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

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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 tindakbalas 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

$A_{260/280}$	-	Absorbance ratio of 260 nm to 280 nm
$A_{260/230}$	-	Absorbance ratio of 260 nm to 230 nm
ALS	-	Alternative Light Source
BSA	-	Bovine Serum Albumin
bp	-	Base pair
^{o}C	-	Degree celcius
cm^3	-	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
$MgCl_2$	-	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

RT-qPCR	-	Real-time quantitative polymerase chain reaction
RFLP	-	Restriction fragment length polymorphism
RNA	-	Ribonucleic acid
rpm	-	Revolution per minute
SD	-	Standard Deviation
STR	-	Short tandem repeat
TBE	-	Tris-borate-ethylene diamine tetra acetic acid
U/ µL	-	Unit per microliter
µL	-	Microliter
µM	-	Micromole
UTM	-	Universiti Teknologi Malaysia
UV	-	Ultraviolet
VNTR	-	Variable number tandem repeat
vs	-	Versus
v/v	-	Volume/Volume
w/v	-	Weight/Volume

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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.

REFERENCES

Akane, A., Matsubara, K., Nakamura, H., Takahashi, S. and Kimura, K. (1993). Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification. *Journal of Forensic Sciences* 39, 362-372.

Amersham Biosciences. (1998). *PCR Product Analysis, A Guide to Using Semidry Flatbed Gel Electrophoresis.*[Brochure]. USA: Gelifesciences.

Andrew, L. R. (1999). *Chelex DNA Extraction*. Polis Diraja Malaysia (PDRM) Forensic Science Centre. Method No. 6A, Version 3.

Apostolov, A. (2012). DNA Identification of Biological Traces on Cigarettes: Vices Reveal. *Biotechnology & Biotechnological Equipment*, 26(3), 2994-2998.

Applied Biosystems. (2012). *AmpFlSTR® Identifiler® Direct PCR Amplification Kit*. Life Technologies Corporation.

Anderson, S., Bankier, A. T., Barrell, B. G., Bruijn, M. H. L., Coulson, A. R., Drouin, J., Eperon, I. C., Nierlich, D. P., Roe, B. A., Sanger, F., Schreier, P. H., Smith, A. J. H., Staden, R. and Young, I. G. (1981). Sequence and organization of the human mitochondrial genome. *Nature* 290, 457-464.

Axelrod, A., Antinozzi, G. and Axelrod, A. (2007). *The complete idiot's guide to forensics*. New York: Alpha Books.

Bali, L. E., Diman, A., Bernard, A., Roosens, N. H. C. and De Keersmaecker, S. C. J. (2014). Comparative Study of Seven Commercial Kits for Human DNA Extraction from Urine Samples Suitable for DNA Biomarker-Based Public Health Studies. *Journal of Biomolecular Techniques*. 25(4), 96–110. National Center for Biotechnology Information, U.S.

Barbaro, A., Staiti, N., Cormaci, N. and Saravo, L. (2004). DNA profiling by different extraction methods. International Congress Series. 1261, 562-564. ScienceDirect.

Briuer, F. L. (1976). New Clues to Stone Tool Function: Plant and Animal Residue. *American Antiquity*. 41(4), 478-484. Society for American Archaeology.

Buttler, J. M. (2001). *Forensic DNA Typing: Biology and Technology Behind STR Markers*. Academic Press, UK.

Casey, L., Engen, S. and Frank, G. (2013). Quantitative Analysis of the DNA Distribution on Cigarette Butt Filter Paper. *Journal of Forensic Sciences*. 58(2). Wiley Online Library.

Castella, V., Simonin, N. D., Casadevall, B. and Mangin, P. (2006). Forensic evaluation of the QIAshredder/QIAamp DNA extraction procedure. *Forensic Science International*. 156(1), 70-73. ScienceDirect.

Catalin, M., Andrei, A. and Mitrasca, O. (2015). *Modern Methods of Collection and Preservation of Biological Evidence for Human Identification by DNA Analysis*. University of Medicine and Pharmacy from Timisoara: Abacus Diagnostics.

Committee on DNA Forensic Science: An Update. (1996). *The Evaluation of Forensic DNA Evidence*. Washington: National Academies Press.

