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Quantification of Touch DNA on Glass, Plastic, and Ceramic Glasses

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A B S T R A C T

In Indonesia, there are many criminal cases. Every criminal offender will certainly bring and leave something at the crime scene that can be used as a trial or evidence. In addition, perpetrators who accidentally or intentionally come into contact with surrounding objects can cause the transfer of trace evidence to these objects. DNA touch left on an object can provide information about the identity of individuals in contact with that object. This study aimed to determine the quantity and quality of DNA Touch in glasses made of glass, plastic, and ceramic touched by one proband and two probands. DNA extraction was carried out using the 5% Chelex method. using Univariate and one-way ANOVA. DNA quality was observed based on the comparison results of Å260 and Å280 on the SimpliNano spectrophotometer. The results showed that the DNA concentration in glass, plastic and ceramics was not significantly different in each treatment. There was no interaction between the type of glass, hand touch, or average DNA concentration. Extracted DNA showed poor quality.

1. INTRODUCTION

1.1. Research Background

In Indonesia, various criminal cases such as terrorism, murder, rape, theft and so on are rampant [2]. An incident can be declared a criminal act through investigation by the police using the methods regulated in Article 1 paragraph (5) of the Criminal Code [8]. A criminal case must be resolved using a criminal case resolution process and mechanism. Examination of a criminal case, whether at the police, prosecutor's office or court, essentially aims to find the material truth about a case. Law enforcement officials must obtain evidence to reveal a case to resolve a criminal case [11].

In forensics, there is a motto that there is no crime that leaves no trace. Perpetrators of criminal acts will bring and leave something at the crime scene that can be used as traces or evidence. Apart from that, perpetrators who accidentally or intentionally come into contact with surrounding objects can cause the transfer of trace evidence to those objects [18]. Fingerprints, blood, sperm, saliva, or other objects such as wood, cloth, hair, iron, glass, and tissue are silent witnesses that can be found at the crime scene [13].

Personal identification is one of the methods used to solve a problem in criminal and civil cases. Identification in forensic medicine includes fingerprints, property examination, medical, dental, serology, and exclusive methods. Currently, identification methods have developed in molecular forensics [9,17]. Molecular forensics is a branch of medical science that utilizes developments in molecular biology technology in solve various forensic cases, such as tracking perpetrators of murder, and rape, searching for missing people, and various other cases using Deoxyribonucleic Acid (DNA) [1].

According to Butler [5], personal identification using DNA analysis involves six stages: examination, extraction, quantification, amplification, electrophoresis and data analysis. In humans, biological parts that can be used as a source of DNA include blood, oral moccasial epithelium, hair follicles, urine, sperm, teeth, bones, and almost all other parts of the human body [10].

DNA touch refers to the DNA left behind from skin cells when a person touches or comes into contact with an object [16]. DNA touch left on an object can provide information about the identity of individuals who have come into contact with that object [14]. According to Burrill [4], DNA touch is a biological sample obtained without visible spots or body fluids being found. This includes sweat DNA left on used clothing or DNA left on the handle of an object.

1.2. Research Objective

This study aimed to determine the quantity and quality of DNA Touch in glasses made of glass, plastic, and ceramic touched by one proband and two probands.

2. MATERIALS AND METHODS

2.1. Place and time of research

This research was conducted from 28 December 2022 to 21 June 2023. Finger and lip prints were taken on Jl. Bukit Dharma II, Jimbaran, Bali. The research was carried out in the form of DNA analysis consisting of DNA extraction with Chelex 5%, and quantity testing with the SimpliNano spectrophotometer which was carried out at the UPT Forensic Laboratory, Udayana University and the Integrated Biomedical Laboratory, Faculty of Medicine, Udayana University.

2.2. Methods

The experimental research design used a factorial design with two factors: type of glass (glass, plastic, and ceramic) and type of touch (hand touch touched by one proband and two probands and mouth touch). The research includes treatment of samples and DNA analysis which is carried out as follows.

Treatment of samples

In the first treatment, the glass was touched by one proband, and in the second treatment, the glass was touched by two probands. In the treatment where two probands touched the glass, the first (female) acted as the provider of tea/coffee and the second (male) as the consumer. So in the first experiment, the samples to be examined were fingerprints and lip prints on the handle and mouth of the glass by the first proband (female). Meanwhile, in the second treatment, the samples to be examined consisted of fingerprints from two probands (male and female) on the handle and lip prints from second proband (male) on the mouth of glasses made of glass, plastic, and ceramic.

