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# The Evaluation of Factors Affecting the Amount of DNA Obtained from Fired Cartridge Cases

## Ateşlenmiş Kovanlar Üzerinden elde edilen DNA Miktarına Etki Eden Faktörlerin Değerlendirilmesi

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### ABSTRACT

**Objective:** It involves the evaluation of the effects of different biological materials deposited on cartridge cases on the amount of DNA recovered post-firing, considering the influence of time and different types of swabs

**Methods:** MKE 9x19 mm brass cartridges were contaminated with blood and epithelial cells, fired, and swabbed at different intervals (1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 2 months, 3 months) to assess DNA quantity. The study compared microfiber and cotton swabs, utilizing the phenol-chloroform method for DNA extraction and the Quantifiler Trio kit with the 7500 real-time polymerase chain reaction system for quantification.

**Results:** According to the AmpFISTR™ Identifiler™ kit, eight cartridge cases fell within the high-quality DNA profile range (0.05-0.125 ng/μL). One of these is an epithelium-contaminated cartridge, while the others are blood-contaminated cartridges. For cotton swabs, the highest degradation rate was obtained in the 4<sup>th</sup> week for blood contaminated cases, 2<sup>nd</sup> weeks for epithelial cell-contaminated cases. For microfiber swabs, the highest degradation rate was obtained in the 3<sup>rd</sup> month for blood contaminated cases, 1st day for epithelial cell-contaminated cases. In a study using cotton and microfiber swabs to collect samples from different biological materials on cartridge cases, no significant difference was found in DNA quantity between the swab types on day 1 and month 3.

**Conclusion:** The microfiber swab, considered an alternative to the routinely used cotton swab, did not demonstrate superiority. DNA sufficient for successful profiling was obtained even from cartridges swabbed three months after firing. Additionally, blood-contaminated cartridges had significantly higher DNA levels than those contaminated with epithelial cells.

**Keywords:** Cartridge case, DNA quantification, microfiber swap, cotton swap, touch DNA



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## ÖZ

**Amaç:** Kovan üzerine yerleştirilen farklı biyolojik materyallerin ateşleme sonrası geri kazanılan DNA miktarı üzerindeki etkilerinin, zaman faktörü ve farklı sürüntü çubuğu türleri dikkate alınarak değerlendirilmesidir.

**Yöntem:** Üzerine kan ve epitel hücre bulaştırılan MKE marka 9x19 mm çapında pirinç fişekler ateşlendikten sonra farklı zaman aralıklarında (1 gün, 1 hafta, 2 hafta, 3 hafta, 4 hafta, 2 ay, 3 ay) üzerlerinden sürüntü alınarak DNA miktarı değerlendirilmesi gerçekleştirilmiştir. Mikrofiber ve pamuk sürüntü çubuğunun mukayese edildiği bu çalışmada DNA izolasyon yöntemi olarak fenol kloroform, miktar ölçümü için ise Quantifiler Trio kiti ile 7500 gerçek-zamanlı polimeraz zincir reaksiyon cihazı kullanılmıştır. Böylece kovan üstüne bulaştırılan kan ve epitel hücrenin ateşleme sonucu farklı zaman aralıklarında analize alınması ve farklı sürüntü çubuğu türlerinin DNA miktarı üzerindeki etkisi ölçülmüştür.

**Bulgular:** Sekiz kovan AmpFISTR™ Identifiler™ kitine göre yüksek kaliteli (0.05-0.125 ng/μL) profil aralığındaydı. Bunlardan biri epitelle kontamine kovan iken diğerleri kanla kontamine kovanlardı. Pamuklu sürüntü çubuğu için en yüksek bozunma oranı kanla kontamine kovanda 4. haftada, epitel hücreyle kontamine kovanlarda 2. haftada elde edildi. Mikrofiber sürüntü çubukları için en yüksek bozunma oranı kanla kontamine kovanlarda 3. ayda, epitel hücreyle kontamine kovanlarda 1. günde elde edildi. Kovanlardaki farklı biyolojik materyallerden örnek toplamak için pamuk ve mikrofiber sürüntü çubukları kullanan bu çalışmada, sürüntü çubukları türleri arasında 1. günde ve 3. ayda DNA miktarında anlamlı bir fark bulunmadı.

