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Research Paper

Comparative Study of Touch DNA from Different Surfaces: Amelogenin Locus Analysis for Forensic Purposes

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ABSTRACT

Background: This study examines the concentration of touch DNA on various surfaces using analysis of the amelogenin locus to identify the donor's gender. Touch DNA, which is transferred through contact, was collected from masks, iron door handles, plastic, and glass. DNA extraction and amplification were performed using PCR.

Methods: Touch DNA samples were collected from masks, iron door handles, plastic, and glass using sterile swabs after contact by male and female donors. DNA extraction was performed using the Chelex method, followed by PCR amplification targeting the amelogenin locus. DNA concentration was measured and compared across different surface types to evaluate variations in DNA transfer.

Results: The findings indicate that masks provided the highest DNA concentration, while plastic and glass had the lowest. Factors such as surface type, individual activity, and environmental conditions significantly influence DNA concentration. Additionally, male donors consistently left more DNA than female donors, consistent with previous studies. The study highlights the importance of surface roughness and porosity in DNA retention, with rougher surfaces generally retaining more DNA.

Conclusion: This research offers valuable insights for forensic investigators by demonstrating the utility of touch DNA in linking individuals to crime scenes. However, more extensive studies with larger sample sizes are needed to confirm these results and strengthen conclusions.

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Introduction

Crime has been deeply ingrained in human civilization, and forensic science serves as a critical tool for collecting evidence that can substantiate or disprove an individual's connection to criminal acts. One of the most significant advancements in this domain is forensic DNA analysis, or DNA fingerprinting, which has transformed criminal investigations worldwide. This methodology has introduced innovative approaches to crime-solving and is now an indispensable asset to both law enforcement agencies and judicial systems. Efforts are currently underway to establish national and regional DNA databanks to store profiles generated by accredited laboratories. The meticulous procedures involved in the collection, preservation, and transportation of DNA samples are fundamental to the accuracy and reliability of DNA fingerprinting analysis (1, 2). The identification of individual traits in forensic investigations involves assessing key parameters such as age, sex, and ethnicity. Among these, the estimation of gender is of significant importance, as it helps refine the scope of the investigation by limiting further analysis to individuals of the determined sex. This process is crucial for enhancing the precision and effectiveness of forensic analyses, ensuring that investigative efforts remain focused and methodologically sound (2, 3).

Forensic genetics is the primary standard in the investigation and judicial systems for identifying a person from DNA recovered from a crime scene, thereby assisting the court in identifying individuals involved in criminal events. The challenge faced by forensic researchers is the scarcity of available samples for analysis, which necessitates an understanding of the relevance of other biological traces to support analysis (4). The DNA from a person found at the crime scene will be matched with someone who is likely responsible for the crime committed. Low DNA concentration in non-body fluid biological samples is often referred to as trace DNA or touch DNA (5).

The amount of DNA evidence depends greatly on the method of transfer. Primary DNA transfer occurs through direct contact between individuals, such as when they speak, cough, or sneeze. Physical contact with any object can also transfer DNA through epidermal cells. This is what is referred to as touch DNA (4).

Touch DNA is DNA derived from shed skin cells or other biological materials that adhere to an object or even a person when in physical touch (6). Although it remains a topic of debate among scientists, numerous studies have shown that this touch DNA is derived from shed keratinocytes. Sources include intact or partial skin cells, epithelial cells with nuclei from fluids or body parts that come into contact with a person's hands (saliva, sebum, perspiration), and free DNA, which can be endogenous or transferred to surfaces that have been in physical contact (7).

The Locard Exchange Principle states that every interaction leaves a trace owing to the shedding of tiny skin cells, which are epithelial cells present in the skin's outermost layer (8, 9). Cell-free DNA is a reliable source of genetic material, often yielding better results than cellular DNA, yet scientists remain skeptical about its origins (7).

Several factors can influence the amount of DNA that adheres to touched objects. Generally, men release more DNA than women; younger men tend to release more than older men; and washing hands too frequently can also affect the amount of available DNA. Physical activities that cause sweating can also result in a high concentration of touch DNA (7).

