Research Paper

Impact of Environmental Exposure on Mitochondrial DNA Degradation in Medical Masks: A Forensic Evaluation of Storage Time and Amplicon Length

Baety Adhayati[1*], Rizqi Fithratullah[2], Luluk Hermawati[3], Yuda Nabella Prameswari [3], Abdul Hadi Furqoni[4], Alvina Setiawardani[5], Zakkiyatus Zainiyah[6]

1. *Department of Humanities and Medicolegal, Faculty of Medicine, Universitas Sultan Ageng Tirtayasa, Banten, Indonesia.*
2. *Forensic Science Program, Postgraduate School, Universitas Airlangga, Surabaya, Indonesia.*
3. *Department of Medical Biology, Faculty of Medicine, Health Sciences, and Nursing, Universitas Sultan Ageng Tirtayasa, Banten, Indonesia*
4. *Center for Biomedical Research, Research Organization for Health, National Research and Innovation Agency (BRIN), Cibinong, Indonesia*
5. *Forensic Science Program, School of Health Science, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia*
6. *Master of Health Administration, Noor Huda Mustofa University, Madura, Indonesia*

Citation Adhayati B, Fithratullah R, Hermawati L, Prameswari YN, Furqoni AH, Setiawardani A, et al. Impact of Environmental Exposure on Mitochondrial DNA Degradation in Medical Masks: A Forensic Evaluation of Storage Time and Amplicon Length. International Journal of Medical Toxicology and Forensic Medicine. 2026; 16:E51909.

https://doi.org/10.22037/ijmtfm.v16.51909

Article info:

Received: 25 February, 2026

Accepted: 01 June, 2026

Published: 21 August, 2026

Keywords:

DNA degradation, Forensic identification, Mask, Mitochondrial DNA (mtDNA)

ABSTRACT

Background: Medical masks recovered from crime scenes are valuable sources of biological evidence, yet the stability of DNA on these porous substrates under environmental stress remains under-researched. This study investigates the degradation profile of mitochondrial DNA (mtDNA) in cotton-polyester blend masks under outdoor conditions, hypothesizing that prolonged exposure accelerates hydrolytic damage and preferentially affects larger amplicons.

Methods: Medical masks worn by six male volunteers were stored outdoors (relative humidity 56–81%) for 1, 7, 14, and 20 days. DNA was extracted using the DNAzol method and quantified by UV spectrophotometry. mtDNA integrity was assessed by amplifying HVS-I (143 bp) and HVS-II (126 bp) regions. Statistical significance was determined using One-way ANOVA.

Results: Total DNA concentration significantly decreased by ~96% from Day 1 (373.58 ± 211.17 ng/µl) to Day 20 (14.07 ± 5.16 ng/µl) ($p<0.05$). DNA purity followed a similar downward trend, dropping from 1.84 ± 0.07 (Day 1) to 0.89 ± 0.43 (Day 20), indicating severe contamination or degradation. Amplification success was length-dependent: the longer HVS-I fragment failed to amplify after Day 1, whereas the shorter HVS-II fragment remained detectable up to Day 14.

Conclusion: Outdoor environmental exposure markedly reduces DNA quantity and purity within 20 days, likely driven by humidity-induced hydrolysis and bacterial growth. The differential survival of amplicons suggests that forensic protocols for degraded mask evidence should prioritize shorter amplicons (e.g., HVS-II) and implement immediate preservation measures, such as desiccation or refrigeration, to halt decay.

*** Corresponding Author:**
Baety Adhayati
Department of Humanities and Medicolegal, Faculty of Medicine, Universitas Sultan Ageng Tirtayasa, Banten, Indonesia.
E-mail: *baety.adhayati@untirta.ac.id*

Introduction

Masks are widely recognized as personal protective equipment designed to protect users from airborne particles, droplets, and aerosols that may threaten human health [1]. Although their use has declined since the peak of the COVID-19 pandemic, masks have become integrated into daily life and are still commonly used in certain settings [2]. Beyond their protective function, masks also carry forensic relevance. Criminals frequently wear masks to conceal their identities during unlawful activities [3], which makes the recovery of masks at crime scenes a plausible scenario in forensic investigations.

