

Original Article

Characterization of DNA Quality, TPOX Locus, TH01 Locus in Seawater Soaked Nails in Forensic Identification

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ABSTRACT

Forensic science is multiplying. One of them is the identification of events at sea. Of course, the victim's body can be submerged in seawater for days so that components in seawater can affect the victim's body. This research aimed to see the effect of storage time on the level and purity of DNA in nail samples. Identification of nail DNA using the Polymerase Chain Reaction (PCR) method. The DNA purity test examines nail samples using a spectrophotometer read at 260 and 280 nm wavelengths. Measurement of DNA levels using the PCR instrument on the STR TPOX locus, the result was 32.02 ug/uL on positive control, 36.73 ug/uL in seawater immersion on the 2nd day, 35.35 ug/uL in seawater immersion on the 7th day, and 34.5 ug/uL in seawater immersion on the 20th day. At the STR TH01 locus, the result was 32.155 ug/uL on positive control, 38.05 ug/uL on the 2nd day of immersion in seawater, 35.18 ug/uL on the 7th day, and 33.88 ug/uL on the 20th day. Results Examination of the Total Plate Number of seawater on the 2nd day, 7th day, and 20th day of immersion found no colonies of germ colony growth. This study obtained sufficient DNA purity from the 2nd day, 7th day and 20th day. So that the samples could be analyzed for DNA levels, the result obtained from the study showed a decrease in DNA levels at the TPOX Locus and TH01 Locus from the 2nd day, 7th day, and 20th day.

Keywords: DNA, Locus, TPOX, TH01, Seawater

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INTRODUCTION

The geographical factors above provide a problem and a challenge in every incident. Of course, fast and precise transportation is needed to connect islands using air and sea transportation, so air and sea traffic will become crowded with this trip ¹. Seawaters have a wide variety of areas and have different characteristics from each of its waters. Of course, this will significantly impact everything that might happen, like the plane crash and ship

accident ². Airplanes often cross the ocean, and the State of Indonesia is an archipelago. However, this flight route has been carefully chosen by experienced experts because there are not just any places that airplanes can fly over ³. Airplane that are usually used as flight routes are usually routes with calm winds, rarely have storms, and are far from dangerous mountains. An airplane crash is an event that is certainly not desirable, the causes of which are, of course, also from many factors. The location of the accident can be in the air, land, or sea; of course,

retrieving the debris from the accident will be more challenging if the accident is at sea ⁴.

In another study, forensic analysis using nail material is an essential source of DNA. This relates to the composition and structure of the nail DNA located in the cells' keratin. The DNA extraction is more complex than those commonly used with fresh somatic cells ⁵.

Seawater's composition, a mixture of 96.5% pure water and 3.5%, consists of dissolved mineral salts such as sodium, calcium, magnesium, Chloride, and sulfate. Seawater tastes salty because it contains a salt/salinity level of 3.5%, and the content of each sea is different. It is from the composition contained in seawater that it is possible to influence the identification of the victim's body parts. This is supported by other studies so that seawater pH levels are 5.50 and NaCl levels are 1,652.93 mg/L ⁶.

Seawater affects the decomposition of corpses and creates a potential difference due to the salt content in the water. Odontological identification can be in the form of DNA analysis of the dental pulp because the dental pulp is protected by hard tissues such as dentine and enamel, which makes the pulp able to protect DNA. Still, enamel is semipermeable, which can affect the degree of extraction of DNA and its purity. Of course, teeth are one of the possible pieces of the victim's body that can be found. This possibility can happen to other body parts, such as nails ⁷.

The percentage of success in DNA analysis of nail samples soaked in distilled water and sea water showed 100% results from 5 individual samples with a research period of 1-3 months ⁸.

The Federal Bureau of Investigation (FBI) and the world's forensic expert community designed 13 locus as a national forensic identification system that synergizes with the Combined DNA Index System (CODIS) database. For personal identification, the researcher chose the TPOX and TH01 locus because several studies have shown that the TPOX and TH01 locus have strong discriminating power in Asian populations ⁹.

This research aimed to see the effect of storage time on the level and purity of DNA in nail samples.

METHOD

The research material comes from the nails of one individual, a living human female. They have collected nail clippings every week for several months. The nail samples were immersed in seawater in glassware at room temperature. On the 2nd day, a portion of the sample was taken as the 2nd day immersion treatment. On the 7th day, a portion of the sample was taken as the 7th-day immersion treatment; on the 20th day, a portion of the sample was taken as the 7th-day immersion treatment 20th. A positive control was also carried out, i.e., nails without seawater immersion. Negative control in this research using seawater. All the test materials above were placed in different mortars and crushed until smooth. The tested materials that had been refined continued to be analyzed quantitatively and molecularly together on the 20th day.

In this study, the spectrophotometer instrument model Maestronano SN 667100070821 brand Maestrogen was used to analyze the purity of DNA. Meanwhile, to analyze the quantity of DNA, a PCR instrument was used with the CFX Opus 96SN 795BR03572 model, the Bio-Rad brand.

