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EFFECT OF STORAGE TIME ON DNA CONTENT AND PURITY IN LIP PRINT

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Abstrak

Pakar forensik dalam mengungkap kebenaran suatu kasus harus melalui identifikasi, dokumentasi, dan pengumpulan bukti. Terkadang di TKP, ditemukan sidik bibir di permukaan suatu benda seperti mulut botol plastik. Penelitian sidik bibir pada mulut botol plastik dilakukan selama 1, 3 dan 7 hari. Terdapat 21 sampel dengan rincian 3 sebagai kontrol, 6 sampel untuk hari pertama, 6 sampel untuk hari ke-3, dan 6 sampel untuk hari ke-7. Ekstraksi DNA menggunakan DNAzol, kuantifikasi menggunakan spektrofotometer UV, dan amplifikasi DNA menggunakan primer STR yaitu lokus TPOX dan TH01. Untuk visualisasi DNA menggunakan gel akrilamida. Hasil rata-rata kadar DNA sidik bibir selama 1, 3, dan 7 hari adalah 369,82 g/ml, 550,72 g/ml, 318,02 g/ml. Hasil rata-rata kemurnian DNA sidik bibir selama 1, 3, dan 7 hari adalah 1,79; 1,78 dan 1,79. Dari hasil kuantifikasi DNA diambil kadar DNA terendah dan tertinggi pada hari ke-1, 3, dan 7. Dari 6 sampel dan 3 kontrol yang diamplifikasi menggunakan lokus TPOX dan TH01, hasilnya terlihat jelas pada pita gel akrilamida.

***Kata Kunci**: DNA, Forensik, Lip Print, STR*

Abstract

Forensic experts in uncovering the truth of a case must go through identification, documentation, and collection of evidence. Sometimes at a crime scene, lip prints are found on the surface of an object such as the mouth of a plastic bottle. Lip print research on plastic bottle mouths was carried out for 1, 3 and 7 days. There are 21 samples with details of 3 as controls, 6 samples for the first day, 6 samples for the 3rd day, and 6 samples for the 7th day. DNA extraction using DNAzol, quantification using UV spectrophotometer, and DNA amplification using STR primers, namely TPOX and TH01 loci. For DNA visualization using acrylamide gel. The average results of lip print DNA levels for 1, 3, and 7 days were 369.82 g/ml, 550.72 g/ml, 318.02 g/ml. The average yield of lip print DNA purity for 1, 3, and 7 days was 1.79; 1.78 and 1.79. From the results of DNA quantification, the lowest and highest DNA levels were taken on days 1, 3, and 7. Of the 6 samples and 3 controls amplified using the TPOX and TH01 loci, the results were clearly visible on the acrylamide gel band.

Keywords: DNA, forensic, lip print, STR,

1. INTRODUCTION

Criminal cases that occur will be processed by the authorities so that the victims and perpetrators in this case can be revealed. Identification is useful for determining the identity of the perpetrator in justice process. Based on Disaster Victim Investigation (DVI) guidelines 2009 was stated that there are two types of identification, namely primary identification and secondary identification. Primary identification covers inspection urine accumulation. (fingerprint), dental record, and inspection of Deoxyribonucleic Acid (DNA). Secondary identification includes medical and property (Prawestiningtyas and Algozi, 2009). At the crime scene, in some medicolegal cases such as rape, robbery and

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hanging, bloodstains may not be found. Saliva is a liquid that is often found on the bodies of criminal victims and also as evidence that found at crime scenes, both in wet and dry form. Saliva is found can from lip prints on plastic packaging of bottled drinking water.

Each individual has characteristics physical that different with other individuals. One physical characteristic that can be identified in an individual is lip print. Lip print is just like fingerprints that has different gene expression in each individual. Lip prints have that structure unique and will not be changed for the whole life. The outer surface of the lips has many elevations and indentations form called feature patterns lips, lip prints can be obtained on clothing, glasses, glasses, cigarettes, windows and doors (Dineshshankaret al., 2013).

