The Use of Touch DNA Analysis in Forensic Identification Focusing on STR CODIS LOCI THO1, CSF1PO and TPOX

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Abstract

Introduction:Identification through DNA analysis is an accurate diagnostic tool. DNA analysis is via Variable Number of Tandem Repeat (VNTR) and Restriction Fragment Length Polymorphisms (RFLP). Up to date, blood spots/blood, sperm spots, vaginal swabs, buccal swabs and bones are specimens that are widely used in the field of forensics for DNA analysis. At the crime scene, the perpetrator's skin may accidentally be in contact with surrounding objects thereby transferring trace evidence to the objects.

Method and Materials: Laboratory observation to demonstrate identification through DNA isolation from the objects that are touched (touch DNA), using the STR CODIS locus, with a momentary research design. DNA was isolated from buccal swabs and swab properties (watches and mobile phones) from volunteers who have signed the consent form. A total of ten samples were used in this study.

Results and Discussion: Mean levels of DNA [UV-Visible Spectrophotometer] buccal swab: 167.89 ± 85.71 µg / ml, watch swab: 59.19 ± 5.58 µg / ml, mobile swab: 38.09 ± 2.12 µg / ml and the purity of the buccal swab DNA: 1.79 ± 0.71, the watch swab: 1.69 ± 0.76, the mobile swab:1,53 ± 0,56. Visualization of PCR products onPolyacrylamide Agarose Composite Gel Electrophoresis [PAGE] stained with Silver and amplified using the standard primers THOI, TPOX and CSF1POfor STR CODIS showed a 100% detection of amplicons. Allele profiles on all samples of STR CODIS were a match or identical to the positive control K562.

Conclusion: Property (handphone and watch) swabs can be used as alternative materials in forensic identification using Touch DNA analysis. It was able to be isolated and amplified by using Polymerase Chain Reaction on the STR CODIS loci THO1, CSF1PO and TPOX

Keywords:*Touch DNA, STR CODIS, Identification*

Introduction

Identification is a way of identifying individuals with characteristics and features to distinguish them from others. Currently the identification method has evolved towards molecular forensic DNA (Deoxyribonucleic acid). DNA is the smallest unit and is present in all living things from microorganisms to higher organisms such as humans, animals and plants.

Identification through DNA analysis is an accurate diagnostic tool. DNA analysis includes analysis of Variable Number of Tandem Repeat (VNTR) and Restriction Fragment Length Polymorphisms (RFLP). DNA analysis through VNTR is a DNA examination method that is based on certain repeated base sequences (core sequences). DNA repeated sequence areas with base size less than 1 kb (kilo base pair), are known as 'microsatellite' or Short Tandem Repeat (STR).[1]

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The Federal Bureau of Investigation [FBI] designed 13 STR locus as a synergistic forensic identification system with the Combined DNA Index System (CODIS) database. The STR locus used by FBI includes TH01, TP0X, CSF1PO, vWA, FGA, D3S1358, D5S818, D7S820, D13S317, D16S539, D8S1179, D18S51, and D21S11, plus amelogenin markers used to determine the sex of the victim.[2,3]

At the crime scene the perpetrator's skin surface or part of his body is often accidentally exposed to the surrounding objects, resulting in the transfer of trace evidence to the objects.In this case one of the technologies namely touch DNA / contact trace DNA can be used, through DNA that is transferred through skin cells when objects are held or touched. Up to date, personal identification through touch DNA testing is not widely known.[4]

Materials and Methods

The type of research was experimental laboratory. Research sample: the watch and handphone used by the respondent and the buccal swab. All respondents used in this study signed the consent form after they freely agreed to participate as subjects of the research. The sample size used was 12.