This touch DNA can be found in various areas, including clothing, weapons, door handles, and personal items. Analysis using touch DNA samples can assist forensic researchers in establishing a connection between a suspect and the crime scene, providing additional evidence for identification, or even eliminating innocent individuals from suspicion (8).

Until now, research on touch DNA with more varied treatments to determine sex has not been widely conducted.

Materials and Methods

Materials used in this study include touch DNA samples from two donors, as well as experimental objects such as masks, iron door handles, plastic, and glass. DNA analysis was conducted with DNAzol (Invitrogen, ThermoFisher Scientific, Waltham, MA, USA), Chloroform (Merck KGaA, 664271, Darmstadt, Germany), Isopropanol (EMSURE®, Merck KgaA, 64271 Darmstadt, Germany), Amelogenin Primer (Promega Corporation, Madison, USA), PCR Mix Promega (Promega Corporation, Madison, USA), Nuclease Free Water (Promega Corporation, Madison, USA), Acrylamide Reagent (Sigma-Aldrich), Tris-Borate-EDTA (TBE) Buffer (Promega Corporation, Madison, USA).

Instrumentation

Instruments used in this study include Centrifuge (Fresco 2 Centrifuge by Thermo Scientific, Geel, Belgium), UV Visible Spectrophotometry (Nanodrop Lite UV Visible Spectrophotometer by Thermo Scientific, Geel, Belgium), PCR (BIO-RAD T100 Thermal Cycler), Electrophoresis (Bio Rad Life Sciences, California), and some laboratory equipment such as laminar air flow (LAF), thermomixer, vortex, pipette, tips, and spin-down.

Ethical Clearance

This research has obtained ethical clearance from the Faculty of Dental Medicine Health Research Ethical Clearance at Universitas Airlangga, with certificate number 1004/HRECC.FODM/X/2024.

Touch DNA Sampling

This work was carried out in the Human Genetic Laboratory at the Tropical Disease Institute, Universitas Airlangga, Surabaya, Indonesia. The materials used in this investigation included contact DNA samples from two donors, as well as experimental objects such as masks, iron door handles, plastic, and glass. To ensure a pure experimental environment, all objects were sterilized using DNAZap solution and isopropanol, as per the procedure. Following sterilization, masks, plastic, and glassware were exposed to UV radiation under laminar airflow for 30 minutes to remove any residual DNA from their surfaces. To avoid cross-contamination, all donors were asked to wash their hands with soap and dry them with sterile tissues. Following that, all donors were allowed to participate in various activities for an hour as part of their daily routine, in different rooms at the same temperature, with explicit instructions not to touch others or to wear gloves. After an hour, each donor was instructed to convene in a room for touch DNA sampling. Each donor held every object for 30 seconds. The goal of this sample-collecting strategy was to acquire as many touch DNA samples as possible from all items and connect them to criminal episodes.

Table 1. DNA Sequence Primer Forward and Reserve.

Primer	Sequence
Forward	5'CCCTGGGCTCTGTAAAGAA-3'
Reserve	5'ATCAGAGCTTAAACTGGGA AGCTG-3'

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Touch DNA Recovery

Touch DNA was recovered from objects using the twofold swab approach. This procedure requires wetting a nylon swab (4N6 FlOQSwabs) with nuclease-free water. The wet swab is then used to capture the initial sample after applying pressure to all objects held by the donor. The same area that was previously tested with the wet swab is then wiped with a dry nylon swab.

DNA Extraction

DNA was extracted using the Phenol-Chloroform-Isoamyl Alcohol (PCI) organic extraction method (Thermo Fisher Scientific, Geel, Belgium). After collecting samples from touched objects, cotton swabs were placed in conical tubes and incubated overnight with 300 µl of aquabidest. After incubation, the sample was sonicated for 45 minutes, then centrifuged at 12,000 rpm for 10 minutes at 4°C. Following centrifugation, the supernatant was extracted. DNAzol (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) in 1000 µL pellets was added, vortexed for 3-5 minutes, then incubated for 15 minutes. 0.2 ml of chloroform (Merck KGaA, 664271, Darmstadt, Germany) was added, vortexed, and incubated overnight. The mixture was then centrifuged at 8,000 rpm for ten minutes. The centrifuged supernatant was collected and placed in a new 1.5 cc microtube. 0.5 ml of isopropanol (EMSURE®, Merck KgaA, 64271 Darmstadt, Germany) was added, mixed, and incubated for 30-60 minutes. Following incubation, the material was centrifuged at 12,000 rpm for ten minutes. The supernatant was removed, and a 0.5 ml pellet containing 70% ethanol was added and incubated for 30 to 60 minutes. Finally, centrifuge the sample at 12,000 rpm for 5 minutes. Remove the supernatant and add 50µl of aquabidest pellets, then vortex.