2. HEADING

According to Tsuchihashi, lip prints can be inherited from parents, but there are similarities in the lip prints of each individual. According to Mc Donell two physically indistinguishable identical twins haveLip Print different (Reddy, 2011; Evirilia, Sam and Oscandar, 2015).

Examination of lip prints using STR primers really needs to be done as a reference if a criminal case occurs and it is difficult to identify the victim or perpetrator.

Research on the TH01 and TPOX loci was carried out by Toetik Koesbardiati et al to detect the Gilimanuk human skeleton, Bali Province, to see genetic variation (Koesbardiatiet al., 2013). These two loci have also been widely studied, for example to find out the frequency of alleles in the Minangkabau ethnicity (Hidayat and Susanti, 2018), Bangladeshi and Japanese ethnicities (Kidoet al., 2003), genetic variation in Thai people (Rerkamnuaychokeet al., 2006), and genetic variation in the Taiwanese population (Puet al., 1998).

Research on the TH01 and TPOX loci was carried out by Toetik Koesbardiati et al to detect the Gilimanuk human skeleton, Bali Province, to see genetic variation (Koesbardiatiet al., 2013). These two loci have also been widely studied, for example to find out the frequency of alleles in the Minangkabau ethnicity (Hidayat and Susanti, 2018), Bangladeshi and Japanese ethnicities (Kidoet al., 2003), genetic variation in Thai people (Rerkamnuaychokeet al., 2006), and genetic variation in the Taiwanese population (Puet al., 1998).

Research by Retno et al (2015) about the effect of storage time on DNA content and purity inearphone with a duration of 1,3, and 5 days show relatively the same results or do not experience a significant difference. These samples were amplified using STR loci D18S51 and D21S11 which also showed clear bands on acrylamide gel (arimurti retno, Yudianto and astuti W, 2015). Another study on the effect of storage time on DNA levels and purity was carried out by Yudianto et al (2016) using samplesearphone for 1, 7, 14, and 20 days which were detected using mitochondrial DNA. The results showed that detection using mitochondrial DNA was found on day 1 (Yudianto, Sispitasri and Margaret, 2016).

3. RESEARCH METHOD

The sample used in the research is Lip print attached on a plastic bottle. Lip print came from 6 female volunteers with the inclusion criteria of healthy conditions, namely not experiencing swelling, lesions, or other pathological conditions in the mouth area. This research has been deemed ethically feasible, issued by the ethics committee of the Faculty of Dentistry, Airlangga University with the number: 002/HRECC.FODM/1/2022. The number of replications was 3 times for each treatment group. Then the total sample size required is 18 replications obtained from the number of replications multiplied by the number of group divisions, 6 x 3 = 18

replications and added 3 samples as controls so that the total is 21 samples. Sample preparation i.e Pellets from sample results lip print which had been incubated for 18 hours were put into a labeled centrifuge tube. Then DNA isolation was carried out using DNAzol or using the phenol chloroform method (Chenet al., 2010). DNA quantification was carried out using a UV-spectrophotometer to determine the level and purity of DNA (Doshiet al., 2010). DNA amplification using STR primers locus TPOX and TH01 with a predetermined protocol (Chunget al., 2004; Coble and Butler, 2005). PCR results will be visualized using acrylamide gel and photographed using a camera (Jainet al., 2010).

4. RESULTS AND DISCUSSION

DNA content was calculated from the multiplication of OD260, dilution factor, and 50 µl/ml measured with a Uv-spectrophotometer, the measurement results expressed in ng/µl or µg/ml with a ratio measuring scale. DNA levels will be described in the control group and also in the long treatment of 1 day, 3 days and 7 days of storage. The results of the description of DNA levels (ng/µl) are presented in Table 4.1

Table 4.1 results of DNA content Lip Print

Perlakuan	*N*	*Minimum*	*Maximum*	*Mean*	*SD*
Control	3	102,07	192,06	152,39	45,93
Day 1	6	172,54	502,95	369,82	117,85
Day 3	6	336,64	1046,47	550,72	251,75
Day 7	6	229,28	454,34	318,02	79,10
Total sample	21	102,07	1046,47	375,64	196,90

Table 4.1 shows that DNA content tends to increase with increasing storage time. In the control, the average DNA content was 152.39 ng/µl, then increased to 369.82 ng/µl after being stored for 1 day, then increased again to 550.72 ng/µl after being stored for 3 days, but when storage time of 7 days, the average DNA content decreased to 318.02 ng/µl. During 1 day of storage, there was an increase in DNA content of 142.7% compared to the initial time, then the DNA content increased again by 48.9% after 3 days of storage, and after that there was a decrease in DNA content of 42.3% after storage for 7 day.