RESULTS

Overview of DNA quality

In testing the purity of the nail DNA samples, the spectrophotometric method was used with a spectrophotometer with the Maestronano model, which was read at a wavelength of 260 nm and 280 nm.

Table 1. DNA Purity Test of nail samples

Treatment	Day	DNA Purity		
		A260	A280	A260/A280
Seawater immersion	2	0.022	0.011	1.886
	7	0.015	0.009	1.819
	20	0.027	0.014	1.839

Quality overview of the TPOX Locus and TH01 Locus

At the STR TPOX locus, the result was 38.93 ug/uL in seawater immersion on the 2nd day, 35.51 ug/uL in seawater immersion on the 7th day and 34.04 ug/uL in seawater immersion

on the 20th. At the STR TH01 locus, the result was 37.09 ug/uL on the 2nd day of immersion in seawater, 34.51 ug/uL on the 7th day of seawater immersion and 34.15 ug/uL on the 20th day.

The positive control (nail without immersion in seawater and examined on the 20th day) for the TH01 locus was 32.15 ug/uL. On the 2nd day of immersion, the results were 38.05 ug/uL, an increase because the nail cells were damaged when exposed to seawater immersion. Even though the DNA content was higher than the positive control, the longer the immersion, the more step by step the DNA content decreased, as was the case on the 7th day of immersion; the DNA level was 35.18 ug/uL, and on the 20th day of immersion, the DNA level was 33.88 ug/uL.

Table 2. STR CODIS TPOX & TH01 nail samples

	Day	DNA level (ug/uL)	
		Locus TH01	Locus TPOX
Positive control		31.73	31.42
		32.57	32.62
Seawater immersion	2	38.93	37.09
		37.17	36.37
	7	35.51	36.20
		34.86	34.51
	20	34.08	34.85
		33.68	34.15

The positive control (nail without immersion in seawater and examined on the 20th day) for the TPOX locus was 32.02 ug/uL. On the 2nd day of immersion, the results were 36.73 ug/uL, an increase; this was also because the nail cells were damaged when exposed to seawater immersion. Even though the DNA content was higher than the positive control, with prolonged immersion, step by step, the DNA content decreased as was the case on the 7th day of immersion; the DNA level was 35.35 ug/uL, and on the 20th day of immersion the DNA level was 34.50 ug/uL.

In this study, a quantitative examination was also carried out on STR TPOX Locus and TH01 STR Locus on distilled water as a negative control and quantitative examination of TPOX STR Locus and TH01 STR Locus on seawater as the control variable of this study.

Table 3. STR CODIS TPOX & TH01 without nail samples in Aqua distillate and Seawater

Sample	DNA Level (ug/uL)	
	Locus TH01	Locus TPOX
Aqua distillate	0,00	0,00
Seawater	0,00	0,00

The table above shows the results of the CODIS TPOX STR and the CODIS TH01 STR, which result in distilled water and seawater with a value of 0.00. This illustrates that aqua distillate as a negative control does not affect the quantitative results of the test material.

Analysis of Total Plate Number as a control variable

In this study, control variables were also examined, such as the presence of bacteria that had the potential to grow in seawater media due to the immersion of the nail samples not in the condition of being directly in the sea but conditioned by immersion in seawater in glassware containers stored in the laboratory at room temperature. Results Examination of the Total Plate Number of seawater on the 2nd day, 7th day and 20th day of immersion found no colonies of germ colony growth.

DISCUSSION

This study uses the time difference on the 2nd day, 7th day and 20th day. The choice of the time difference is based on the 2nd day, namely the start of the identification process at the crime scene. The 2nd day is part of the Submerged Fresh phase, at this stage, the period between the body sinking in the water until it starts to float. Water is at this stage that the body is likely to be found. The 7th day is the latest (maximum) deadline for investigators/police to carry out a series of identification processes at the crime scene after conducting a preliminary examination. This is by the Regulation of the Head of the State Police of the Republic of Indonesia Number 14 of 2012 concerning Criminal Investigation Management ¹⁰.

Meanwhile, the 20th day is the time limit for investigators to conduct investigations. Still, if the case still needs to fill out the complete files, it can be extended for the investigation process at the TKP for a maximum of 40 days with the permission of the public prosecutor ¹¹.

Short Tandem Repeats (STR) is an uncoding region found in the core DNA and consists of 2-7 nucleotide sequences arranged repeatedly. The size of STR fragments is usually at most 500 bp, therefore, STR can be amplified using a relatively small amount of template DNA and can be used to analyze degraded DNA samples. Using 13-20 STR locus, a person's identity can be determined¹².

DNA in nails is very easy to extract with commercial DNA extraction kits. Effects of the environment such as exposure to seawater can affect the quantity of DNA in nails. This is because nails become more weathered when submerged in seawater. The cell structure in nail DNA extraction is a separation of DNA from other sample contents to produce pure DNA. Extracted DNA can be sourced from blood, sperm, bones, teeth, hair, saliva, urine, feces, and nails¹³.