**Sonuç:** Rutin analizlerde kullanılan pamuk sürüntü çubuğuna alternatif olarak düşünülen mikrofiber sürüntü çubuğunun pamuk sürüntü çubuğuna karşı bir üstünlüğü olmadığı görülmektedir. Ateşleme vesürüntü alma arasında 3 aygeçen kovanlardan dahi başarılı profil sağlanabilecek DNA miktarı elde edilmiştir. Ayrıca kan bulaştırılmış kovanlar epitel bulaştırılmışlara göre daha yüksek seviyede DNA barındırmaktadır.

**Anahtar Kelimeler:** Kovan, DNA miktarı, mikrofiber sürüntü çubuğu, pamuk sürüntü çubuğu, dokunma DNA'sı

## INTRODUCTION

Violent crimes, including murder, theft, and terrorism, have emerged as a growing concern and social issue in Turkey. A prevailing characteristic of these crimes is the frequent use of firearms. The rising rates of unlicensed firearm usage further support this belief (1).

When a gun is fired, the bullet travels toward its target, while the cartridge case, often a ballistic clue found at crime scenes, is ejected from the weapon and falls near the shooter (2). While perpetrators may attempt to clean traces and evidence from the weapon, they often overlook materials like the ejected cartridge case. Consequently, this high-potential evidence should be meticulously examined in laboratories.

When loading a round into the magazine, the shooter applies pressure to the cartridge case, causing fluids such as sweat, oil, and blood from the shooter's hand to transfer onto the surface. Additionally, epithelial cells are also deposited. These biological materials can identify the shooter's DNA on the cartridge case (3). DNA evidence is a primary tool in suspect identification, and analyzing such evidence can increase the success rate of identifying perpetrators, thereby enhancing the deterrent effect of firearms-related crimes.

In cases of homicide or assault, the assailant may be injured by the victim, and if the shooter reloads the magazine with a bloody hand, blood can transfer onto the cartridge. Another common type of biological material found on the cartridge is sweat and epithelial cells from routine contact, known as touch DNA (4,5). These two types of biological materials are crucial in DNA quantification and have been compared in this study.

Cartridges collected from crime scenes may not be analyzed immediately due to various reasons, such as delayed crime reporting, high laboratory workloads, or procedural issues in different departments. Over time, sweat evaporates, decreasing DNA quantity (6). This study investigates the temporal changes in DNA quantity in cartridge cases.

The amount of cells deposited by touch is typically low, and depending on the DNA isolation method, up to 70% of DNA may be lost during the isolation process (7). These challenges have led scientists to seek more sensitive techniques for identification (8). One such method is using different types of swabs for sample collection. Forensic laboratories in Turkey commonly use cotton swabs, which bind biological materials tightly to fibers, making it challenging to release the sample (9). Samples collected with these swabs must be wet; if not, the swab is moistened with a solution before collecting the sample. However, this can create an environment conducive to bacterial growth, so the swab must be dried before packaging to prevent hydrolysis, oxidation, and DNA degradation (10). However, drying the swab is time-consuming. Microfiber swabs, made of parallel nylon threads around a plastic rod, contain antimicrobial agents, eliminating the need for drying after sample collection. The capillary action in microfiber swabs allows for easier release of cells into the extraction solution compared to cotton swabs (11,12). This study compares the DNA yield of cotton and microfiber swabs.

Temperatures of up to 1800 °C can be observed when a firearm is discharged (13). The hot gases produced by the propellant combustion cover most of the cartridge's side surface, as the chamber does not fully seal the cartridge. This pressure causes

the cartridge to expand, transferring some cells containing DNA onto the barrel surface. Additionally, the composition and quantity of propellant gases vary depending on the cartridge type (14,15).

Given these factors, obtaining DNA from a cartridge case depends on numerous variables. The study focuses on the time elapsed between the deposition and analysis of the biological sample, the type of biological material containing DNA, and the type of swab used for sample collection. Consequently, this study elucidates the evidentiary value of frequently analyzed cartridge cases.