DNA Content and Purity Measurement

The DNA concentration and purity were measured using UV-Visible Spectrophotometry (Nanodrop Lite UV Visible Spectrophotometer by Thermo Scientific, Geel, Belgium) at 260 and 280 nm wavelengths.

DNA Amplification

PCR (BIO-RAD T100 Thermal Cycler) was carried out with the amelogenin genes (Promega Corporation, Madison, USA) and acrylamide gel electrophoresis. In a vortex tube, add 12.5 µl PCR Mix Promega (Promega Corporation, Madison, USA), 2.5 µl forward and reverse primer, and 7.5 µl target DNA. Mix thoroughly. The tube was filled to 25 µl with Nuclease Free Water (Promega Corporation, Madison, USA) at pH 8.5.

The PCR technique consisted of an initial

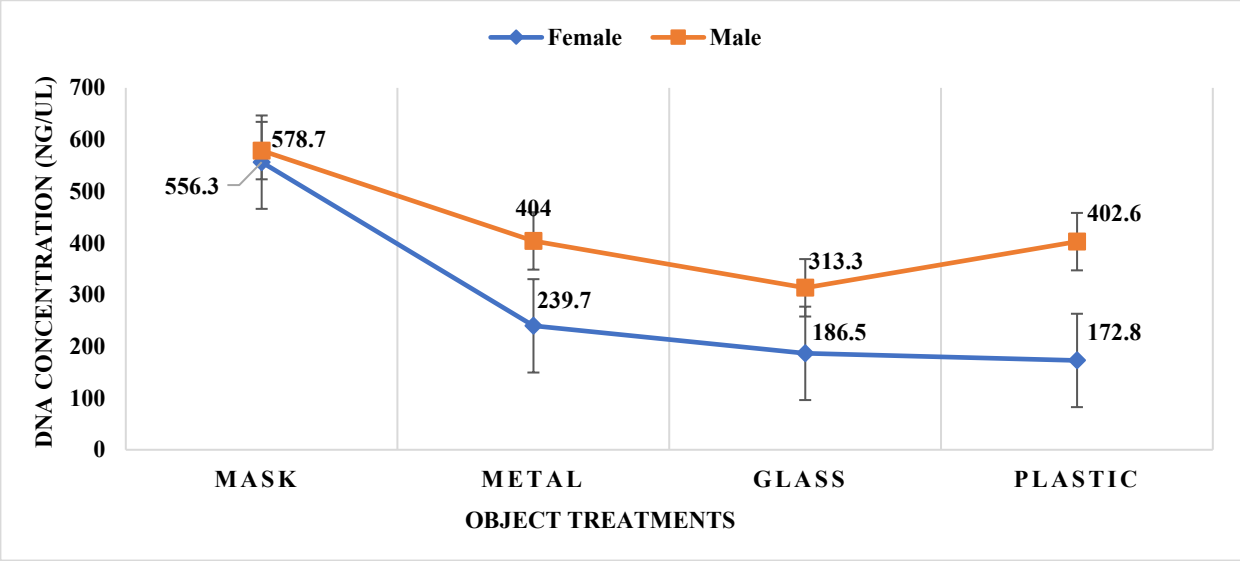

Figure 1. Mean of DNA Concentration.

denaturation at 95°C for 11 minutes, followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 59°C for 45 seconds, and extension at 70°C for 45 seconds. A one-hour extension at 60°C was done as the final step. The primers used are listed in Table 1.