Research Materials: DNAzol Reagent, 100% & 70 % ethanol solution. PCR Mix [ATP,CTP,TTP GTP, MgCl2,TaqPolymerase], Nuclease Free water, STR CODIS primers of locus:

[TH01:-CTGGGCACGTGAGGGCAGCGTCT-/TGCCGGAAGTCCATCCTCACAGTC-,

TP0X:-ACTGGCACAGAACAGGCATCTAGG-/-GAGGAACTGGGAACCACACAGGT-,

CSF1PO:-AACCTGAGTCTGCCAAGGACTAGC-/-TCCACACACCACTGGCCATCTTC]Results

Table 1. Average amount and purity of DNA samples

Sample	Average amount of DNA (x ± SD) (mg/ml)	Average purity of DNA (λ260 nm/ λ280 nm)
Buccal Swab	167,89 ± 85,71	1,79 ± 0,71
Watch Swab	59,19 ± 5,58	1,69 ± 0,76
Handphone Swab	38,09 ± 2,12	1,53 ± 0,56

Key, x: mean and SD: Standard Deviation

PCR products were visualised using Polyacrylamide Agarose Composite Gel Electrophoresis [PAGE] and the gel was stained with silver. To amplify the STR CODIS locus, the standard primers [THO1, TPOX and CSF1PO] were used and all buccal swab and property [watch and handphone] swab samples showed positive results signifying a 100% detection. Allele profiles in all samples were a match with the positive control, K562, as shown in both Figure 1 and their purity profiles were also shown on table 1.

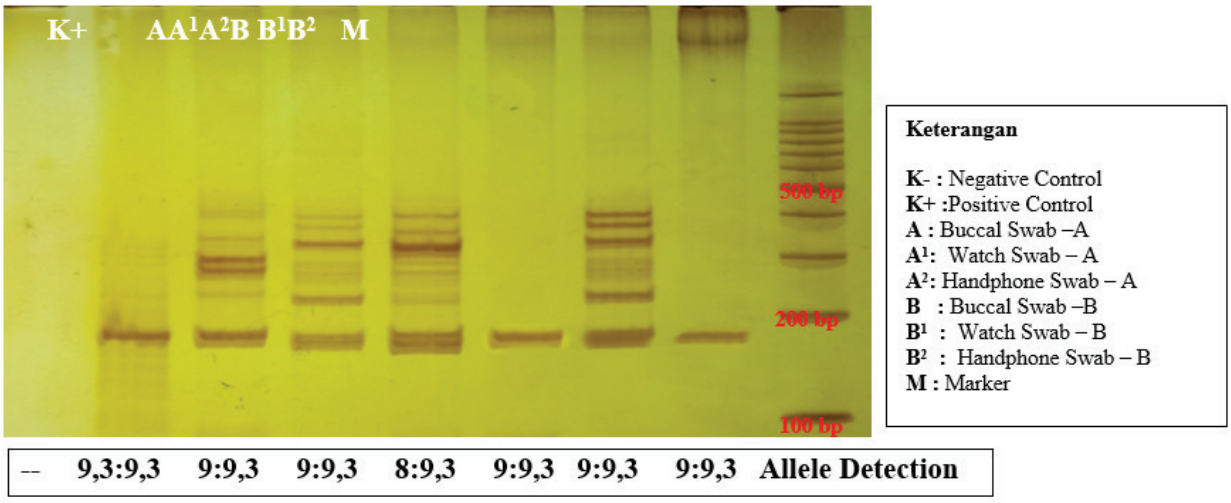

Figure 1. Visualization of STR CODIS PCR product using at locusTHO1 [156 – 195 bp]

The figure 2 below shows the visualization of PCR products with PAGE stained with Silver. The samples were amplified using the primer CSF1PO [amplicon product 321–357 bp] and all the results were positive as shown by the matching bands with the positive control [K562] : 9 : 10.

