



RESEARCH ARTICLE

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The effect of fingerprint enhancement methods applied on adhesive surfaces on DNA recovery: a preliminary study

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Abstract

The presence of body fluids such as blood, saliva, semen or urine during fingerprint research on the evidence taken from the crime scene makes it necessary to protect biological materials to examine the evidence in multiple ways. Therefore, it is crucial that fingerprint development (FD) techniques do not disrupt the structure of biological materials during FD procedures. In this sense, it is essential to determine whether biological material or fingerprints should be the priority during the collection of evidentiary materials, to determine the systematic order and to determine whether the FD methods to be applied cause damage to the genetic material used in the identification of individuals and to evaluate them in terms of their evidentiary quality. This study investigated the effects of the application of trace detection methods on DNA profiling processes in evidence where fingerprints and biological samples are found at the same time. In this study, blood, saliva, semen and urine samples were taken from a male individual who signed an informed consent form at the laboratory stage. The samples were applied 50 μ L on the adhesive tape surface. After application, the samples were treated with crystal violet (CV) and sticky side (SS) fingerprint development chemicals suitable for the surface type. The prepared samples were dried under room conditions. After 1 day and 45 days under normal room conditions, silica-based DNA extraction was performed. After extraction, DNA quantification was performed using the fluorimetry method. In the study, biological samples with known DNA content were used to focus on DNA quantification. Among the fresh samples prepared in the study, DNA recovery was higher in the SS-treated urine, blood and saliva samples and in the CV-treated semen sample group compared to the other groups. This shows that chemical treatment of some biological samples on adhesive tape increases the efficiency of DNA recovery. When the 45-day waiting samples were compared with the control group samples, DNA recovery decreased in CV-treated urine and blood samples, while DNA recovery increased in SS-treated urine and blood samples. In semen samples, both CV and SS treatment negatively affected DNA recovery. In saliva samples, DNA recovery increased ~2-fold in the CV-treated sample group, while SS treatment caused a ~75% decrease in DNA recovery. The results show that the non-porous adhesive tape does not adversely affect the amount of DNA in terms of STR profiling of latent FD chemicals used on the surfaces and that adhesive tape treated with fingerprint enhancement chemicals can actually be used for advanced forensic genetic analyses for DNA extraction on surfaces.

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Keywords: Fingerprint enhancement; Adhesive surface; DNA extraction; Biological fluids; Forensic genetics

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1. Introduction

In crime dynamics, no matter how professional the perpetrators are, a suspect leaves something at the crime scene or the victim and takes something away from the crime scene or the victim. With the current technology, it is possible to identify and analyse the visible/invisible evidence left at the crime scene [1], [2]. Forensic scientists investigate human biological fluids (such as blood, saliva, semen, and urine) and comparable fingerprints as the most reliable types of evidence, which have the characteristics of certainty, uniqueness (being unique to the individual) and high distinctiveness, to provide objective evidence to the courts regarding the elucidation of crimes and the identification of criminals.

Fingerprints are one of the oldest forms of forensic evidence linking the crime scene to the offender and are based on the assumption that everyone has a unique set of patterns on the fingertip. The residues that make up the fingerprint pattern are a mixture of secretions from various glands in the skin (sudoriferous eccrine, apocrine, and sebaceous), but also a complex formation mixed with environmental substances that come into contact with the person's skin [3]. The condition and structure of the surface (such as rough-smooth, porous-non-porous, absorbent-non-absorbent) on which the fingerprint is found, whether there is any residue affecting the fingerprint (such as oil, blood), environmental factors (such as wetting, drying), the possible age of the fingerprint (duration of stay on the surface) make it necessary for the methods used in fingerprint research to be different [4]. Among the FD methods, Iodine Vapour, Ninhydrin, DFO (9- Diazaflueron), Indanedione, 5-MTN (5-(methylthio)ninhydrin), Termanin and Silver Nitrate are used on porous surfaces (such as raw wood, paper) and on non-porous surfaces (metal, glass, plastic, Cyanoacrylate and colouring methods (such as Rhodamine-6G, Ardrex, Nile Red, Yellow Basic), Sudan Black, Amido Black, Hungarian Red, SPR and especially Crystal Violet, Sticky Side chemicals are used on adhesive surfaces. The main goal of FD is to take advantage of the adhesion and colouring properties of the chemicals used for the sweat and sweat-containing substances in the fingerprint [5].