Daquila, R. T., Bechtel, L. J., Videler, J. A., Eron, J. J., Gorczyca, P. and Kaplan, J. C. (1991) Maximizing sensitivity and specificity of PCR by preamplification heating. *Nucleic Acid Research.* 19, 3749-3749

Dettmeyer, R. (2013). *Forensic medicine*. Berlin: Springer-Verlag Berlin and Heidelberg GmbH & Co KG.

Elkins, K., Kadunc, R., Mann, G. and McLaughlin, S. (2009). Comparison of Quantity and Quality of DNA Recovered from Burn Samples in which Burn Temperatures and Conditions were Varied. *The Internet Journal of Forensic Science*. 4(2).

Elkins, K. M. (2013). *Forensic DNA Biology: A Laboratory Manual*. United States: Elsevier.

Foster, A. and Laurin, N. (2012). Development of a fast PCR protocol enabling rapid generation of AmpFℓSTR® Identifiler® profiles for genotyping of human DNA. 3(6). National Center for Biotechnology Information, U.S.

Gan, W., Zhuang, B., Zhang, P., Han, J., Li, C. X. and Liu, P. (2014). A Filter Paper-Based Microdevice for Low-Cost, Rapid, and Automated DNA Extraction and Amplification from Diverse Sample Types. *Royal Society of Chemistry*. 14(19), 3719-3728.

Gyllensten, U. B. and Erlich, H. A. (1988). Generation of single-stranded DNA by the polymerase chain reaction and its application to direct sequencing of the

HLA-DQA locus. *Genetics*. 85, 7652-7656. Proceedings of the National Academy of Sciences, U.S.A.

Goodwin, W., Linacre, A. and Hadi, S. (2007). *An Introduction to Forensic Genetics*. England: John Wiley & Sons, Ltd.

Grskoviic, B., Zrnec, D., Popovicc, M., Petek, M. J., Primorac, D. and Mrsic, G. (2013). Effect of Ultraviolet C Radiation on Biological Samples. *Croatian Medical Journal*. 54(3), 263-271. National Center for Biotechnology Information.

Hedman, J. (2011). DNA Analysis of PCR-Inhibitory Forensic Samples. Sweden: Media-Tryck, Lund.

Hochmeister, M. N., Budowle, B. and Jung, J. (1991). PCR - Based Typing of DNA Extracted from Cigarette Butt. *International Journal of Legal Medicine.* 104, 229-233.

Horn, G. T., Richards, B. and Klinger, K.W. (1989). Amplification of a highly polymorphic VNTR segment by polymerase chain reaction. *Nucleic Acids Research*. 17(5), 2140. National Center for Biotechnology Information, U.S.

Horswell, J. (2004). *The Practice of Crime Scene Investigation*. London: Taylor & Francis.

Houck, M. and Siegel, J. (2010). *Fundamentals of Forensic Science*. Amsterdam: Academic Press.

Institute for Public Health. (2012). *Report of the Global Adult Tobacco Survey (GATS) Malaysia*. Ministry of Health Malaysia.

Kamel, A. M., Mossallam, G. I., Mahmoud, H. K., Hamdy, N., Haddad, A. E., Fahmy, O. and Nassar, A. A. (2002). Variable Number Tandem Repeat Polymorphism as a Tool of Chimerism Detection in Allogeneic Stem Cell Transplantation. *Journal of the Egyptian National Cancer Institution*. 14(4), 275-287. Academia.

Kasai, K., Nakamura, Y. and White, R. (1990). Amplification of a variable number of tandem repeats (VNTR) locus (pMCT118) by the polymerase chain reaction (PCR) and its application to forensic science. Journal of Forensic Science. 35(5), 1196-200. National Center for Biotechnology Information, U.S.

Khare, P., Raj, V., Chandra, S. and Agarwal, S. (2014). Quantitative and qualitative assessment of DNA extracted from saliva for its use in forensic identification.

Journal of Forensic Dental Sciences. 6(2), 81–85. National Center for Biotechnology Information, U.S.

Krishnan, S. *Determination of Genetic Relatedness in Kelampayan Plantlets Regenerated from Tissue Culture using Damd Markers*. Bachelor of Science Honours. Universiti Malaysia Sarawak; 2013.