DNA purity is generated by calculating the OD ratio260 day OF280 which is measured withUv-spectrophotometer. DNA purity will also be described in the control group and also in the long treatment of 1 day, 3 days and 7 days of storage. The results of the description of DNA purity are presented in Table 4.2.

Tabel 4.2 Result of DNA Purification

Treatment	*N*	*Minimum*	*Maximum*	*Mean*	*SD*
Control	3	1,56	1,68	1,62	0,06
Day 1	6	1,57	1,90	1,79	0,11
Day 3	6	1,61	1,86	1,78	0,11
Day 7	6	1,74	1,88	1,79	0,05
Total sample	21	1,56	1,90	1,76	0,10

Table 5.2 shows that the purity of DNA tends to increase with increasing storage time, although the changes are relatively small. In the control, the average DNA purity was 1.62, then increased to 1.79 after being stored for 1 day, then slightly decreased to 1.78 after being stored for 3 days, and increased again when the storage time was 7 days to 1 ,79. During 1 day of storage, there was an increase in DNA purity of 10.5% compared to the beginning, then the DNA content decreased slightly by only 0.6% after 3 days of storage, but after that there was an increase in DNA purity of only 0.6% after stored 7 days.

The samples analyzed for locus were samples with the highest and lowest DNA levels on each treatment day. So that for one locus there are seven (7) samples examined

with details of six (6) samples and one (1) control examined.

The visualization results of the PCR locus TPOX products are shown in Figure 4.1

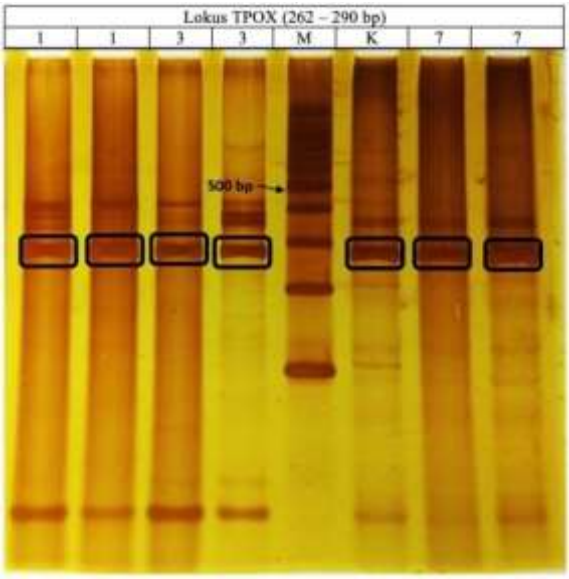

Figure 4.1 results of visualization of TPOX locus PCR products

PCR product DNA size (bp) for the TPOX locus: 262 - 290 bp. The picture above shows the results of the PCR amplification of the TPOX locus on Lip Print samples attached to plastic bottles from the 1st, 3rd, and 7th days, bands andband DNA. From the picture it can be seen that the DNA bands of each sample are at the position of the TPOX locus which can be said that the DNA was successfully amplified using PCR

The visualization results of the TH01 locus PCR products can be seen in Figure 4.2