In forensic cases such as murder, sexual assault, and theft, efforts are made to determine whether there is a connection between the suspects and the incident by analysing genetic information obtained from biological materials (such as blood, semen, saliva, and urine) found at the crime scene. In criminal investigations, biological samples collected from the crime scene are identified by DNA analysis. The DNA molecule is found in the cell, which is the building block of human beings, and the DNA of all people except identical twins is different from each other. Another important feature of DNA is that it shows the same structural features in all human cells. In addition, it is known that DNA is inherited from parents and maintains its structure, except for some rare negative effects, such as mutations and external factors. Mutations, while sometimes considered negative, are indeed a natural part of evolution and contribute to genetic diversity. Additionally, sometimes external factors can induce adverse effects on DNA. These scientific facts have made DNA-based identification one of the most valid and precise methods [6].

In some incidents, findings can yield more than one type of evidence group, such as physical, chemical, biological, and trace evidence. One of the most critical findings that contain different evidence groups in criminal investigations is adhesive surfaces. Biological and trace evidence obtained from the adhesive surfaces of the tapes makes an essential contribution to the rapid resolution of forensic incidents. In particular, adhesive surfaces are actively used in the creation of terrorist attacks and bomb devices, in the packaging used in the transport of narcotic substances, in the detection of kidnapped persons or in the identification of the number plates attached to stolen motor vehicles. The importance of adhesive surfaces in terms of criminal investigations stems from the fact that these surfaces have any connection with the environment in which the event took place and that they contain DNA or fingerprints of the people who carried out the event [7], [8]. Judicial authorities may request forensic scientists to perform both fingerprint and DNA analyses on such evidence from the crime scene in criminal laboratories [9].

However, fingerprints obtained from crime scenes may be dirty or partial and may not be suitable for identification. DNA profiling of these prints, which are not suitable for identification, can be used to identify this evidence [10]. Thus, combining partial results of fingerprints and DNA can increase confidence in the identification of the suspect. However, the concern that FD may reduce DNA recovery also raises concerns about the simultaneous use of these complementary analyses [11]. Although this concern contributes to the development of analyses to determine the DNA yield on the surface of the finding by using latent trace development techniques on the finding, the data obtained may contain complex and variable results since biological samples containing non-standardised and uncertain amounts of DNA are used in these studies [12], [13]. Using a starting material with known amounts of DNA can eliminate the variability in DNA recovery and allows for statistical analysis between methods for DNA recovery [11]. Many studies have been conducted to evaluate the effects of FD techniques on subsequent DNA profiling, depending on the quantity and quality of DNA present in the fingerprint [14]–[18]. However, research on the recovery of DNA on surfaces containing different biological fluids and treated with FD chemicals is at the theoretical level and is very limited. Since criminal investigations are multi-faceted and the evidence obtained is valuable not only in terms of fingerprint research but also in terms of biological investigations, it is important to investigate whether the applied FD techniques disrupt the genetic material of the perpetrator of the incident. This aims to investigate the effects of FD methods applied to biological samples on adhesive surfaces on which fingerprints and biological fluids are found at the same time on the recovery of DNA from the target surface, and the results obtained are aimed to be used for the reorganisation of criminal analysis applications and obtaining results.

2. Materials and methods

2.1. Fingerprint development reagent and chemicals

The formation procedures of the fingerprint development/staining methods used in the study were performed according to the formulations of Bleay et al.[5], [19]:

Crystal Violet: 1 g Crystal Violet (CV) (Meck, Germany) was weighed and dissolved in 1000 mL distilled water to prepare a 1000 mL working solution. Sticky Side (SS) (Sirchie, USA) FD was used as a ready solution.