Linch, C. A. and Prahlow, J. A. (2008). Microscopical Examination of Particles on Smoked Cigarette Filters. *Forensic Science, Medicine and Pathology*. 4(4), 228-233. Humana Press Inc.

Malaysians smoked eight billion contraband cigarettes in 2012. (2014, 3rd January). *The Star Online*.

Mangin, P. and Ludes, B. (1994). Acta Medicinæ Legalis. *XVIth Congress of the International Academy of Legal Medicine and Social Medicine*. 31st May 1994. Strasbourg, France.

McClintock, J. (2014). *Forensic Analysis of Biological Evidence*. CRC Press.

Mozayani, A. and Noziglia, C. (2006). *The Forensic Laboratory Handbook Procedures and Practice*. New Jersey: Humana Press Inc.

Nandy, A. (2010). *Principles of Forensic Medicine: Including Toxicology*. (3rd ed.) Kolkata: New Central Book Agency (P) Ltd

National Oceanic and Atmospheric Administration. (2010). *Polymerase Chain Reaction (PCR)*. USA.

Nicklas, J.A. and Buel, E. (2003). Quantification of DNA in Forensic Samples. *Analytical and Bioanalytical Chemistry*. 376, 1160-1167.

Noren, L.,Hedell, R., Ansell, R. and Hedman, J. (2013). Purification of Crime Scene DNA Extracts using Centrifugal Filter Devices. *Investigative Genetics*. 4(8). BioMed Central.

Novotny, T. E., Hardin, S. N., Hovda, L. R., Novotny, D. J., McLean, M. K. and Khan, S. (2011). *Tobacco and cigarette butt consumption in humans and animals*. National Center for Biotechnology Information.

Ohmori, T.,Toyoda, H., Toida, T., Imanari, T. and Sato, H. (2001). Comparison of oligosaccharides derived from salivary mucin of Japanese secretor and non-secretor individuals of blood group type-A. *Glycoconjugate Journal*. 18(8), 635-640. Springer Link.

Old, J. B., Boonlayangoor, P. W. and Reich, K.A. (2009). Developmental Validation of RSID™-Saliva: A Lateral Flow Immunochromatographic Strip Test for the

Forensic Detection of Saliva. *Journal of Forensic Science*. 54(4), 866-873. American Academy of Forensic Sciences.

Oleski, K., Campbell, C., Patel, R. and Welker, J. (2010). DNA Extraction for obtaining DNA from cigarette filter paper collected in Paris and East Lansing for PCR amplification detecting p53 mutations.

Oorschot, R.A., Ballantyne, K. N. and Mitchell, R. J. (2010). Forensic Trace DNA: A Review. *Investigative Genetics*. 1(14). National Center for Biotechnology Information, U.S.

Oswald, N. (2010). Determining DNA Concentration and Purity. *Bitesize Bio*. Retrieved October 26, 2015, from http://bitesizebio.com/13501/dna-concentration-purity/

Park, S. J., Min, W. S., Yang, I. H., Kim, H. J., Min, C. K., Eom, H. S., Kim, D. W., Han, C. W., Lee, J. W. and Kim, C. C. (2000). Effects of Mixed Chimerism and Immune Modulation on GVHD, Disease Recurrence and Survival after HLA-identical Marrow Transplantation for Hematologic Malignancies. *Korean Journalof International Medicine*. 15(3), 224-231. National Center for Biotechnology Information, U.S.

Pizzamiglio, M., Marino, A., Maugeri, G. and Garofano, L. (2003). STRs typing of DNA extracted from cigarette butts soaked in flammable liquids for several weeks. Forensic Science International. 136(Supplement 1):56.

Promega Corporation. *GoTaq® Flexi DNA Polymerase*. U.S.A: Usage Information. 2014

Rarick, J. (2012, June 12). Malaysia releases its first Global Adult Tobacco Survey. World Health Organization. Retrieved 27 October, 2015, from http://www.wpro.who.int/mediacentre/releases/2012/20120613/en/

Register, K. (2000). Cigarette Butts as Litter - Toxic as Well as Ugly?. *Underwater Naturalist*. 25(2). American Littoral Society.