## MATERIALS AND METHODS

To prevent contamination before the study, the cartridges, firearms, magazines, and consumables were cleaned with Zefiran IM (Molteni, Switzerland). The consumables were autoclaved at 121 °C for 20 minutes. Two women and two men over the age of 18 contaminated MKE 9x19 mm brass cartridges with blood and epithelial cells. Blood-contaminated cartridges were prepared by pricking the alcohol-cleaned middle finger with a sterile single-use lancet needle using a home glucose meter under the supervision of the researcher. Approximately 10 µL of blood from the middle finger was transferred to the thumb and index finger and applied to the cartridge with pressure. Participants washed their hands with soap and water for one hour before contaminating the cartridges with epithelial cells. During daily activities, without any additional requests (except the request not to wear gloves to increase sweating), the participants applied pressure to the cartridges with their thumb and index finger. Care was taken to ensure that finger contact with the cartridge did not exceed 10 seconds during sample preparation. The cartridges, loaded into the weapon by the participants, were fired by a professional shooter using a Sarsılmaz Kılınc Mega 2000 model firearm at a licensed private shooting range. A tarp was spread where the cartridge cases would fall, and it was replaced after each shot. The cartridge cases that fell onto the tarp were collected by a researcher

wearing double gloves and placed in paper evidence bags. Gloves were changed after handling each different cartridge case. The evidence bags were then transported to the laboratory.

### Control Samples

As a control group, each participant contaminated one cartridge with epithelial cells and another with blood, using cartridges identical to those used in the experiment. Control samples were not fired. A total of 8 cartridges were sampled the day after contamination using cotton swabs, and DNA analyses were conducted (Table 1).

Positive control samples were used to verify the accuracy of the analyses and evaluate the effectiveness of the kit. Negative control samples do not contain template DNA and are used to detect contamination.

### DNA Isolation

To investigate the effect of swab type on DNA yield, COPAN brand (Italy) microfiber (FLOQ) swabs and BeyanLab brand (Turkey) cotton swabs were used. The microfiber swab was cut with scissors at the breakpoint and transferred whole into an Eppendorf tube. The cotton swab was cut into small pieces with scissors and placed in the tube. The scissors, previously disinfected with bleach, were changed for each swab (12). After firing, the cartridge cases were stored in sterile storage containers under laboratory conditions until analysis (up to 3 months later). Cartridge cases swabbed with microfiber swabs were analyzed 1 day and 3 months later, while those swabbed with cotton swabs were analyzed 1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 2 months, and 3 months later. A total of 80 swabs were subjected to DNA analysis, including 64 cotton swabs (8 control + 56 fired) and 16 microfiber swabs (Table 1). DNA isolation was performed using the organic isolation (phenol-chloroform) method (9). Unlike Semizoğlu's (9) protocol, 10 µL of proteinase-K was added to the fired samples, 5 µL to the control samples, and 3 µL to the isolation negative. Additionally, after evaporating the alcohol in the experimental tubes, 30 µL of Te<sup>+4</sup> buffer was added to each sample, followed

**Table 1. Number of swabs used to evaluate the effect of time factor on DNA amount**

| Biological sample                      | Surface type         | Sample amount | Waiting time of samples                   |   |         |         |         |         |         |
|--|----------------------|---------------|---|---|---------|---------|---------|---------|---------|
|  |                      |               | Cotton swab                               |   |         |         |         |         |         |
| Blood                                  | Brass cartridge case | ~10 µL        | Day 1                                     | Week 1  | Week 2  | Week 3  | Week 4  | Month 2 | Month 3 |
|  |                      |               | Epithelial cell                           | Amount accumulated by pressing for 10 seconds | 4 swabs | 4 swabs | 4 swabs | 4 swabs | 4 swabs |
| Periodic interim total for cotton swab |                      |               | 8 swabs                                   | 8 swabs                                       | 8 swabs | 8 Swabs | 8 swabs | 8 swabs | 8 swabs |
| Periodic subtotal for microfiber swab  |                      |               | 8 swabs                                   | x   | x       | x       | x       | x       | 8 swabs |
| Number of control samples              |                      |               | 8 swabs                                   |   |         |         |         |         |         |
| Total number of samples                |                      |               | 80 swabs (40 blood + 40 epithelial cells) |   |         |         |         |         |         |

by a quick spin (short centrifugation, approximately 5 seconds) to ensure that no  $Te^{+4}$  buffer containing DNA remained on the cap's inner surface or tube walls.

### DNA Quantification

Quantification was carried out using the Quantifiler Trio kit (ThermoFisher, Waltham, MA) on the 7500 real-time polymerase chain reactionsystem (PCR) system (ThermoFisher, Waltham, MA). Table 2 shows the mixtures and DNA quantities distributed in each tube for quantification.