Acrylamide Gel Electrophoresis

An Erlenmeyer flask was filled with 3 cc of acrylamide reagent (Sigma-Aldrich) and 8 cc of 0.5x Tris-Borate-EDTA (TBE) Buffer (Promega Corporation, Madison, USA). Following homogenization, 100 µL of ammonium persulfate (APS) solution was added, and the mixture was stirred. The gel mixture was placed in a mold and allowed to set. Once established, the gel was placed in an electrophoresis chamber containing 0.5x TBE solution, and PCR samples were loaded. Electrophoresis was performed at 100 volts for 60 minutes, followed by gel staining.

Results

This study focused on amelogenin amplification as a reliable and widely accepted marker for sex determination, enabling assessment of DNA recovery success across different substrates. The results of amelogenin amplification serve only as a preliminary indication of sex determination, not as full genetic profiling. Amplification was performed using the PCR method on the amelogenin locus to determine sex. Another study used string objects to analyze Limit Detection using touch DNA samples. The results of the research indicate limitations in touch DNA detection, specifically within the concentration range of DNA (>0.25, 0.0625–0.25, <0.0625 ng) (10).

The concentration of DNA was measured using a UV-Visible Spectrophotometer (Nanodrop Lite UV-Visible Spectrophotometer by Thermo Scientific, Geel, Belgium) at a wavelength of λ260 nm. At the same time, DNA purity was assessed at 260 nm and 280 nm.

Figure 1 and 2 shows that the samples with mask objects from both donors have the highest DNA concentration compared to samples with other objects. Meanwhile, the lowest DNA concentrations in female and male donors were observed in samples containing plastic and glass objects, respectively.

The findings from this research illustrate the relationship between touch DNA concentration and the success of amplification at the amelogenin locus, enabling the identification of an individual's sex. The selection of objects used in this research is based on the types of evidence commonly found at crime scenes.

Discussion

Touch DNA is obtained from skin cells left behind when someone touches an object. This touch DNA is often used in many criminal case investigations because, during the commission of a crime, it can be used to ensure that the suspect touched an object at the crime scene (11). Because touch DNA is a visible material, a process of rubbing, scraping, or applying adhesive to the object is necessary to collect and analyze skin cells (11, 12). Nuclease-free water is also