Roberts, K. A. and Johnson, D. J. (2012). Investigations on the Use of Sample Matrix to Stabilize Crime Scene Biological Samples for Optimized Analysis and Room Temperature Storage. National Criminal Justice Reference Service.

Roussel, Y., Wilks, M., Harris, A., Mein, C. and Tabaqchali, S. (2005). Evaluation of DNA extraction methods from mouse stomachs for the quantification of H. pylori by real-time PCR. *Journal of Microbiol Methods*. 62, 71-81.

Rudin, R. and Inman, K. (2002). *An Introduction to Forensic DNA Analysis*. (2nd ed.). Washington, D.C.: CRC Press LLC.

Saiki, R. K., Scharf, S., Faloona, F., Mullis, K. B., Horn, G. T., Erlich, H. A. and Arnheim, N. (1985). Enzymatic amplification of beta-globin genomic sequence and restriction site analysis for diagnosis of sickle cell anemia. *Science.* 230, 1350-4.

Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullis, K. B. and Erlich, H.A. (1988). Primer-directed enzymatic amplification of DNA with Thermostable DNA-polymerase. *Science.* 239, 487-491.

Sambrook, J. and Green, M. R. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbour Laboratory Press.

Scollo, M. M. and Winstanley, M. H. (2015). *Tobacco in Australia: Facts and issues.* Melbourne: Cancer Council Victoria.

Sharma, R. (2008). *Concise Textbook Of Forensic Medicine & Toxicology*. (2nd ed.). New Delhi: Elsevier

Shepherd, C., Harbison, S. and Saul, D. J. (2012). Extraction of DNA from cigarette butts using forensicGEM® Cigarette. *ZyGEM Application Note 102*. New Zealand: ZyGEM Corporation Ltd.

Thacker, C. R., Oguzturun, C., Ball, K. M. and Court, D. S. (2006). An investigation into methods to produce artificially degraded DNA. *Elsevier*. International Congress Series 1288, 592-594.

Thermo Fisher Scientific. (2008). *260/280 and 260/230 Ratios* [Brochure]. USA: Life Technologies.

Thermo Fisher Scientific. (2010). *Nucleic Acid Thermo Scientific NanoDrop Spectrophotometers.* [Brochure]. USA: Thermo Fisher Scientific Inc.

Thermo Fisher Scientific (2015). *AmpFlSTR®Identifiler® Direct PCR Amplification Kit* [Brochure]. USA: Life Technologies.

Thomson, G., Wilson, N., Bushell, L., Al Matar, W., Ball, B., Chiu, J., Culliford, N., Gately, C., Gibson, K., Hudson, J., Hunt, P., Rangamuwa, K., Tapp, D., Wickramaratne, H. and Young, V. (2008). *Butt lengths differ by area deprivation level: a field study to explore intensive smoking*. 10(5):927-31. National Center for Biotechnology Information.

Till, B. J., Zerr, T., Comai, L. and Henikoff, S. (2006). A protocol for tilling and ecotilling in plants and animals. *Nature Protocols*. 1, 2465-2477.

Trepat, E. V. and Castello, A. (2015). More about RSID-saliva: the effect of sample age and the environment on the test's efficacy. *Australian Journal of Forensic Sciences*. 47(4), 450-455. Taylor & Francis Online.

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(1-3), 1-17. Science Direct.

Web of Science. (2015). Thomson Reuters Scholarly and Scientific Research.

Witt, S., Neumann, J., Zierdt, H., Gebel, G. and Roscheisen, C. (2012). Establishing a novel automated magnetic bead-based method for the extraction of DNA from a variety of forensic samples. *Forensic Science International*. 6(5), 539-547. ScienceDirect.

Yudianto, A. (2009). Effectiveness of Cigarette Butts as an Alternative Material for Forensic DNA Identification with Polymerase Chain Reaction (PCR) in Short Tandem Repeat (STR) Loci. *Folia Medica Indonesiana*. 45(2), 112-114.

Zacny, J. P. and Stitzer, M. L. (2012). *Human Smoking Patterns*. Monograph 7. National Cancer Institute.

Zakaria, A., Sharif, R., Embong, A. H., Omar, S. H. and Ali, R. (2015). *Dato' Seri Anwar Ibrahim V Public Prosecutor*. Press Summary.