The number of hand touches during the experiment was ten times, and the lips were touched seven times, with the details being that in the glass treatment being touched by one proband, all touches were carried out by first proband (female), while in the glass treatment being touched by two probands then the first proband (female) was the provider tea/coffee will touch the handle of the glass three times (without any lips touching the mouth of the glass) and second proband (male) as the consumer will touch the handle of the glass seven times.

Fingerprints and lip prints were collected using the double swab method using a cotton swab where the cotton swab was moistened with ddH2O and then rubbed on the handle and mouth of the glass. After that, the swab is continued with a new cotton swab. The cotton swab was then cut and placed in a 2 mL tube for DNA analysis.

2.3. DNA analysis

2.3.1. Touch DNA extraction

DNA extraction from fingerprint and lip print samples was performed using the 5% Chelex method. The tube containing fingerprint and lip print samples on a cotton swab was added with 300 µL Chelex 5% and 7.5 µL proteinase-K 10 mg/mL, then vortexed low spin for 10 seconds. Next, the samples were incubated in a water bath at 56ºC for 60 minutes and vortexed for 10 seconds. The sample was continued by incubation using a hot plate at 100ºC for 8 minutes then vortexed for 10 seconds. The sample was centrifuged at 8000 rpm for 7 minutes and the supernatant was taken via a cotton swab. The sample is then continued with DNA quantity and quality testing.

2.3.2. DNA touch quantification

Quantification of extracted DNA was carried out using a SimpliNano spectrophotometer. Testing begins by turning on the device selecting DNA mode and setting the wavelength to 260 nm and 280 nm. Then a blank test was carried out using Chelex 5% and sterilized using H2O. The extracted DNA was then dropped in 1 µL. The measurement results will appear in ng/µL concentrations, and DNA purity can be seen directly in Å260/Å280 (BioChrom, 2015).

2.4. Data analysis

Quantitative data obtained was DNA concentration (ng/µL) from DNA touch extraction in each treatment. Quantitative data analysis was done using the IBM SPSS For Windows Version 25 application with Univariate tests on fingerprint samples and one-way ANOVA on lip print samples. If there is a significant difference ($p<0.05$), proceed with the Duncan Test. DNA quality was observed based on the comparison results of Å260 and Å280 on the SimpliNano spectrophotometer, where pure or good isolates showed comparison results of ≥1.8 and ≤2.0.

3. RESULT AND DISCUSSION

3.1. DNA fingerprint concentration Water Content

DNA isolates from 36 samples were tested using a SimpliNano spectrophotometer. Based on the Table 1, it is known that the highest average concentration of fingerprint DNA on glasses touched by one proband was obtained on plastic glasses (178.1 ng/µL), followed by ceramic glasses (174.27 ng/µL), and glass glasses. (170.63 ng/µL). The same results were also found in the average lip print DNA concentration of proband one (female) where the highest average DNA concentration was obtained on a plastic cup (196.03 ng/µL), followed by a ceramic cup (175.9 ng/µL). , and glass beakers (134.2 ng/µL).

In addition, the highest average concentration of fingerprint DNA on glasses touched by two probands was obtained on plastic glasses (197.6 ng/µL), followed by ceramic glasses (182.4 ng/µL), and glass glasses (175. 1 ng/µL). Meanwhile, the average DNA concentration of lip prints of proband two (male) was found to be the highest DNA concentration on ceramic glasses (186.80 ng/µL), followed by

plastic glasses (180.73 ng/µL), and glass glasses (123. 7 ng/µL).

Table 1. Results of statistical analysis of the average DNA fingerprint concentration in the quantity test using the SimpliNano spectrophotometer with Univariate test

Hand touch Type	Average DNA concentration (cup type)		
	Glass	Plastic	Ceramic
Touched by one proband	170.63 ± 32.65[a]	178.1 ± 51.55[a]	174.27 ± 49.58[a]
Touched by two probands	175.1 ± 82.23[a]	197.6 ± 13.07[a]	184.2 ± 64.31[a]

Note: The letter a notation after the number indicates there is no significant difference between columns and rows at the 5% level (P>0.05); Numbers after ± indicate standard deviation; Data on average DNA concentration in ng/µL units.

Statistical analysis of the One-way ANOVA test shows that the type of glass and lip touch are not significantly different from the average lip print DNA concentration (ng/µL) as presented in Table 2.

Based on Table 2, it is known that the highest average lip print DNA concentration in the glass used by proband one was obtained in a plastic glass (196.03 ng/µL), followed by a ceramic glass (175.9 ng/µL), and a glass glass. (134.2 ng/µL). while the average lip print DNA concentration on the glass used by proband two with the highest to lowest average was obtained on the ceramic glass (186.80 ng/µL), followed by the plastic cup (180.73 ng/µL), and the glass cup. (123.7 ng/µL). DNA isolation from 36 samples was then followed by DNA quantity and quality testing using a SimpliNano spectrophotometer with 260 nm and 280 nm wavelengths.