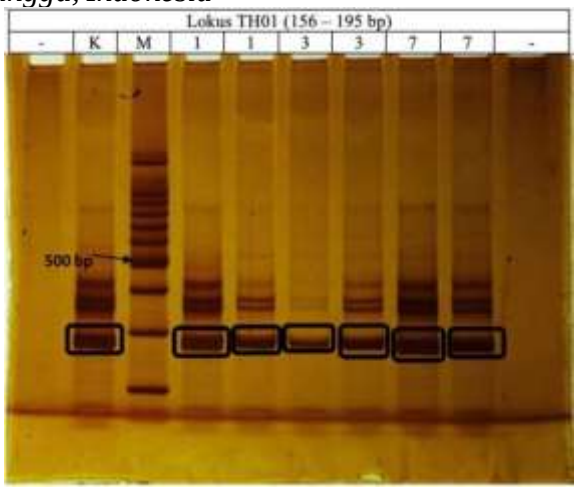

Figure 4.2 results of visualization of PCR products TH01 locus

PCR product DNA size (bp) for the TH01 locus: 156-195 bp bp. Figure 5.5 above shows the results of the TH01 locus PCR amplification of the Lip Print sample attached to a plastic bottle from the 1st, 3rd and 7th day, bands andband DNA. From Figure 5.5 it can be seen that the size of the DNA bands for each sample is different, but it can be said that the DNA was successfully amplified using PCR.

The research results obtained from the effect of differences in sample storage times of 1, 3, and 7 days on the average DNA purity between 1.62 – 1.79. With these results, the requirements for DNA purity for DNA amplification have been fulfilled. DNA purity in this study increased on day 1, decreased on day 3 and increased on day 7. The average DNA purity on the first day was 1.79; third day 1.78; and seventh day 1.79. Although there was a decrease on day 3 and an increase on day 7, the purity of the DNA was still in the category of pure DNA, so it could be used for further analysis.

DNA damage caused by abnormal exposures such as high temperatures results in irreversible damage to the purine – pyrimidine wet hydrogen bonds in DNA (Kusumadewi, Kusuma and Yudianto, 2012).

Damage to DNA from the outside can cause damage to the DNA chain,

damage to DNA bases (Base Damage), Sugar Damage. In addition, DNA damage can be affected by the degradation process by bacteria. Research conducted by Chen showed that the number of bacteria in air-conditioned and ventilated rooms ranged from 50-130 colonies/m², whereas in non-air-conditioned and ventilated rooms it ranged from 7-25 colonies/m². Air circulation in air-conditioned rooms The air conditioner tends to be closed, this condition prevents air from leaving the room and vice versa, so that the bacteria in the air remain in the room (Chenet al., 2010).

In another study, it was found that the required DNA level in the amplification examinationPolymerase Chain Reaction (PCR) The minimum STR is in the range of 0.5 – 2.5 ng (sosiawan, 2007). In addition to the level of sample DNA in PCR-based DNA testing, it is also necessary to consider sufficient DNA quality. The quality of the DNA in question is that the DNA used in the analysis must be in a non-degradable condition. If the DNA is severely degraded, it will result in the primer not being able to attach to the processannealing) on the DNA target to be multiplied (Kusumadewi, Kusuma and Yudianto, 2012).

5. CONCLUSIONS AND SUGGESTIONS

The conclusion of this study is the sample lip print can be detected using primers TPOX and TH01. Samples that have low grades and purity are influenced by external and internal factors so that they will experience DNA degradation. Suggestions for other researchers are to be able to use other STR loci to examine samplesLip Print so that it can add to the literature for further research in the field of forensics.

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BIBLIOGRAPHY

arimurti retno, anindita riesti, Yudianto, A. and astuti W, S. puji (2015) 'Identifikasi DNA Dari Swab Earphone Dengan Teknik Str (Short Tandem Repeat) Untuk Kepentingan Forensik', *Jurnal Biosains Pascasarjana*, 17(1), p. 26. doi: 10.20473/jbp.v17i1.2015.26-32.

Butler, J. M. (2006) 'Genetics and Genomics of Core Short Tandem Repeat Loci Used in Human Identity Testing', 51(2). doi: 10.1111/j.1556-4029.2006.00046.x.

Butler, J. M. (2011) *Advanced Topics in Forensic DNA Typing : Methodology*. U.S.A.

Chen, H. *et al.* (2010) 'Evaluation of five methods for total DNA extraction from western corn rootworm beetles', *PLoS ONE*, 5(8), pp. 1–6. doi: 10.1371/journal.pone.0011963.