### Statistical Analysis

IBM SPSS 29.0 software was used for the statistical analyses. The alpha level was set at 0.05, and the confidence interval was 95%. Descriptive statistics were provided for the collected data. Arithmetic means, and standard deviations were calculated for quantitative data. The normality of data distribution was assessed using the Shapiro-Wilk test. Parametric methods were used for data with normal distributions, while non-parametric methods were applied to data that did not follow a normal distribution. For comparisons between the two groups, the Student's t-test was used for parametric data, and the Mann-Whitney U test was used for non-parametric data. Comparison of blood and epithelium contaminated cartridges cases in terms of DNA amount and degradation, comparison of cotton and microfiber swab types in terms of DNA amount and degradation showed normal distribution, while comparison of cartridges cases in terms of DNA amount and degradation over time did

not show normal distribution. Repeated measurements over time were analyzed using repeated measures ANOVA. Results with a p-value less than 0.05 were considered statistically significant. Descriptive statistics are presented in Tables 3-5 and Figure 1.

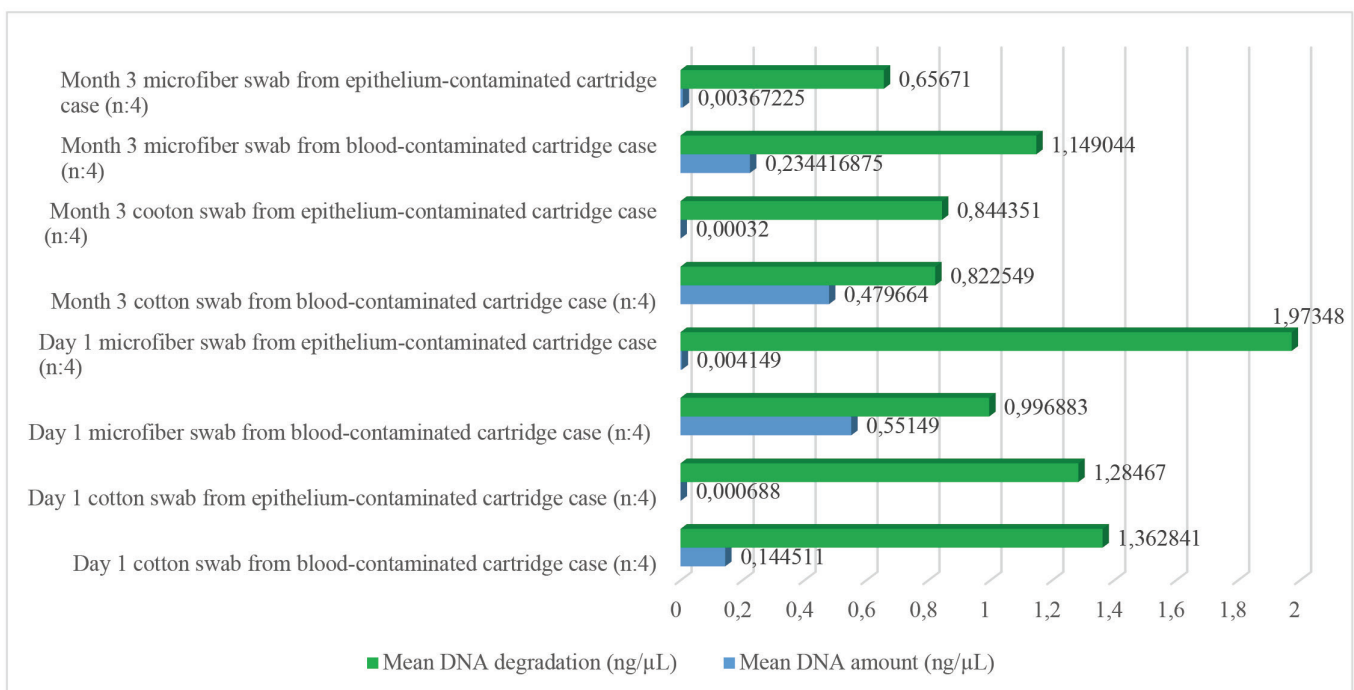
## RESULTS

The reliability of the measurements conducted with the real-time PCR system was assessed, yielding an  $R^2$  value of 0.999, an average IPC value of 27.89519, and a slope of -3.363. According to the Quantifiler Trio kit, a degradation index greater than 10 or a value of 0 indicates that the sample has undergone degradation. Values between 1 and 10 represent partial degradation, while values less than 1 indicate that the sample has not degraded (16).

The amount of DNA obtained after isolation provides insight into the quality of the resulting electrophoresis profile. According to the AmpFISTR™ Identifier™ kit, a high-quality profile is achieved with DNA quantities between 0.05 and 0.125 ng/ $\mu$ L (17).