2.2. Preparation of biological samples

All procedures performed in this human participant study complied with the ethical standards of the institutional and/or national research committee, the 1964 Declaration of Helsinki and its subsequent amendments, or comparable ethical standards. 70 mL venous blood, 50 mL saliva, 100 mL urine, and 40 mL semen samples were obtained from a 35-year-old healthy man on the same day, who signed an informed consent form. Biological samples (venous blood, saliva, urine and sperm) were stored in a refrigerator (Vestel, Turkey) at +4 °C until the study was performed. No special instructions were given to the volunteers to obtain realistic data on forensic cases. The surface and consumables were exposed to UV light for 30 minutes before the experimental work to prevent possible contamination. 50 µl of biological sample was transferred onto adhesive tape (Pattex, Germany) using an automatic pipette (Eppendorf, Germany) (Figure 1). Samples of the volunteer applied on the adhesive surface and not treated with any FD were used as control samples. A blind sample for negative control was run with each group of samples to eliminate the risk of contamination.

2.3. Application of FD chemicals

In the study, 2 types of FDs were applied on the adhesive tape surface to which blood, saliva, urine and semen samples were transferred. CV and SS were applied 100 µl with an automatic pipette (Eppendorf, Germany) and allowed to dry in a fume hood.

The prepared samples were analysed at 2 different time intervals (day 1 and day 45) to determine the possible change in the DNA amount of biological samples treated with fingerprint chemicals over time. The prepared samples were kept for 45 days in sterile evidence storage cabinets where the evidence obtained from the crime scene was kept. At the end of the process, DNA extraction was performed from both freshly prepared samples and samples kept for 45 days.

2.4. DNA extraction and DNA quantification

The adhesive tape bearing the biological sample was utilised in its entirety as the starting material for the extraction. The entire adhesive tape, approximately 1.0 cm wide and 3.0 cm long, was placed in a 1.5 mL microcentrifuge tube. DNA extraction of FD chemicals-treated blood, saliva, urine, and semen samples was performed following the manufacturer's instructions and extracted using the QIAamp® DNA Micro Kit (Qiagen, Hilden, Germany). To a 1.5 mL microcentrifuge tube containing the sample, 20 µL proteinase K solution and 400 µL AL kit lysis buffer were added. After vortexing for 15 s, the tube was incubated at 60°C for 30 min for protein digestion. After a brief centrifugation, the resulting mixture was transferred to a 2 mL QIAamp micro-spin column and centrifuged at 8000 rpm for 1 min. After discarding the collection tube and replacing it with a new collection tube, 500 µL of AW1 solution was added to the column and then centrifuged at 8000 rpm for 1 min. After changing to a new collection tube, 500 µL of AW2 solution was added to the column, followed by centrifugation at 14000 rpm for 3 min. After transferring the QIAamp micro-spin column to a new 1.5 mL microcentrifuge tube, 20 µL of AE buffer was added to the column and incubated for 10 min at room temperature. Finally, DNA was extracted by centrifugation at 8000 rpm for 1 min [9], [20].

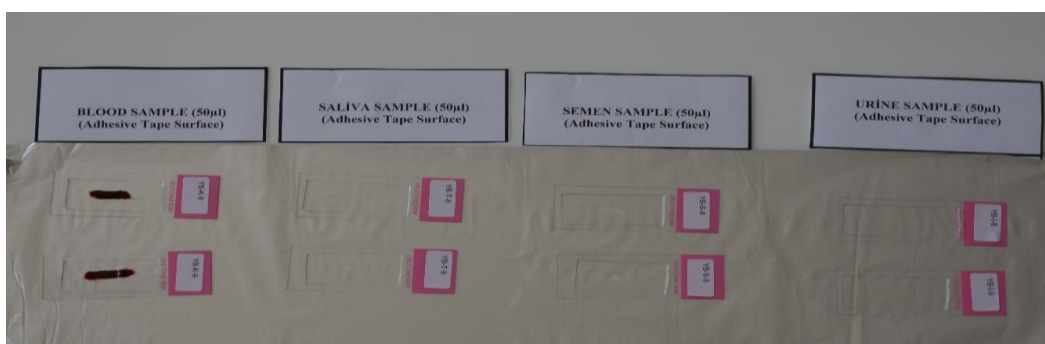


Fig. 1. Biological samples applied on adhesive tape in a volume of 50 µL.