Forensic scientists can exploit masks as valuable sources of biological traces. DNA can be obtained from saliva stains deposited through breathing or speaking, sweat absorbed into the mask material, or touch DNA transferred from the skin [4, 5]. Among different genetic markers, mitochondrial DNA (mtDNA) is often targeted in forensic analysis, particularly when nuclear DNA is insufficient or degraded. This preference arises from the higher copy number of mtDNA per cell and its greater stability under adverse conditions compared to nuclear DNA [6, 7].

Despite its advantages, mtDNA is not immune to degradation. Environmental factors such as ultraviolet (UV) radiation, temperature fluctuations, humidity, and prolonged storage can lead to progressive DNA damage [8, 9]. Such degradation reduces DNA concentration and purity, which in turn compromises the success of downstream analyses such as amplification and sequencing [10].

The substrate of the evidence also plays a critical role in DNA retention and stability. The medical masks used in this study are composed of a polyester blend with over 50% cotton. Recent literature suggests that fabric type influences DNA recovery; cotton has better absorbency and holds moisture longer than pure synthetic fibers like polyester. This moisture retention in outdoor settings may accelerate hydrolytic damage compared to non-absorbent surfaces [10, 11]. While previous studies have examined DNA on various fabrics, limited research has specifically addressed the temporal stability of mtDNA on medical masks exposed to outdoor environmental stress

Therefore, this study addresses this gap by evaluating the degradation profile of mtDNA on masks. We hypothesized that prolonged outdoor storage would significantly accelerate the reduction of DNA quantity and purity due to the combined effects of humidity and temperature. Furthermore, we predicted that degradation would affect amplicon success in a length-dependent manner, with longer mtDNA targets (HVS-I) failing to amplify earlier than shorter targets (HVS-II). By establishing the relationship between storage duration and DNA integrity, these findings aim to inform forensic protocols on the viable time window for recovering mask evidence.

Materials and Methods

Ethical Approval and Sample Collection

This study was designed as a preliminary investigation and received ethical approval from the Health Research Ethical Clearance Commission of the Faculty of Dental Medicine, Universitas Airlangga (No. 1229/HRECC.FODM/XI/2023). Medical masks were collected from six male volunteers aged 20–30 years. To simulate realistic usage, volunteers wore the masks for a duration of eight hours prior to collection. While the sample size (n=6) limits broad generalization, it was deemed sufficient for this pilot analysis to establish degradation trends.

Environmental Exposure and Storage

To evaluate the impact of environmental stress, the mask samples were divided into four groups corresponding to storage durations of 1, 7, 14, and 20 days. Unlike in controlled indoor storage, samples were stored outdoors to expose them to natural fluctuations in temperature and humidity. The environmental conditions during the storage period showed a relative humidity range of 56–81%, which facilitated hydrolytic degradation and potential bacterial growth.

DNA Extraction and Quantification

DNA extraction was performed using the DNAzol method following standard protocols [9]. To ensure the validity of the extraction process and monitor for cross-contamination, negative extraction blanks (negative controls) were processed alongside the samples. The concentration and purity of the extracted DNA were quantified using a UV spectrophotometer [12]. Purity was assessed based on the absorbance ratio, with values between 1.8 and 2.0 considered ideal for downstream PCR applications.

Table 1. Average Total DNA Concentration and DNA Purity in Mask Samples.

Storage Time (Days)	Mean DNA Concentration (ng/µl) ± SD	Mean DNA Purity ± SD
1	373.58 ± 211.17	1.84 ± 0.07
7	348.87 ± 51.15	1.78 ± 0.01
14	315.98 ± 3.007	1.72 ± 0.02
20	14.07 ± 5.16	0.89 ± 0.43

International Journal of
Medical Toxicology & Forensic Medicine

PCR Amplification

Mitochondrial DNA amplification was carried out using Polymerase Chain Reaction (PCR) targeting the Hypervariable Region I (HVS-I) and Hypervariable Region II (HVS-II) loci [13, 14]. The specific target sizes were 143 bp for HVS-I and 126 bp for HVS-II. The annealing temperature was set at 60°C for both loci. While this temperature follows standard protocols, it is noted that high annealing temperatures may affect the yield of degraded samples.