### Comparison of DNA Quantity and Degradation Rates in Control Samples

No contamination was detected in the negative control samples during isolation. The average DNA quantity in positive control samples was 0.17991128 ng/ $\mu$ L for blood-contaminated cartridge cases and 0.00016103 ng/ $\mu$ L for epithelial cell-contaminated cartridge cases. The average degradation indices



**Figure 1.** Descriptive statistics of samples taken with cotton and microfiber swabs from blood and epithelium contaminated casings, on the 1<sup>st</sup> day and after the 3<sup>rd</sup> month post-firing

were 0.828337327 for blood-contaminated cartridge cases and 0.31648314 for epithelial cell-contaminated cartridge cases (Table 3). Three of the epithelial cell-contaminated cartridge cases showed a degradation value of 0. The amount of DNA each individual leaves on a surface varies, affecting the recoverable DNA quantity and the quality of the resulting profile (18).

**Table 2. Reaction and primer mix and DNA amount dispensed**

|                  |       |
|------------------|-------|
| Reaction Mixture | 10 µL |
| Primer Mixture   | 8 µL  |
| DNA Amount       | 2 µL  |

### Comparison of DNA Quantity and Degradation Rates in Blood and Epithelial Cell- Contaminated Cartridge Cases

The average DNA quantity in samples that were contaminated with blood, fired, and swabbed at different times using cotton and microfiber swabs was found to be 0.321917729 ng/µL, with an average degradation rate of 1.191024206 ng/µL. According to the AmpFISTR™ Identifiler™ kit, 7 of these samples fall within the high quality profile range. None of the samples showed any signs of degradation (Table 4).

In contrast, the average DNA quantity in samples that were contaminated with epithelial cells fired and swabbed

**Table 3. Descriptive statistics of DNA amounts and DNA degradation rates of control samples**

| Blood-Contaminated Cartridge Case (n:4) |                    |                          |                    | Epithelium-Contaminated Cartridge Case (n:4) |                    |                          |                    |
|---|--------------------|--------------------------|--------------------|--|--------------------|--------------------------|--------------------|
| DNA amount (ng/µL)                      |                    | Degradation rate (ng/µL) |                    | DNA amount (ng/µL)                           |                    | Degradation rate (ng/µL) |                    |
| Average                                 | Standard deviation | Average                  | Standard deviation | Average                                      | Standard deviation | Average                  | Standard deviation |
| 0.179911280                             | 0.089574456        | 0.828337327              | 0.142089117        | 0.00016103                                   | 0.000138195        | 0.31648314               | 0.632966280        |

**Table 4. Descriptive statistics of DNA amounts and degradation rates from cartridge cases contaminated with blood and epithelium cells**

| Blood contaminated cartridge cases (n:36) |                    |                          |                    | Epithelium-contaminated cartridge cases (n:36) |                    |                          |                    |
|---|--------------------|--------------------------|--------------------|--|--------------------|--------------------------|--------------------|
| DNA amount (ng/µL)                        |                    | Degradation rate (ng/µL) |                    | DNA amount (ng/µL)                             |                    | Degradation rate (ng/µL) |                    |
| Average                                   | Standard deviation | Average                  | Standard deviation | Average  | Standard deviation | Average                  | Standard deviation |
| 0.321917729                               | 0.465012326        | 1,191024206              | 0.591666243        | 0.007079351                                    | 0.018902583        | 1.72939042               | 2.971484060        |

**Table 5. Descriptive statistics of the changes in DNA quantity and degradation rates over time in cartridge cases contaminated with blood and epithelial cells**