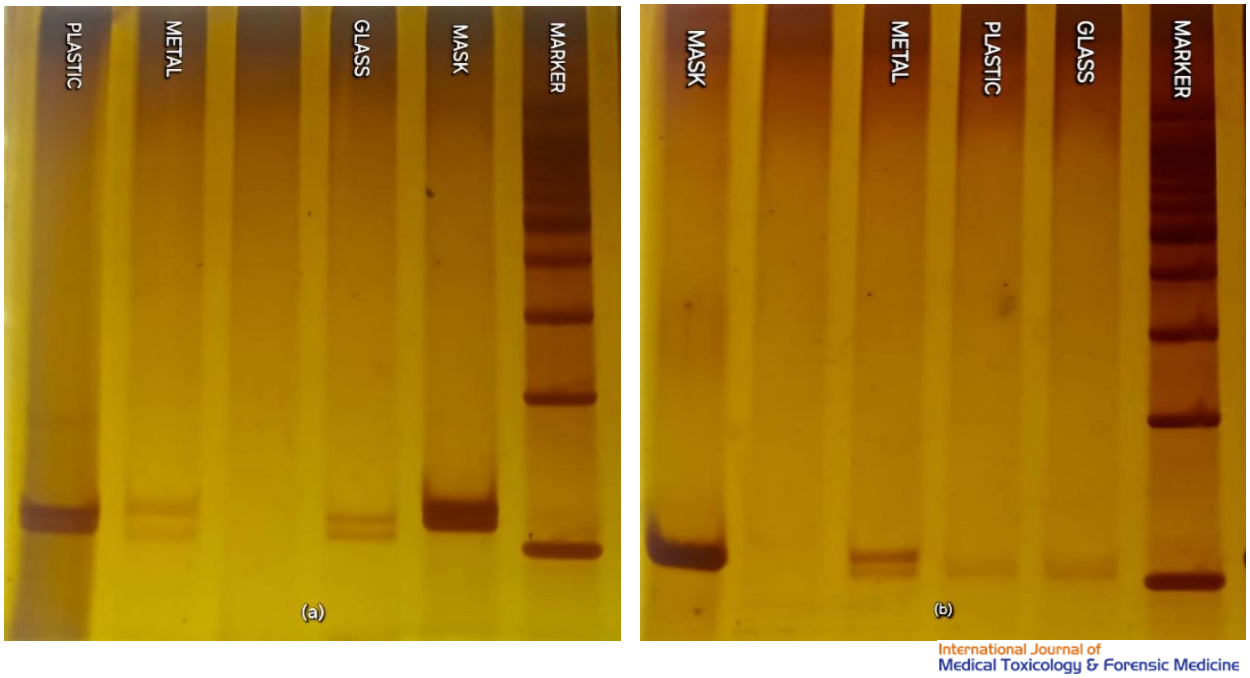

Figure 2. Visualization of Acrylamide Electrophoresis Band using Amelogenin loci compared to marker (100 bp ladder). (a) Male donor and (b) Female Donor.

required to resuspend the cells and absorb the DNA. A cotton swab moistened with the solution is specifically used to dissolve the cells, thereby increasing the concentration of the obtained DNA (12).

Touch DNA sources include anucleate corneocytes, fragmented cells, nucleated epithelial cells, and free cell DNA. Anucleate corneocytes are the outermost layer of the epidermis that has undergone keratinization and lost its nucleus and organelles; approximately 10^9 corneocytes are shed by an adult human during active desquamation. The number of nucleated cells transferred is greater than the number of anucleate corneocytes. azCell-free DNA is a highly promising source of touch DNA, though it remains challenging to understand fully. This cell-free DNA is produced because many cell types actively release DNA to some extent, so its presence is not surprising. This free cell DNA can be found in sweat, saliva, semen, urine, and also on objects that have been touched (5).

The concentration of DNA obtained during analysis is determined by the DNA precipitation process. This process is influenced by several factors, including the individual's tendency to release DNA, the activities performed before DNA deposition, the type of surface from which the DNA is collected, and the quality of physical contact during DNA deposition. Some individuals are considered better sources of touch DNA than others. This is because the intensity of skin cell shedding varies among individuals. In addition, individual habits of touching the face, eyes, nose, hair, and so on increase the likelihood of concentrating touch DNA. Direct skin contact on the surface can also increase the amount of touch DNA deposited on that object (13–15).

In this study, the mask object had the highest touch DNA concentration among the research objects. This result is consistent with Alketbi's (16) findings: rough, porous surfaces retain DNA more effectively than smooth surfaces. This occurs because the abrasive nature of rough surfaces tends to shed skin cells, thereby increasing the likelihood of DNA retention. It is also reported that the amount of touch DNA recovered from cotton substrates is much higher than on plastic, averaging 11.68 ng, while the average on plastic is only 0.4 ng. Other researchers have noted that the substrate surface roughness can affect the number of skin cells that adhere. Regarding clothing materials (porous or non-porous, smooth or rough), higher concentrations of touch DNA are obtained than with touch DNA collected directly from human skin (17).

Pesaresi et al. (18) conducted a study using touch DNA samples on different objects, and the results showed that smooth, non-porous surfaces are more likely to retain DNA than rough, porous surfaces. The reason is that smooth, non-porous surfaces can increase the rate of sweat during interaction, thereby increasing the amount of DNA deposited. The findings of Pesaresi et al. (18) contradict those of this study. This may be due to differences in individuals' abilities to produce sweat, which will serve as a source of touch DNA (16). This is why, in this study, male donors had higher touch DNA concentrations in all object samples than female

donors (7). Young adult men aged 18-45 experience a higher rate of skin sagging compared to women of the same age (17).