Chung, D. T. *et al.* (2004) 'A Study on the Effects of Degradation and Template Concentration on the Amplification Efficiency of the STR Miniplex Primer Sets', *Journal of Forensic Sciences*, 49(4), pp. 1–8. doi: 10.1520/jfs2003269.

Coble, M. D. and Butler, J. M. (2005) 'Characterization of New MiniSTR Loci to Aid Analysis of Degraded DNA ∗', 50(1).

Dineshshankar, J. *et al.* (2013) 'Lip prints: Role in forensic odontology', *Journal of Pharmacy and Bioallied Sciences*, 5(SUPPL.1), pp. 95–98. doi: 10.4103/0975-7406.113305.

Doshi, R. *et al.* (2010) 'Spectrophotometric analysis of nucleic acids: Oxygenation-dependant hyperchromism of DNA', *Analytical and Bioanalytical Chemistry*, 396(6), pp. 1–19. doi: 10.1007/s00216-010-3461-x.

Evirilia, E., Sam, B. and Oscandar, F. (2015) 'Lip print pattern identification of Deutromalayan subrace using a modification of lip print formulation technique as forensic odontology application', *Padjadjaran Journal of Dentistry*, 27(1), pp. 60–65. doi: 10.24198/pjd.vol27no1.26680.

Hidayat, T. and Susanti, R. (2018) 'Analisis genetik lokus CSF1PO, TH01, dan TPOX short tandem repeats pada etnis minangkabau', *Majalah Kedokteran Andalas*, 41(3), pp. 94–100. doi: 10.25077/mka.v41.i3.p94-100.2018.

Jain, S. *et al.* (2010) 'Diagnosis of down syndrome and detection of origin of nondisjunction by short tandem repeat analysis', *Genetic Testing and Molecular Biomarkers*, 14(4), pp. 489–491. doi: 10.1089/gtmb.2009.0191.

Kido, A. *et al.* (2003) 'Allele frequency distributions of four STR loci vWA , TH01 , TPOX and F13A01 in three Asian populations (Japanese , Bangladeshis and Indonesians)', 1239, pp. 105–108.

Koesbardiati, T. *et al.* (2013) 'Loci STR CODIS (TH01,TPOX) Genetic Variation on Gilimanuk Man (Bali Islands)', 33(2), pp. 133–150.

Krenke, B. E. *et al.* (2002) 'Validation of a 16-Locus Fluorescent Multiplex System *', 47(4), pp. 1–13.

Kusumadewi, A., Kusuma, S. E. and Yudianto, A. (2012) 'The Analyze of Human DNA Soft Tissue that Contaminated Formalin During 1 Month and 6 Month at Locus D13S317 using STR-PCR', *JBP*, 14(2), pp. 115–121.

Prawestiningtyas, E. and Algozi, A. M. (2009) 'Forensic Identification Based on Both Primary and Secondary Examination Priority in Victim Identifiers on Two Different Mass Disaster Cases', *Jurnal Kedokteran Brawijaya*, 25(2), pp. 87–94.

Pu, C. E. *et al.* (1998) 'DNA short tandem repeat profiling of Chinese population in Taiwan determined by using an automated sequencer', *Forensic Science International*, 97(1), pp. 47–51. doi: 10.1016/S0379-0738(98)00131-5.

Reddy, L. V. K. (2011) 'Lip prints: An Overview in Forensic Dentistry', *Journal of Advanced Oral Research*, 2(1), pp. 17–20. doi: 10.1177/2229411220110104.

Rerkamnuaychoke, B. *et al.* (2006) 'Thai population data on 15 tetrameric STR loci - D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818 and FGA', *Forensic Science International*, 158(2–3), pp. 234–237. doi: 10.1016/j.forsciint.2005.05.020.

Yudianto, A., Sispitasri, Y. E. and Margaret, N. (2016) 'Analysis of Earphone Swab Mitochondrial Dna As an Alternative Material for Identification Examination', *Folia Medica Indonesiana*, 52(3), pp. 169–173. doi: 10.20473/fmi.v52i3.5446.