Isolated DNA was quantified by the fluorimetric method using fluorescence technology, which provides high accuracy and sensitivity. In this method, a Qubit® Fluorimeter (Invitrogen by Thermo Fisher Scientific, USA) measuring at 260 nm wavelength was used, and the standard procedure steps for the Quant-iT™ dsDNA HS (Invitrogen by Thermo Fisher Scientific, USA) kit were followed [21]. DNA extraction and DNA quantification were performed for each sample type in 3 repeats.

2.5. Statistical analysis

All statistical evaluations were performed using IBM SPSS Statistics v25.0 software. The differences between the two study groups, such as those based on time, were examined using an independent sample t-test or a Wilcoxon signed-rank test, depending on whether the data followed a normal distribution. For differences between three or

more groups, such as different biological samples or fingerprint development chemicals, one-way ANOVA and post hoc tests were applied. A p-value < 0.05 was considered statistically significant.

3. Results and discussion

Biological samples of various body fluids and evidentiary quality are frequently encountered at the crime scene. Developing fingerprints contaminated with biological samples with different lifetimes and obtained using different methods is challenging for forensic scientists. Furthermore, the concern that FD procedures may adversely affect DNA recovery may prevent the simultaneous use of complementary analyses. Scientists are investigating the effect of chemical agents used to develop fingerprints on DNA yield in biological findings. In this sense, this study tried to determine how the techniques used in the development of latent fingerprints affect DNA recovery, to determine the order of application of fingerprint development processes with biological evidence on adhesive surfaces in terms of evidence security, and to identify and investigate the effects of these FD agents that affect forensic DNA analysis.

The presence of body fluids such as epithelial cells, blood, saliva, urine and semen, as well as fingerprints on the evidence taken from the crime scene, makes it necessary to protect biological materials in the development of latent fingerprints. Therefore, this study will contribute to the determination of the order of examination in the criminal laboratory to prevent the destruction of biological or fingerprint evidence and prevent contamination of evidence. According to studies, the vast majority of studies in the field of fingerprinting have focused on the development of methods to make fingerprints visible on various surfaces and conditions [22]–[24]. However, the limited number of studies discussing the impact of FD methods on other types of evidence often focus on the development of strategies for the recovery of the touch DNA contained in the fingerprint itself and transferred from fingerprints by epithelial cells [2], [25]. Accordingly, the number of studies on the extent to which FD methods change the structural properties of biological materials (such as blood and saliva) or the level of DNA degradation is also limited [11], [26].

DNA extraction and quantification were performed in repeats of 3 from each sample in this study. The average DNA amounts obtained by Qubit Fluorimeter after DNA extraction are given in Table 1. The amount of DNA observed from the blind samples studied in each group to observe the presence of contamination was <0.05 ng/μL. The degradation index was calculated by the ratio of the amount of DNA obtained from the control sample to the amount of DNA obtained from the FD-treated sample (Suppl 1). In addition, in contrast to the studies in the literature, this study focused on DNA quantification and used starting material with known amounts of DNA [26], [27]. The presence of contamination was checked by using a negative control (NC) to ensure internal control throughout the study. No DNA presence was observed in the negative controls used (<0.05 ng/μL). Among the fresh samples prepared in the study, DNA recovery was higher in the SS-treated urine, blood and saliva samples and in the CV-treated semen sample group compared to the other groups (Table 1). Surprisingly, DNA recovery in SS and CV-treated saliva samples, CV-treated semen samples, and SS-treated blood samples increased significantly after chemical treatment compared to the control group. This shows that chemical treatment of some biological samples on adhesive tape increases the efficiency of DNA recovery. When the 45-day waiting samples were compared with the control group samples, DNA recovery decreased in CV-treated urine and blood samples, while DNA recovery increased in SS-treated urine and blood samples. In semen samples, both CV and SS treatment negatively affected DNA recovery. In saliva samples, DNA recovery increased ~2-fold in the CV-treated sample group, while SS treatment caused a ~75% decrease in DNA recovery. It should be noted that oral hygiene or oral microbial content is a factor affecting saliva’s DNA content especially.