Electrophoresis and Visualization

The PCR products were analyzed using 1% agarose gel electrophoresis and visualized under a UV transilluminator [15]. The interpretation of amplification success was qualitative; samples were categorized as "positive" if a distinct DNA band was visible and "negative" if no bands were observed. A positive control (known DNA) and a negative control (PCR mix without template) were included in every run to verify the efficacy of the PCR and electrophoresis conditions.

Statistical Analysis

Data regarding DNA concentration and purity were expressed as Mean ± Standard Deviation (SD). Statistical differences between storage durations (Day 1 vs. Days 7, 14, and 20) were analyzed using One-Way ANOVA, with a p-value < 0.05 considered statistically significant, to confirm the degradation trends observed over the 20-day period.

Results

Total DNA Concentration and Purity

The quantification of DNA extracted from mask samples revealed a time-dependent degradation profile (Table 1). On Day 1, the mean total DNA concentration was recorded at 373.58 ± 211.17 ng/µl. This yield gradually decreased on Day 7 (348.87 ± 51.15 ng/µl) and Day 14 (315.98 ± 3.007 ng/µl). A marked reduction was observed by Day 20, where the concentration dropped significantly to 14.07 ± 5.16 ng/µl ($p<0.05$). The high standard deviation observed on Day 1 reflects inter-individual variability in shedding rates (saliva/touch DNA) among the volunteers.

DNA purity, measured by the A260/A280 ratio, followed a similar downward trend. Samples stored for 1, 7, and 14 days maintained purity ratios within the optimal range for PCR amplification (1.84 ± 0.07, 1.78 ± 0.01, and 1.72 ± 0.02, respectively). However, by Day 20, the purity ratio declined sharply to 0.89 ± 0.43, indicating the presence of contaminants such as proteins or phenols, likely exacerbated by bacterial growth in the outdoor environment.

Mitochondrial DNA Amplification (HVS-I and HVS-II)

The success of mitochondrial DNA amplification varied with amplicon length and storage duration. For the longer HVS-I locus (143 bp), a positive band was detected in only one sample (M6) on Day 1, which had the highest DNA concentration. No amplification bands were visible for HVS-I in samples stored for 7, 14, or 20 days, suggesting rapid degradation of this fragment length.

In contrast, the shorter HVS-II locus (126 bp) demonstrated greater persistence. Positive bands were observed in samples stored for 1, 7, and 14 days (Samples M5/M6 Day 1; M2/M3 Day 7; M1/M5 Day 14). However, by Day 20, no bands were detected for HVS-II, consistent with the significant drop in DNA quantity and purity observed at this time point. The negative controls included in the electrophoresis run showed no bands, confirming the absence of contamination during the PCR process.

Discussion

Degradation Dynamics of Total DNA Quantity and Purity

The present study revealed a distinct time-dependent degradation profile of DNA recovered from medical masks stored outdoors. While the initial total DNA yield on Day 1 was notably high (373.58 ng/µl), it is crucial to interpret this value with caution. As noted by Burrill et al. (2021) and Tozzo et al. (2022), DNA yield from worn items originates from

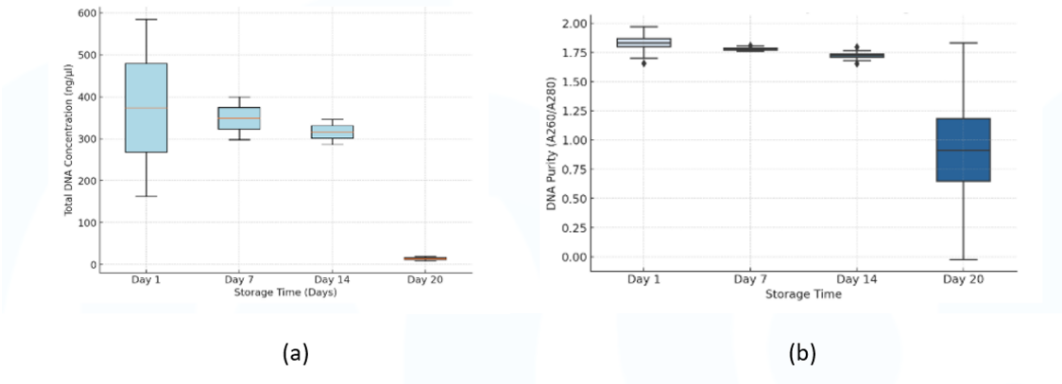

Figure 1. (a). Box Plot of Total DNA Concentration in Mask Samples. (b) Box Plot of Total DNA Purity in Mask Samples.