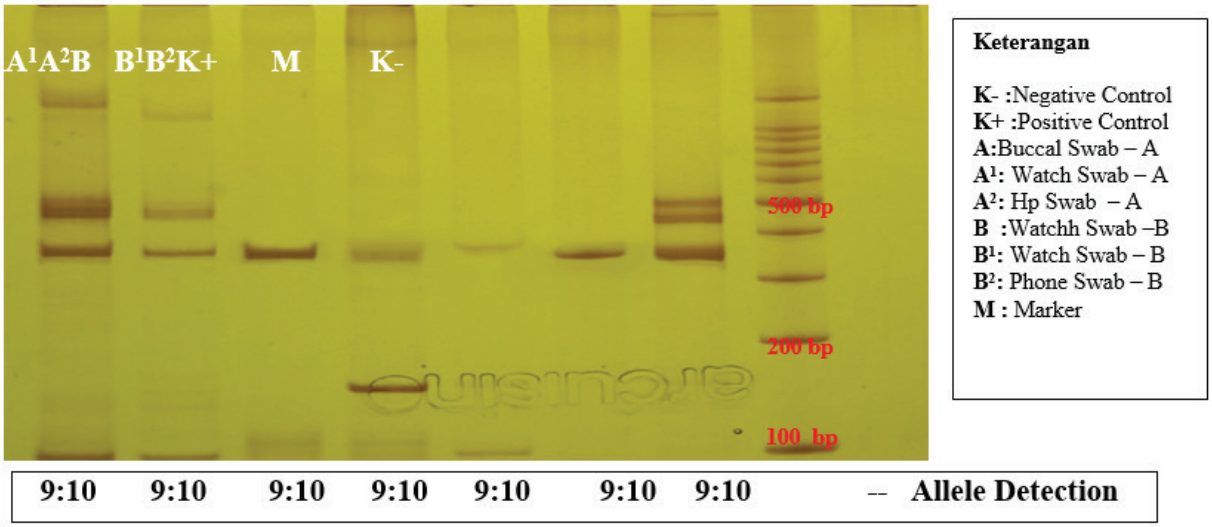

Figure 2. Visualization of STR CODIS PCR product using at locusCSF1PO [321 – 357 bp]

Discussion

The results were obtained by measuring the average amount of DNA using a UV-Visible Spectrophotometer. The average DNA level of all samples was at a minimum amount of 0.25 ng with a purity of 1.8-2.[2,3]

The above Figure shows the visualization of PCR products on Polyacrylamide Agarose Composite Gel Electrophoresis [PAGE] stained with silver. Repeat - Combined DNA Index System [STR- CODIS] locus THO1 [amplicon product 156 – 195 bp] showing that all samples were detected as compared to the positive control [K562]: 9,3:9,3.[5,6]

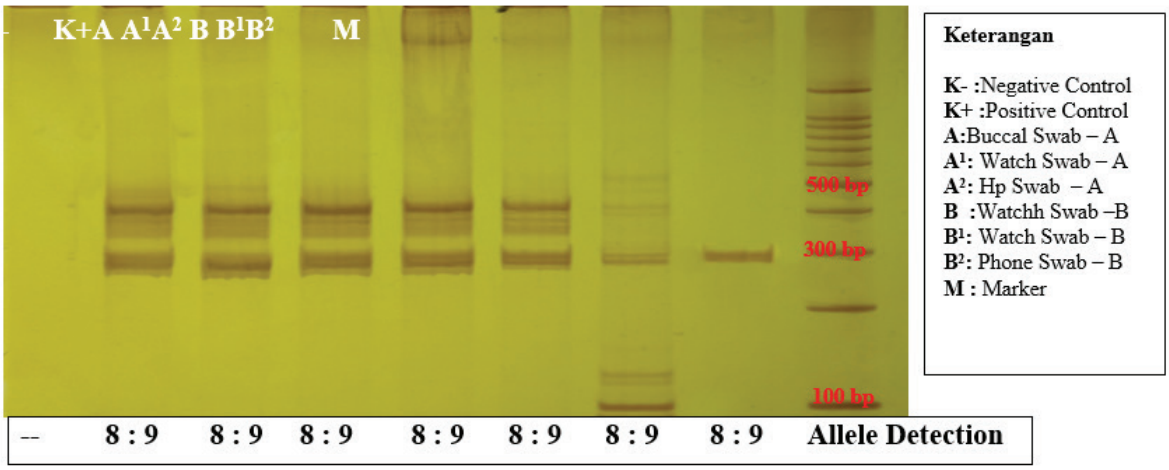

Figure 3. Visualization of STR CODIS PCR product using at locus TPOX [262 – 290 bp]

Figure 3 above, shows the visualization of PCR products with PAGE stained with silverand the amplicons were amplified using the CODIS STR TPOX locus [amplicon product 262 - 290 bp] and all samples were detected as positive [detection +] when compared with the positive control [K562]: 9: 10. For adequate visualization of the results,sufficient levels andpurity of the DNA is needed in orderfor the DNA to be used

as an examination material, including in this case of identification and paternity test.[7]