Table 1. Time-dependent DNA quantification comparison independent sample T-test data obtained from adhesive tape surface treated with fingerprint development reagents.

Sample	Fingerprint development	Time (day)	P values
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chemicals		1 day		45 days		
		DNA quantity (ng/μL)*		DNA quantity (ng/μL)*		
		Mean	SD	Mean	SD	
Urine	Control	0,523	0,111	0,249	0,034	0,015
	Crystal violet	0,074	0,008	0,120	0,016	0,011
	Sticky side	0,485	0,019	0,342	0,027	0,002
Blood	Control	2,137	0,086	1,127	0,150	0,001
	Crystal violet	3,067	0,120	0,217	0,070	<0,001
	Sticky side	7,370	0,285	3,193	0,316	<0,001
Semen	Control	4,850	0,719	10,937	0,627	<0,001
	Crystal violet	6,790	0,403	10,403	0,425	<0,001
	Sticky side	4,730	0,205	6,177	0,265	0,002
Saliva	Control	3,830	0,519	8,820	0,390	<0,001
	Crystal violet	24,153	0,243	16,793	0,627	0,001
	Sticky side	47,973	1,830	2,430	0,415	<0,001

* Sample repeats n=3

In the comparison between groups (Figure 2), when biological samples treated with FD and kept for 1 to 45 days were compared according to sample type, DNA recovery decreased in urine, blood and saliva samples kept for 45 days, while DNA recovery increased in semen samples. These different observations can be explained by the amount of nucleated and DNA-containing cells that biological samples contain by their nature.

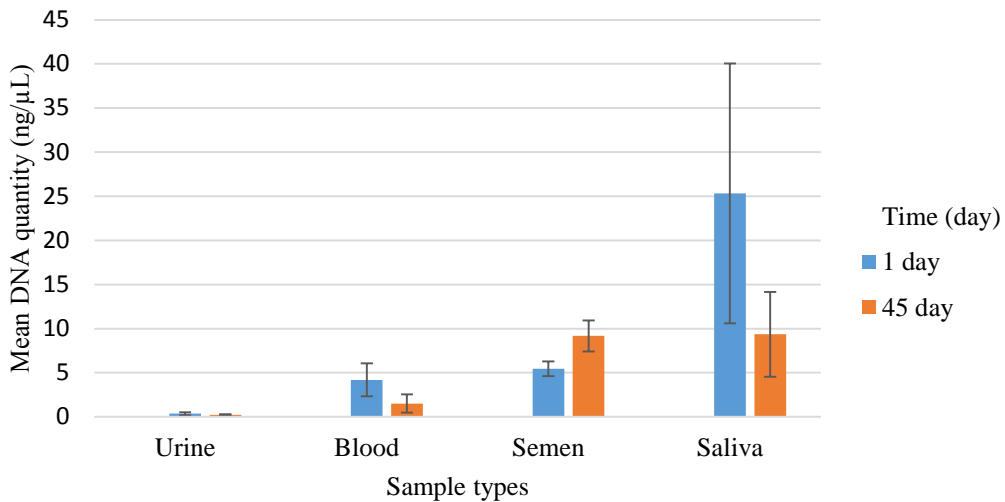


Fig. 2. Time-dependent DNA amount (ng/μL) obtained from different biological fluids regardless of FD difference.

When the DNA amounts (ng/μL) obtained from samples containing different biological fluids, treated with CV and SS FD techniques and kept for 1 to 45 days were evaluated in terms of chemical exposure (Figure 3), compared to the control group, both CV and SS treatment caused an increase in DNA recovery in 1-day samples, while CV

treatment caused an overall increase in DNA recovery in samples kept for 45 days, while SS treatment decreased DNA recovery. While more DNA amount was obtained with SS in fresh findings, the amount of DNA obtained at the end of the 45th day decreased significantly. This decrease in DNA recovery is not expected to significantly affect the quality of DNA profiles.