The SimpliNano spectrophotometer was used to determine the accuracy of DNA isolates in the form of DNA concentration and purity (ng/µL) [3]. Apart from that, examining DNA quantity is to determine the right amount of DNA to be amplified. The minimum DNA level for amplification to be carried out is 20 ng/mL or 0.02 ng/µl.

According to Daly [6], the type or substrate of the media influences the amount of DNA transferred. In this research, there were variations in the types of glass, namely glass, plastic and ceramic. However, these three types of glass have the same type of surface texture, namely smooth or non-porous, so the results of the Univariate statistical test on fingerprint samples and the results of the One-way ANOVA statistical test show that the variables in this study, namely the glass material and the type of touch, both have no influence. significantly different from the average DNA concentration (P>0.05). Apart from that, there was no interaction between the type of glass and hand touch, the average DNA concentration. The purity of the DNA obtained in this study ranged from 1.123 to 1.709.

Table 2. Results of statistical analysis of the average DNA concentration in the lip print quantity test using the SimpliNano spectrophotometer with one-way ANOVA test

Lip touch	Average DNA concentration (cup type)		
	Glass	Plastic	Ceramic
First Proband	134.2 ± 49.79[a]	196.03 ± 33.79[a]	136.6 ± 92.35[a]
Second Proband	123.7 ± 53.18[a]	180.73 ± 94.95[a]	186.8 ± 85.14[a]

Note: The letter a notation after the numbers indicates there is no significant difference between columns at the 5% level (P>0.05); Numbers after ± indicate standard deviation; Data on average DNA concentration in units of ng/µL.

According to Daly [6], the type or substrate of the media influences the amount of DNA transferred. In this research, there were variations in the types of glass, namely glass, plastic and ceramic. However, these three types of glass have the same type of surface texture, namely smooth or non-porous, so the results of the Univariate statistical test on fingerprint samples and the results of the One-way ANOVA statistical test, show that the variables in this study namely the glass material and the type of touch, both have no influence. significantly different from the average DNA concentration (P>0.05). Apart from that, there was no interaction between the type of glass, hand touch, or average DNA concentration.

3.2. Purity of the DNA

The purity of the DNA obtained in this study ranged from 1.123 to 1.709. The purity of the DNA obtained in this study ranged from 1.123 to 1.709. According to Biochrom [3], good DNA purity standards using the SimpliNano spectrophotometer are ≥1.8 and ≤2.0. Based on this, the DNA isolates in this study were not good or not pure. Good DNA purity standards using the SimpliNano spectrophotometer are ≥1.8 and ≤2.0. Based on this, the DNA isolates in this study were not good or not pure. According to Deveruex and Sherry [8], DNA purity of less than 1.8 will indicate the presence of potential inhibitors such as protein, phenol, and/or remaining cell debris. The presence of protein compounds and/or remaining cell debris can be caused by the difficult supernatant

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extraction process, where the extraction results leave a small amount of supernatant and must be taken or pipetted using a micropipette through a cotton swab.

Table 3. DNA purity results in fingerprint and lip print samples using the SimpliNano spectrophotometer

Sample	Purity
$TCP1_1$	1.126
$TCP1_2$	1.316
$TCP1_3$	1.187
$TCP2_1$	1.324
$TCP2_2$	1.194
$TCP2_3$	1.327
$TPP1_1$	1.320
$TPP1_2$	1.230
$TPP1_3$	1.161
$TPP2_1$	1.170
$TPP2_2$	1.123
$TPP2_3$	1.201
$TRP1_1$	1.504
$TRP1_2$	1.179
$TRP1_3$	1.238
$TRP2_1$	1.265
$TRP2_2$	1.176
$TRP2_3$	1.307
$M1C_1$	1.709
$M1C_2$	1.362
$M1C_3$	1.137
$M2C_1$	1.314
$M2C_2$	1.468
$M2C_3$	1.379
$M1P_1$	1.447
$M1P_2$	1.259
$M1P_3$	1.216
$M2P_1$	1.308
$M2P_2$	1.320
$M2P_3$	1.211
$M1R_1$	1.311
$M1R_2$	1.200
$M1R_3$	1.305
$M2R_1$	1.186
$M2R_2$	1.236
$M2R_3$	1.242

CONCLUSION

Statistical analysis of Univariate and One-Way ANOVA tests showed that the type of glass and the type of touch did not differ in DNA purity (P>0.05). DNA quality (Å260/Å280) produces impure or bad DNA isolates <1.8.

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