THESIS

ANALYSIS OF AMELOGENIN GENE TO IDENTIFY SEX FROM TOOTHBRUSH AS A FORENSIC EVIDENCE

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HTET HTET AUNG

NIM 091814653016

MASTER STUDY PROGRAM
FORENSIC SCIENCE
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Submitted to Postgraduate School of the Universitas Airlangga

in fulfillment of the requirements for the degree of

Master of Forensic Science

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CERTIFICATE OF APPROVAL

THIS THESIS WAS APPROVED ON

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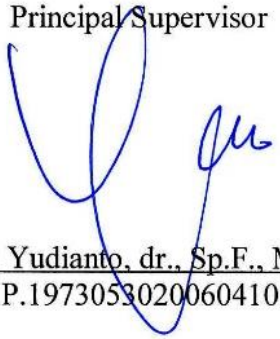
By:

Principal Supervisor



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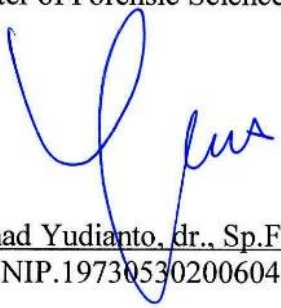

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DECLARATION STATEMENT

Here undersigned;

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Thesis Title : Analysis of Amelogenin Gene to Identify Sex from Toothbrush as a Forensic Evidence

Stating exactly that my thesis is original (the work itself) is not the result of imitation or plagiarism of the work other people. This thesis has never been submitted to obtain an academic degree.

In this thesis there are no opinions that have been written or published other people, except in writing which are clearly stated as reference with the author's name is mentioned and listed in the bibliography. Thus, this statement is made without coercion from any party, if this statement is not true, so I am willing to accept sanctions in accordance with norms and regulations that apply at Airlangga University.

Surabaya, 28 January 2020

Htet Htet Aung

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SUMMARY

Analysis of Amelogenin Gene to Identify Sex from Toothbrush as a Forensic Evidence

Introduction

Sex determination is of great importance as additional information in criminal investigations as well as in identification of missing persons, no suspect cases, and ancient DNA studies (Andreasson and Allen, 2003). There are many biological samples which can be isolated DNA. However, crime scenes are different from each other and the evidences that can be found at the crime scene is different too. In such cases, toothbrush can be used as an alternative to get the DNA. Sex determination is also a part of forensic biological evidence and an essential priority when traditional identification of the deceased becomes impossible. Amelogenin-based gender tests are part of various PCR multiplex reaction kits from different manufacturers which are widely used for DNA typing of both reference samples and casework samples in the forensic field (Steinlechner *et al.*, 2002). This research aims to determine Sex by analysis of the amelogenin gene using Polymerase Chain Reaction (PCR) method on Deoxyribonucleic acid (DNA) isolated from toothbrushes. This testimony was done by using the toothpaste which included 0.221% sodium fluoride.

Methods

To attain the aimed objectives, the study utilized experimental laboratory design with the control group. The experimental process was carried out at the Human Genetic Laboratory of the Institute of Tropical Diseases (ITD) at Airlangga University in November 2019. The experiment research used a total of 16 toothbrush samples (4 control and 12 studied). The process went through sample exposure and DNA typing. The exposition was through the combined effect of sodium fluoride 0.221% containing toothpaste at an interval of day 1, 3, and 7 days duration. The experimental procedure was by DNA profiling to analyze the exposure effect extent by quality and quantity of DNA through spectrophotometric measurement and electrophoretic reaction under amelogenin gene locus primers. Then obtained results were analyzed by SPSS through ANOVA and Kruskal Wallis to establish statistical significance, other analytical methods included Excel, observation.

Results and Discussion

The aims of this study was to analyze the effect of exposure time and men and women (sex) tooth brushing on the quality of DNA from the toothbrush. The results of the average toothbrush rate measurement using UV spectrophotometer from men for 1, 3, and 7 days were 943.3 μg/ml, 385 μg/ml, 530.3 μg/ml, while from women for 1, 3, 7 days were 922.3 μg/ml, 619.5 μg/ml, and 631.8 μg/ml. The results of the Two Way Anova statistical test have shown there was an influential relation between the sodium fluoride containing toothpaste effect on toothbrush and exposure time on day 1, 3 and 7 with a significance value = 0.044 through the amelogenin gene locus. The influential effect is assessed by DNA quality and quantity through spectrophotometer measurement and electrophoretic visualization. Through the parameters, it was found that the toothbrush DNA purity levels and DNA concentration levels are randonmed. There was no impact on DNA quality on toothbrush from the difference of men and women (sex) with a significance value = 0.389 through the amelogenin gene locus. There was no interaction between the effect of long exposure and gender difference on DNA quality found in toothbrush with a significance value = 0.672 through amelogenin gene locus. The average of purity levels in the range between 1.18 - 1.23 μg/ml, so that the purity of DNA has obtained a good value. Moreover, in this study, the DNA extra bands were found from the X-106 bp from women. According to the Carlsson et al., (1995) in their study, the results exclude that the extra bands are caused by intermolecular cross-linking. Incubation of the samples for increasing times before electrophoresis makes the bands move closer and closer to each other as the dye molecules become more homogeneously distributed among the DNA molecules. Finally, the two bands merge into one at an intermediate position (Carlsson et al., 1995).

The visualization of electrophoresis at the Amelogenin gene locus XY (106,112 bp) for men and XX (106 bp) for women produce a band of 100% positive in the total sample. Based on these results, it can finally be concluded that toothbrush can be used as one of the very valuable sources for gender identification for forensic evidence and personal identification.