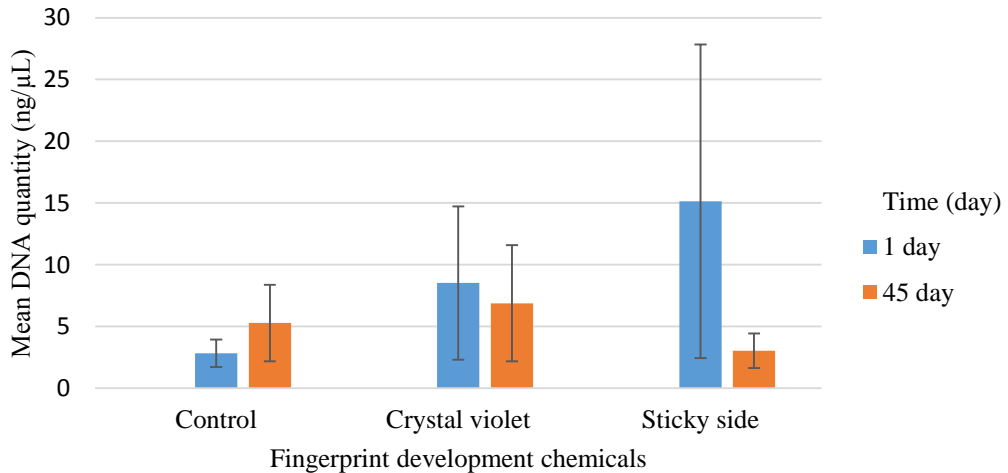


Fig. 3. Variation of DNA amount (ng/μL) depending on fingerprint development methods and time.

The SS method, which is effective on adhesive surfaces, has been used to enhance fingerprints on the adhesive surfaces of tapes [28]. SS combined with Un-du reagent has been observed to negatively affect the amount of DNA in blood fingerprints. However, if SS is applied alone, it is possible to obtain a profile [29]. In a study where bloodstains left on different surfaces at different time intervals were treated with fingerprint chemicals, it was noted that SS can be used for fingerprint development when it is ensured that there is sufficient DNA for analysis [30]. Au et al. developed bloody fingerprints on dark surfaces with white SS powder. They observed that SS reduced the amount of DNA [27]. In this study, similar to Au et al., it was found that the SS application and the 45-day waiting period negatively affected DNA recovery from biological samples.

CV, which becomes visible by attaching to fatty compounds in latent fingerprints, gives the fingerprint a purple appearance as a result of the process. In a study by Lennard et al., fingerprints and bloodstains were left on tape, and fingerprints were made visible by CV [28]. It was observed that CV did not have a negative effect on DNA typing. In particular, no negative effects were observed for DNA extraction, DNA quantification or typing in samples stored dry at room temperature [31]. Treatment of traces on adhesive surfaces with crystal violet did not affect STR analyses [28]. In a study in which dried bloodstains were treated with various reagents, it was observed that crystal violet did not reduce the amount of DNA [32]. PCR-based DNA typing of a single bloody fingerprint developed with crystal violet was successful [29]. The data obtained are parallel to this study. In the present study, CV application increased the DNA recovery rate in both fresh and aged samples. Furthermore, this study shows that in biological fluids other than semen (urine, blood and saliva), the amount of DNA required for identification can be obtained, although there is a decrease in DNA recovery over time.

Due to the sensitivity of biological samples, biological examinations are usually given priority in the examination of evidence. In such evidence, the biological sample taken in order not to damage the fingerprints on the evidence may be insufficient, and a sufficient amount of DNA cannot be obtained for genetic analyses. To obtain results from DNA analyses performed on the evidence, efforts to collect a substantial amount of biological samples often lead to

the compromising or damaging of fingerprint samples present on the same piece of evidence. For this reason, various precautions and caution should be taken when collecting fingerprints contaminated with other body fluids from the crime scene.