The time factor between deposition and recovery also affects the concentration of touch DNA. There is a significant decrease in the amount of DNA recovered over a given period. Bille et al. (19) reported that the decrease in DNA recovered from samples collected and analyzed at 7 and 10 days, respectively, yielded average concentrations of 0.34 ng/µL and 0.038 ng/µL, respectively. Based on this study, it can be concluded that minimizing the time from DNA collection to analysis is an important factor in achieving high-touch DNA results. Different parts of the hand can also increase the likelihood of touch DNA transfer to objects that have been touched. Especially at the fingertips, it has been shown to release DNA in larger quantities (17).

Environmental factors such as high temperatures, humidity, and exposure to UV light also affect the persistence of touch DNA. In humid environments, DNA is susceptible to hydrolytic cleavage and oxidative base damage, leading to base loss during purification and DNA scoring. UV radiation from sunlight can also cause cross-linking between adjacent thymine nucleotides, thereby hindering the progress of DNA polymerase during PCR (16). Raymond et al. (20) reported that the low concentration of touch DNA on objects exposed to humid environments is due to increased degradation rates. However, high humidity can also enhance DNA transfer.

PCR-amplified samples are visualized using acrylamide gel electrophoresis because the polyacrylamide matrix has a greater ability to separate smaller molecules, owing to its smaller pore size, compared to agarose gel (21). The lengths of X and Y are 106 base pairs and 112 base pairs, respectively (22). This is because women have XX chromosomes, and each X chromosome is 106 base pairs long, resulting in only one band. This is different in samples with XY chromosomes, as differences in base-pair length between the X and Y chromosomes result in electrogram traces showing two bands (23). Several factors contribute to differences in band thickness across samples, including DNA concentration and degradation. The DNA concentration significantly affects band thickness in polyacrylamide gel electrophoresis. Thicker bands indicate a high concentration of DNA, while thinner bands indicate a low concentration of DNA in the sample. In addition, DNA degradation can cause bands to thin or even disappear, depending on the level of degradation (24).

The number of donors in this study was intentionally controlled to ensure experimental consistency. While the sample pool is modest, it allows for precise observation of substrate-dependent DNA retention, the core analytical focus of this research. In this study, the statistical strength and generalizability of the findings. Future research with larger and more diverse donor populations is essential to validate the observed trends.

This study applied a uniform extraction approach across all samples to ensure methodological consistency. Although forensic-grade kits were not available, the organic method used remains a recognized and reliable approach for touch DNA recovery and has been employed in multiple published studies. Commercial kits typically incorporate silica columns or magnetic beads to improve the recovery of low-template DNA and reduce the effects of inhibitors. In contrast, the organic PCI method may have contributed to reduced yields and variability. Comparative studies using validated forensic kits are recommended to strengthen the methodological robustness.

The study scope was deliberately limited to amelogenin analysis to evaluate the relationship between DNA concentration and amplification success. This focused design supports a clear interpretation of substrate performance, free from the confounding variables inherent to multiplex STR systems. These markers provide higher discriminatory power and are standard in forensic casework. The absence of these analyses means that the study can only confirm biological sex and cannot contribute to individual identification. Future studies should incorporate multiplex STR kits to strengthen the evidentiary value.

The use of UV spectrophotometry provided consistent comparative quantification across all samples. Although qPCR offers enhanced sensitivity, the selected method was adequate for confirming the presence of measurable DNA and enabling substrate-based evaluation. Sensitive quantification of degraded or low-template DNA was unavailable in this laboratory setting. Reliance on spectrophotometry may not accurately reflect the true amount of amplifiable DNA. Implementation of qPCR-based quantification is strongly encouraged for future work.

Conclusion

This study found that the type of object used has a substantial impact on the concentration of touch DNA retrieved, with masks having the highest concentration and plastic and glass having the lowest. The roughness and porosity of an object's surface affect DNA

retention: rougher or more porous surfaces retain more DNA. Donor features, activity level, and environmental factors such as temperature and humidity all play an important role in DNA degradation and recovery. Male donors often released more DNA than female donors. Proper handling and reducing the period between DNA deposition and analysis are critical for increasing DNA recovery. These discoveries can help forensic investigators identify suspects or victims more accurately using touch DNA analysis. However, more research with larger sample sizes is needed to confirm these results and provide more robust conclusions.

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Conflicts of Interest

The authors report there are no competing interests to declare.

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