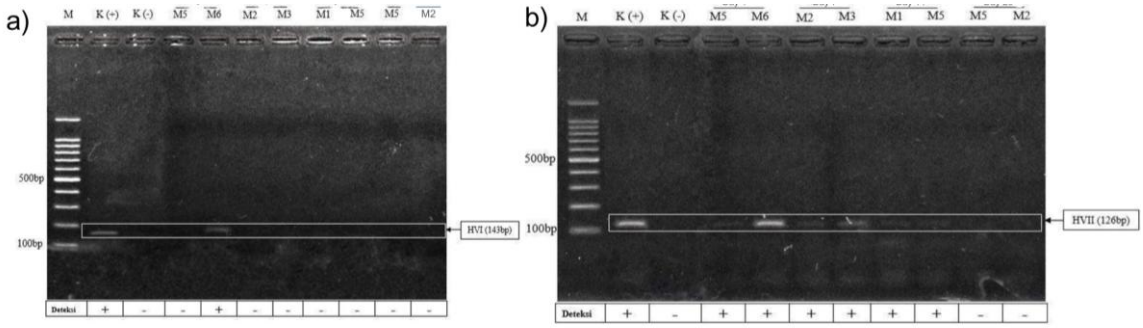

Figure 2. Image of mtDNA visualization results. (A) Locus HVI (Hypervariable Region I). (B) Locus HVII (Hypervariable Region II). The PCR visualization of HVI and HVII loci in mask samples over different storage durations. A faint band for HVI was detected only in the M6 day-1 sample, while HVII bands appeared in several samples up to day 14 but were absent on day 20.

Description: M: 1kb Marker, K (+): Positive Control, K (-): Negative Control, M5 Day 1: Sample with the lowest total DNA concentration and purity on Day 1, M6 Day 1: Sample with the highest total DNA concentration and purity on Day 1, M2 Day 7: Sample with the lowest total DNA concentration and purity on Day 7, M3 Day 7: Sample with the highest total DNA concentration and purity on Day 7, M1 Day 14: Sample with the lowest total DNA concentration and purity on Day 14, M5 Day 14: Sample with the highest total DNA concentration and purity on Day 14, M5 Day 20: Sample with the lowest total DNA concentration and purity on Day 20, M2 Day 20: Sample with the highest total DNA concentration and purity on Day 20

a mixture of keratinocytes, saliva, and sweat. However, the unusually high concentration coupled with the subsequent drop in purity to 0.89 on Day 20 strongly suggests a significant contribution from non-human DNA, particularly bacterial growth facilitated by the mask's moisture retention [16, 17].

Statistical analysis confirmed a significant reduction in DNA concentration by Day 20 ($p<0.05$) compared to Day This decay aligns with the degradation pattern observed in other forensic substrates, where environmental exposure leads to the rapid breakdown of cellular material. The purity drop observed on Day 20 indicates the accumulation of contaminants, such as phenols or protein byproducts from bacterial activity, that inhibit PCR amplification.

Mechanisms of Environmental and Fabric-Induced Degradation

The observed degradation can be attributed to the synergistic effects of the outdoor environment and the substrate's properties. The relative humidity recorded during the study (56–81%) likely promoted hydrolytic degradation, a primary mechanism where water molecules attack the glycosidic bonds of DNA, leading to depurination and single-strand breaks. This process is accelerated in outdoor settings compared to controlled indoor environments due to temperature fluctuations. As noted by Howlett et al. (2014), DNA degradation becomes significant at elevated

temperatures, particularly in samples lacking protective agents [18].