Several DNA extraction methods are commonly used, although the basic principle of all these methods is the same: to separate proteins and other materials from DNA molecules. In addition, the basic steps in DNA extraction are, first, releasing DNA molecules by lysing cells, second, separating DNA molecules from other cellular material, third, isolating DNA so that it allows Polymerase Chain Reaction (PCR) amplification to be carried out¹⁴.

The PCR method can amplify the target DNA sequence in tiny amounts even from a single cell. The results of the above study showed that the quantity of DNA content in nails decreased in proportion to the length of immersion in seawater, this was due to abnormal exposures from the surrounding environment, resulting in irreversible damage to DNA hydrogen bonds. This condition damaged the purine-pyrimidine pair in DNA, where this purine-pyrimidine pair is the main component of the DNA structure¹⁵.

In this study, the Total Plate Number was also analyzed as a control variable. This was intended because immersion in seawater was carried out in glassware containers stored in the laboratory at room temperature, not in seawater conditions by general field conditions. The total plate number did not find the growth of bacterial colonies, this proves that bacteria have no effect on immersion in seawater with glassware containers stored in the laboratory: 1 mL or 1 gram of sample examined. The Total Plate number uses a pour plate technique

because, in this technique, counting the number of bacterial colonies in nutrient media is more straightforward. It does not collect on one side only when compared to spread technique (spread plate)¹⁶.

CONCLUSION

It is concluded that there is an overview of the pattern of degradation of the quality of DNA in nails with time of immersion in seawater, which was analyzed using the spectrophotometric method. There was an illustration of a decreasing pattern of nail DNA levels, amplified at the STR TPOX Locus & STR Locus TH01 by the PCR method.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Viatte C, Clerbaux C, Maes C, Daniel P, Garello R, Safieddine S, Arduin F. Air pollution and sea pollution seen from space. *Surveys in Geophysics*. 2020 Nov;41:1583-609.
2. Stramski D, Boss E, Bogucki D, Voss KJ. The role of seawater constituents in light backscattering in the ocean. *Progress in Oceanography*. 2004 Apr 1;61(1):27-56.
3. Buntoro K. Rethinking Nusantara Indonesia: Legal Approach. *Indonesian J. Int'l L.* 2015;13:468.
4. Alberini A, Austin DH. Strict liability as a deterrent in toxic waste management: Empirical evidence from accident and spill data. In *Economics and Liability for Environmental Problems 2018* Jan 12 (pp. 397-425). Routledge.
5. Bukyya JL, Tejasvi MLA, Avinash A, P. CH, Talwade P, Afroz MM, et al. DNA Profiling in Forensic Science: A Review. *Glob Med Genet*. 2021 Dec;08(04):135-43.
6. Masri M. Desalination Of Sea Water

- Using Activated Zeolite By Chloride Acid In Tropical Area Based On Column Ion Exchange Method.
7. Thaiyibah N, Ightikhoma AR, Yudianto A. The Effect of Seawater on the Quantity of Dental Pulped DNA in Forensic Odontology Identification. In Scitepress; 2019. p. 440–5.
 8. Nakanishi A, Moriya F, Hashimoto Y. Effects of environmental conditions to which nails are exposed on DNA analysis of them. Available from: www.elsevier.com/locate/legalmed
 9. Yudianto A, Indah Nuraini M, Furqoni AH, Manyanza Nzilibili SM, Harjanto P. The use of touch DNA analysis in forensic identification focusing on Short Tandem Repeat-Combined DNA Index System loci THO1, CSF1PO and TPOX. *Infect Dis Rep.* 2020;12.
 10. Riyadi BS, Bambang Hermanto A, Harlina I, Purnomo H. Discretion of Power of the Indonesian National Police Impacts the Abuse of Power in the Case of Letter Forgery of Red Notice “Fugitive Djoko Tjandra” [Internet]. Vol. 9, *International Journal of Criminology and Sociology.* 2020. Available from: <https://kompas.id/baca/polhuk/2020/07/>
 11. Yanto M. Juridical study of the criminal act of defamation article 310 paragraph (1) of the Indonesian criminal law code (KUHP). 5(1).
 12. Tang H, Kirkness EF, Lippert C, Biggs WH, Fabani M, Guzman E, et al. Profiling of Short-Tandem-Repeat Disease Alleles in 12,632 Human Whole Genomes. *Am J Hum Genet.* 2017 Nov 2;101(5):700–15.
 13. Preuner S, Danzer M, Pröll J, Pötschger U, Lawitschka A, Gabriel C, et al. High-quality DNA from fingernails for genetic analysis. *Journal of Molecular Diagnostics.* 2014;16(4):459–66.
 14. Antoniou A, Magoulas A. Application of Mitochondrial DNA in Stock Identification. In: *Stock Identification Methods: Applications in Fishery Science: Second Edition.* Elsevier Inc.; 2013. p. 257–95.
 15. Jäger R. New Perspectives for Whole Genome Amplification in Forensic STR Analysis. Vol. 23, *International Journal of Molecular Sciences.* MDPI; 2022.
 16. Sanders ER. Aseptic laboratory techniques: Plating methods. *Journal of Visualized Experiments.* 2012 May 11;(63):1–18.