Failed PCR amplification is characterized by the absence of bands on agarose gel. Incomplete PCR cycles were minimized by PCR optimization for all the respective primers used.[8]

In this study, 3 STR CODIS loci were used, showing that all the samples for buccal swab and property swab (watch and handphone) were detected positive, as well as the allele profile of each locus in each sample showing matched results. Matched results have the understanding that the allele profile on the buccal swab is identical to the allele profile on the swab property. Only one sample showed a different allele, the sampleis A^2 (handphone] [allele 8 : 9,3]. This could be due to contamination during DNA analysis checking process, starting from the process of sample collection.[9]

Table 2. Allele STR CODIS's profile

LOCUS	SAMPLE											
	A	**A1**	**A2**	**B**	**B1**	**B2**	**C**	**C1**	**C2**	**D**	**D1**	**D2**
CSF1PO	9;9.3	9;9.3	8;9.3	9;9.3	9;9.3	9;9.3	9;9.3	9;9.3	9;9.3	9;9.3	9;9.3	9;9.3
THO1	9;10	9;10	9;10	9;10	9;10	9;10	9;9	9;9	9;9	9;9	9;9	9;9
TPOX	8;9	8;9	8;9	8;9	8;9	8;9	9;9	9;9	9;9	8;9	8;9	8;9

key:A,B,C,D : buccal swab sample

A^1,B^1,C^1,D^1 : watch swab

A^2,B^2,C^2,D^2 : handphone swab

As a positive control, that is K562 which is a positive control in the examination of DNA analysis with STR CODIS and 100 bp ladder markers. DNA analysis tests have a 100% accurate value, when done correctly. This DNA analysis test gave a results probability of 100%.[9]

The following is a profile table of STR CODIS allele detection results [THO1, CSF1PO, TPOX] on DNA samples [watch swabs, handphone swabs and buccal swabs].[10]

Research on STR CODIS as a whole has not yet been reported, only a few primers. Some studies have been conducted from several STR CODIS focus areas. The accuracy of research at the loci THO1, TPOX, CSF1PO, and has been reported in several studies including: chromosome populations and allele sequences at the THO1 locus, population in Thailand with 8 STR loci included THO1, TPOX, CSF1PO and vWA. Research on Chinese population in Taiwan with STR research on genetic variation in Caucasia revealed that genetic variation in the population of Filipinos and Thais living in Taiwan using 9 STR loci. Whereas in Indonesia, a research on the THO1 allele pattern in the Batak population in Surabaya and locus D1S80 and D17S5 populations in Surabaya. STR loci typing method especially the THO1 locus is a reasonable, strong and efficient method making it a useful method in forensic cases. 5 to 6 STR loci has a ratio of 1: 100 billion so that in principle regarding the number of loci examined, the more loci used the better the accuracy value.[11]

Conclusion

Property (handphone and watch) swabs can be used as alternative materials in forensic identification using Touch DNA analysis with an average DNA yield of: 59,19 ± 5,58 **mg/ml** and: 38,09 ± 2,12**mg/ml** for watch and handphone swabs respectively. Both the buccal swab, watch swab and handphone swabs had trace amount of DNA that was able to be isolated and amplified by using Polymerase Chain Reaction on the Short Tandem Repeat locus - Combined DNA Index System [STR-CODIS] loci THO1, CSF1PO and TPOX.

Conflict of Interests: The authors declare that they have no conflict of interest in publishing this article.

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Ethics Approval and Consent to Participate

This study has been agreed by Faculty of Dentistry Universitas Airlangga which number of ethical clearance is 033/HRECC.FODM/IV/2018.

List of Abbreviations

CODIS : Combined DNA Index System

DNA : Deoxyribonucleic acid

FBI : Federal Bureau of Investigation

PAGE : Polyacrylamide Agarose Composite Gel Electrophoresis

PCR : Polymerase Chain Reaction

RFLP : Restriction Fragment Length Polymorphisms

STR : Short Tandem Repeat

VNTR : Variable Number of Tandem Repeat

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