Furthermore, the substrate material played a critical role. The masks used were a polyester blend with over 50% cotton. Schulte et al. (2023) and Bhandari (2023) highlight that cotton possesses higher absorbency than synthetic fibers like polyester. While this absorbency initially traps biological material (saliva/sweat), it also retains environmental moisture, thereby creating a microenvironment conducive to hydrolytic damage and microbial proliferation. This explains the rapid decline in DNA quality relative to non-absorbent surfaces, as discussed in other studies [10, 11]. Additionally, although not quantified in this study, outdoor exposure implies UV radiation, which induces the formation of cyclobutane pyrimidine dimers (CPDs), further blocking polymerase progression during PCR [10, 11].

Impact of Amplicon Length and PCR Conditions

The differential amplification success between HVS-I (143 bp) and HVS-II (126 bp) supports the "mini-amplicon" strategy in forensics. The longer HVS-I fragment failed to amplify in almost all samples after Day 1, whereas the shorter HVS-II fragment remained detectable up to Day 14. Statistically, longer DNA fragments are more likely to contain random lesions (breaks or dimers) than shorter ones. Therefore, targeting shorter amplicons improves the probability of successful recovery from degraded samples, consistent with findings by Yudianto & Setiawan (2020) regarding mini-primers [19].

However, it must be acknowledged that the failure of HVS-I was likely exacerbated by the PCR conditions. The annealing temperature of 60°C used in this study may have been too stringent for degraded templates, as high temperatures can destabilize primer binding on fragmented DNA. Future optimization using gradient PCR or lower annealing temperatures (e.g., 54–56°C) could potentially improve recovery rates for longer fragments.

Study Limitations and Forensic Implications

Several limitations in this study must be noted. First, the sample size (n=6) was small, limiting the generalizability of the statistical findings. Second, the quantification method measured total DNA rather than human-specific DNA; thus, the values reported likely include bacterial DNA, especially in the later stages of storage. Third, gel electrophoresis provides qualitative data; future studies should employ qPCR for precise quantification of human vs. bacterial DNA ratios.

Despite these limitations, the findings provide actionable forensic insights. Evidence recovery from masks exposed to outdoor conditions should be prioritized within the first 14 days. Beyond this window, the likelihood of obtaining a viable mtDNA profile drops significantly due to the combined effects of hydrolysis and contamination. For samples collected after prolonged exposure, forensic laboratories should prioritize targeting short amplicons (<130 bp) and consider additional purification steps to remove bacterial inhibitors.

Conclusion

This study demonstrates that exposure to outdoor environments significantly accelerates the degradation of mitochondrial DNA on medical masks. Quantitative analysis revealed a ~96% reduction in total DNA concentration over the 20-day period (declining from 373.58 ng/µl on Day 1 to 14.07 ng/µl on Day 20), accompanied by a critical drop in purity to 0.89 due to environmental contamination. The degradation profile was length-dependent: while the longer HVS-I amplicon (143 bp) failed to amplify reliably after Day 1, the shorter HVS-II amplicon (126 bp) remained detectable for up to 14 days. Consequently, forensic recovery of mask evidence is recommended within 14 days post-deposition. To mitigate hydrolytic and microbial damage observed in this study, recovered masks should be immediately preserved via desiccation or refrigeration. For samples collected beyond this timeframe, molecular strategies must prioritize short amplicons (<130 bp) to maximize profiling success.

Acknowledgment

None.

Funding

None.

Conflicts of Interest

The authors report that there are no competing interests to declare.

References

[1] Humphreys J. The importance of wearing masks in curtailing the COVID-19 pandemic. J Fam Med Prim Care. 2020;6(2):2606-7. [DOI: 10.4103/jfmpc.jfmpc]

[2] Annita, Silitonga LL, Sayekti FDJ, Taufiq M, Hermawati L, Rosyidah RA, et al. Dasar-Dasar Biomedik: Teknologi dan Aplikasi dalam Kesehatan. PT Bukuloka Literasi Bangsa; 2024. [Link]

[3] Vrij A, Hartwig M. Deception and lie detection in the courtroom: the effect of defendants wearing medical face masks. J Appl Res Mem Cogn. 2021;10(3):392-9. [DOI: 10.1016/j.jarmac.2021.06.002]

[4] Butler JM. DNA extraction methods. In: Advanced Topics in Forensic DNA Typing. 2012. p. 29-47. [DOI: 10.1016/B978-0-12-374513-2.00002-6]