Based on the data obtained from the study, the extraction kit employed has demonstrated its capability to isolate a sufficient amount of DNA from adhesive surfaces treated with FD chemicals, suitable for forensic genotyping and phenotyping analyses. It was determined with the analyses that the latent FD chemicals used on adhesive tape surfaces, which are non-porous surfaces, do not affect DNA recovery at a level that would prevent STR profiling. According to the results obtained, it was determined that DNA recovery was high for all biological sample types obtained from adhesive tape in terms of surface type. These results indicate that forensic genetic analyses for DNA recovery can be performed on adhesive tape surfaces treated with fingerprint enhancement chemicals. Over the past two decades, most studies have used biological fluids, such as bloodstains or saliva, as DNA sources to examine the effect of fingerprint treatments on DNA analysis [33]–[35], while other more recent studies have used volunteer fingerprints as sources traces [13], [36]–[39]. On the contrary, there is no study in which blood, urine, semen and saliva samples were used together. In this sense, the results do not sufficiently overlap with similar findings in the literature because a study has not yet been to determine DNA recovery at two different time intervals after applying FD to four different body fluids on the adhesive surface.

4. Conclusion

It is essential to investigate whether the fingerprint development processes performed on biological evidence obtained in crime scene investigation disrupt the structure of biological materials and to determine which of the fingerprint development methods that provide the same function while providing fingerprint development can be used without disrupting the structure of DNA. This scientific gap in the literature also prevents criminal laboratories from establishing standardised methods for the genetic examination of FD-applied adhesive surfaces, which are also valuable biological evidence due to the DNA they contain. When considered worldwide, it is seen that there are few studies targeting this issue [2]. Moreover, these studies are far from being systematic, and they will unlikely standardise the data they present. Therefore, in the present study, basic steps towards system validation have been taken as a reference and guideline for all future studies in this field.

This study paves the way for an approach to determine how latent fingerprinting chemicals affect DNA recovery from different biological fluids. DNA was successfully extracted from all FD-treated adhesive tape surfaces containing different biological fluids, and DNA amounts were measured in all of them. Longer-term investigations are also required to address how other factors may affect DNA recovery, including the time between latent fingerprinting and DNA analysis, as well as the modification of initial DNA amounts by surface types and the determination of this change.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this article. No financial or personal relationships with third parties exist that could unduly influence, or be perceived to influence, the content of this article.

Author contributions

The authors declare that they have all made equal contributions to the work presented in this article.

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Ethical approval

All experimental protocols were conducted in accordance with relevant guidelines and regulations and were approved by the Ethics Committee of Kutahya Health Sciences University (Approval No: 2020/09-05).

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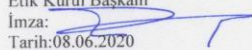
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Appendix

T.C
KÜTAHYA SAĞLIK BİLİMLERİ ÜNİVERSİTESİ REKTÖRLÜĞÜ
GİRİŞİMSEL OLMAYAN KLİNİK ARAŞTIRMALAR ETİK KURULU
KARAR FORMU

ARAŞTIRMANIN AÇIK ADI		Parmak İzi Geliştirme Yöntemleri Uygulanan Biyolojik Materyallerin Bütünlüğünün Değerlendirilmesi ve Söz Konusu Yöntemlerin DNA Analizine Etkilerinin Araştırılması
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	KOORDİNATÖR/SORUMLU ARAŞTIRMACININ BULUNDUĞU MERKEZ	Kütahya Sağlık Bilimleri Üniversitesi Mühendislik ve Doğa Bilimleri Fakültesi
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ARAŞTIRMANIN TÜRÜ	Gözlemsel, Tanımlayıcı	
KARAR BİLGİLERİ	Karar No: 2020/09-05	Tarih: 04.06.2020
	Başvuru dosyası ile ilgili belgeler araştırmanın gerekçe, amaç, yaklaşım ve yöntemleri dikkate alınarak incelenmiş ve uygun bulunmuş olup araştırmanın başvuru dosyasında belirtilen merkezde gerçekleştirilmesinde etik ve bilimsel sakınca bulunmadığına oy birliği ile karar verilmiştir.	

Prof.Dr.Duygu PERÇİN RENDERS
Etik Kurul Başkanı
İmza: 
Tarih:08.06.2020