tinggi ($A_{260/280}$ 1.76 – 1.91) daripada ketulenan eksperimen luaran ($A_{260/280}$ 1.26 – 1.65) puntung rokok. Kepekatan DNA yang ditemui pada kesan air liur pada puntung rokok sangat berbeza dalam struktur eksperimen luar (377.99 – 585.83 ng/µL) berbanding dengan dalaman (266.38 – 290.18 ng/µL); berpunca daripada perbezaan pada kelembapan serta suhu. Kesimpulannya, julat ketulenan yang diperolehi dalam kajian ini adalah dari rendah ke tinggi dan sampel dengan ketulenan perantaraan ke ketulenan tinggi telah terbukti untuk membolehkan pemprofilan DNA. Oleh kerana kepekatan DNA yang dilaporkan di dalam kajian ini boleh terdiri daripada DNA manusia dan bukan manusia, maka tafsiran ketulenan DNA dalam aspek ini adalah lebih baik untuk menjelaskan nilai potensinya dalam aspek forensik berbanding dengan kepekatan DNA.
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LIST OF ABBREVIATIONS

A<sub>260/280</sub> - Absorbance ratio of 260 nm to 280 nm
A<sub>260/230</sub> - Absorbance ratio of 260 nm to 230 nm
ALS - Alternative Light Source
BSA - Bovine Serum Albumin
bp - Base pair
°C - Degree celcius
cm<sup>3</sup> - Centimeter cube
CODIS - Combined DNA Index System
DMSO - Dimethyl Sulfoxide
dNTP - Deoxynucleotide triphosphate
DNA - Deoxyribonucleic acid
g - Gram
gDNA - Genomic Deoxyribonucleic acid
m - Meter
mA - Milliampere
MgCl<sub>2</sub> - Magnesium Chloride
mg/mL - Milligram per milliliter
mL - Milliliter
mm - Millimeter
mM - Millimole
MSDS - Material Safety Data Sheet
ng - Nanogram
nm - Nanometer
OD - Optical density
PCR - Polymerase Chain Reaction
qPCR - Quantitative Polymerase Chain Reaction
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<td>Restriction fragment length polymorphism</td>
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<td>RNA</td>
<td>Ribonucleic acid</td>
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<td>rpm</td>
<td>Revolution per minute</td>
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<td>SD</td>
<td>Standard Deviation</td>
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<td>STR</td>
<td>Short tandem repeat</td>
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<td>TBE</td>
<td>Tris-borate-ethylene diamine tetra acetic acid</td>
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<td>U/µL</td>
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<td>Micromole</td>
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<td>UV</td>
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<td>VNTR</td>
<td>Variable number tandem repeat</td>
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CHAPTER 1

INTRODUCTION

1.1 Background

Cigarette butts are one of the most common carriers of saliva traces in forensic practice. Today, approximately 1.2 billion people worldwide smoke tobacco (Oleski et al., 2010) and many people smoke when they are nervous (e.g., when involved in a crime) (Axelrod et al., 2007). Hence, cigarette butts found at a crime scene can give information of the smokers. For instance, with the presence of lipstick, information of one or more female and/or male smokers may be known. Besides, fingerprints may be present and Deoxyribonucleic Acid (DNA) analysis can be done to aid in personal identification (Horswell, 2004). Personal identity plays a large role in civil or criminal cases whereby a mistake can lead to wrong convictions or fatality in a judicial process. Cigarette butts found at crime scene may contain traces of saliva and attached mucosal epithelium cells from the lips of the smoker, which allows for DNA identification by profiling (Yudianto, 2009). About 2 to 160 ng of DNA extracted from cigarette butts is adequate for typing (Hochmeister et al., 1991).

DNA samples recovered from a crime scene are frequently exposed to damaging environmental conditions such as light, heat and bacterial decomposition before they are collected for analysis. Hence, generating an evidentially valuable profile from these quality-compromised samples is a great challenge to the forensic scientist (Thacker et al., 2006). In a study by Casey and co-workers, DNA distribution was quantified using real-time quantitative polymerase chain reaction
(RT-qPCR) on smoked cigarette butts obtained from external sources. The study found that there was an average more DNA per cigarette butts found indoors than that found outdoors (Casey et al., 2013).

1.2 Problem Statement

There is a growing need to perform trace analyses such as DNA analyses on saliva on cigarette butts found at a crime scene for identification purpose. However, the examination of saliva traces left on cigarette butts as evidences are complicated due to the availability of the biological material in small amounts for analysis and its rapid degradation due to effects of environmental factors such as heat and humidity. This study was therefore embarked to answer the following research questions.

(i) Can sufficient DNA be recovered for DNA profiling, from saliva traces on cigarette butts exposed to different environmental conditions over a period of time (i.e., up to a week)?
(ii) Are the DNA recovered from saliva traces on cigarette butts of good quality or the quality compromised?
(iii) Are the DNA preserved in saliva found on cigarette butts subjected to various environmental conditions amplifiable by PCR?

1.3 Objectives

The objectives of this study are as follows:

(i) To recover DNA from saliva traces on cigarette butts for DNA extraction for evaluating its usefulness for human identification.
(ii) To compare the quality and amplifiability of DNA from saliva traces on cigarette butts exposed to the varying heat and humidity in the field and indoor conditions over a period of time.
1.4 Scope of Study

The scope of study encompasses the estimation of DNA purity and quantity from saliva traces found on cigarette butts exposed to varying conditions of indoor and outdoor environments over a period of up to 1 week (Day-1, 3, and 7). The locations for the indoor and outdoor experiments were both within the Universiti Teknologi Malaysia (UTM) Johor Bahru campus. The data on ambient temperature and relative humidity at which the cigarette butts were exposed were also collected. Comparison on the quality of DNA (purity) as well as the possible amount of DNA recovered among the different groups of cigarette butts was attempted, in view of its practical value for DNA profiling.

1.5 Hypothesis

It was hypothesised that the quality and quantity of DNA recovered from the indoor experiment as well as those with a shorter span of exposure period would be better than that of outdoor experiment and those subjected to a longer period of exposure.

1.6 Significance of Study

In many instances (e.g., Prosecutor versus Anwar Ibrahim), the purity of DNA relating to its possible profiling has been questioned in court since they are prone to environmental degradation (Zakaria, 2015). Because DNA evidence from cigarette butts can be of significance for human identification especially in forensic context, while in most cases they have been recovered in dirty and wet locations, often after certain period of exposure time; the findings reported here may prove useful in elucidating the feasibility of recovering suitable purity and amount of DNA for DNA profiling.
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