[5] Alketbi SK. The affecting factors of touch DNA. J Forensic Res. 2018;9(3):1-4. [DOI: 10.4172/2157-7145.1000424]

[6] Saamia V, Yudianto A, Nurjayadi M, Novitasari N, Furqoni AH. Limit detection of short tandem repeats analysis on touch DNA samples. Indones J Chem. 2017:1-12. [DOI: 10.22146/ijc.94081]

[7] Furqoni AH, Luh N, Megasari A, Yudianto A, Azizah F, Sari ET, et al. Amplification of vWA, FGA, and TH01 loci of DNA samples isolated from ring stored at room temperature. Malays J Med Health Sci. 2023;19(5):97-101. [DOI: 10.47836/mjmhs19.5.14]

[8] Divya K, GK M, Umema A, SS D. Environmental factors affecting the concentration of DNA in blood and saliva stains: a review. J Forensic Sci Res. 2024;8(1):009-15. [DOI: 10.29328/journal.jfsr.1001057]

[9] Salazar Roa AM, Huerta P, Montoya LF, Coliboro V, Castro AF, Barbaro A. DNA evaluation in teeth subjected to various thermal conditions: a preliminary study. Forensic Sci Int. 2026;382:112869. [DOI: 10.1016/j.forsciint.2026.112869]

[10] Schulte J, Rittiner N, Seiberle I, Kron S, Schulz I. Collecting touch DNA from glass surfaces using different sampling solutions and volumes: immediate and storage effects on genetic STR analysis. J Forensic Sci. 2023;68(4):1133-47. [DOI: 10.1111/1556-4029.15305]

[11] Bhandari D. Touch DNA: revolutionizing evidentiary DNA forensics. Int J Forensic Sci. 2023;8(3):1-8. [DOI: 10.23880/ijfsc-16000314]

[12] Dewanata PA, Mushlih M. Differences of DNA purity test using UV-VIS spectrophotometer and Nanodrop spectrophotometer in type 2 diabetes mellitus patients. Indones J Innov Stud. 2021;15:1-6. [DOI: 10.21070/ijins.v15i.553]

[13] Cuenca D, Battaglia J, Halsing M, Sheehan S. Mitochondrial sequencing of missing persons DNA casework by implementing Thermo Fisher's Precision ID mtDNA Whole Genome Assay. Genes (Basel). 2020;11(11):1303. [DOI: 10.3390/genes11111303]

[14] Al-Zubaidi MM, Mahdi AAL, Al-Tabra RH, Al-Sammaraie HKI, El-Jabbar BAA, Jawad A, et al. Mitochondrial DNA in forensics: principles, applications, and limitations. Al-Nahrain J Sci. 2024;27(2):50-62. [DOI: 10.22401/anjs.27.2.05]

[15] Yudianto A, Nzilibili SMM, Harjanto P, Setiawan F. The use of touch DNA analysis in forensic identification focusing on STR CODIS loci THO1, CSF1PO and TPOX. Indian J Forensic Med Toxicol. 2020;14(3):1692-6. [DOI: 10.37506/ijfmt.v14i3.10667]

[16] Burrill J, Rammenou E, Alawar F, Daniel B, Frascione N. Corneocyte lysis and fragmented DNA considerations for the cellular component of forensic touch DNA. Forensic Sci Int Genet. 2021;51:102428. [DOI: 10.1016/j.fsigen.2020.102428]

[17] Tozzo P, Mazzobel E, Marcante B, Delicati A, Caenazzo L. Touch DNA sampling methods: efficacy evaluation and systematic review. Int J Mol Sci. 2022;23(24):15541. [DOI: 10.3390/ijms232415541]

[18] Howlett SE, Castillo HS, Gioeni LJ, Robertson JM, Donfack J. Evaluation of DNAstableTM for DNA storage at ambient temperature. Forensic Sci Int Genet. 2014;8(1):170-8. [DOI: 10.1016/j.fsigen.2013.09.003]

[19] Yudianto A, Nuraini I, Furqoni AH, Nzilibili

SMM, 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(Suppl 1):8716. [DOI: 10.4081/idr.2020]