| Cotton swab (n:56)     |   |                    |                          |                    |  |                    |                          |                    |
|------------------------|---|--------------------|--------------------------|--------------------|--|--------------------|--------------------------|--------------------|
|                        | Blood contaminated cartridge cases (n:28) |                    |                          |                    | Epithelium-contaminated cartridge cases (n:28) |                    |                          |                    |
|                        | DNA amount (ng/µL)                        |                    | Degradation rate (ng/µL) |                    | DNA amount (ng/µL)                             |                    | Degradation rate (ng/µL) |                    |
|                        | Average                                   | Standard deviation | Average                  | Standard deviation | Average  | Standard deviation | Average                  | Standard deviation |
| Day 1                  | 0.144511                                  | 0.129889           | 1.362841                 | 1,031031           | 0.000688                                       | 0.000402           | 1.284669                 | 1,658824           |
| Week 1                 | 0.219841                                  | 0.192935           | 1.051482                 | 0.36252            | 0.008093                                       | 0.008505           | 5.998846                 | 7,590053           |
| Week 2                 | 0.068113                                  | 0.084367           | 1.16114                  | 0.253217           | 0.001602                                       | 0.000864           | 1.258784                 | 1,065084           |
| Week 3                 | 0.266884                                  | 0.347783           | 1.064055                 | 0.42249            | 0.006802                                       | 0.006417           | 0.908431                 | 0.178131           |
| Week 4                 | 0.612452                                  | 0.813952           | 1.668352                 | 0.988973           | 0.028711                                       | 0.055386           | 0.489401                 | 0.588986           |
| Month 2                | 0.329916                                  | 0.579138           | 1.44287                  | 0.694358           | 0.005743                                       | 0.009915           | 2.149843                 | 2,54247            |
| Month 3                | 0.479664                                  | 0.699184           | 0.822550                 | 0.179915           | 0.000320                                       | 0.000261           | 0.844351                 | 0.920234           |
| Microfiber swab (n:16) |   |                    |                          |                    |  |                    |                          |                    |
|                        | Blood-contaminated cartridge cases (n:8)  |                    |                          |                    | Epithelium-contaminated cartridge cases (n:8)  |                    |                          |                    |
|                        | DNA amount (ng/µL)                        |                    | Degradation rate (ng/µL) |                    | DNA amount (ng/µL)                             |                    | Degradation rate (ng/µL) |                    |
|                        | Average                                   | Standard deviation | Average                  | Standard deviation | Average  | Standard deviation | Average                  | Standard deviation |
| Day 1                  | 0.551490                                  | 0.632598           | 0.996883                 | 0.463034           | 0.004149                                       | 0.004663           | 1.973480                 | 1,561424           |
| Month 3                | 0.224389                                  | 0.281223           | 1.149044                 | 0.479686           | 0.007607                                       | 0.00845            | 0.656710                 | 0.827597           |



at different times using cotton and microfiber swabs was 0.007079351 ng/ $\mu$ L, with an average degradation rate of 1.72939042 ng/ $\mu$ L (Table 4). Ten of the epithelial cell-contaminated cartridge cases had a degradation value of 0. Only one sample falls within the high quality profile range according to the AmpFISTR™ Identifiler™ kit, and most of these samples exhibited partial degradation. The DNA quantity obtained from blood-contaminated cartridge cases is statistically significantly higher than that from epithelial cell-contaminated cases ( $p < 0.001$ ). However, there is no significant difference in degradation rates between the types of biological samples on the cartridge cases ( $p = 0.937$ ).

### Comparison of DNA Amount and Degradation Rate on Fired Cartridge Cases Based on Time and Biological Material Types

DNA analyses were conducted on blood and epithelial cells deposited on cartridge cases at various intervals (1 day, 1 week, 2 weeks, 3 weeks, 4 weeks, 2 months, and 3 months) after firing, using cotton swabs. The highest DNA quantity was obtained in the 4<sup>th</sup> week, regardless of the type of biological sample. Degradation rates were lowest in the 3<sup>rd</sup> month for blood-contaminated cases and in the 4<sup>th</sup> week for epithelial cell-contaminated cases (Table 5).

DNA samples were collected from blood and epithelial cell-contaminated cartridge cases at 1 day and 3 months after firing using microfiber swabs. One blood-contaminated cartridge case and two epithelial cell-contaminated cartridge cases had a degradation value of

0. The highest DNA quantity for blood-contaminated cases was obtained on day 1, while it was obtained in the 3<sup>rd</sup> month for epithelial cell-contaminated cases. Degradation was more pronounced in the 3<sup>rd</sup> month for blood-contaminated cases and on day 1 for epithelial cell-contaminated cases.

### Effect of Different Swab Types on DNA Quantity and Degradation Rates

In a study using cotton and microfiber swabs to collect samples from different biological materials on cartridge cases, no significant difference was found in DNA quantity between the swab types on day 1 ( $p = 0.574$ ) and month 3 ( $p = 0.721$ ) (Figure 1). Similarly, there was no statistically significant difference in degradation between microfiber and cotton swabs on day 1 ( $p = 0.797$ ) and month 3 ( $p = 0.721$ ).

## DISCUSSION

Casings obtained as a result of firing are a frequently overlooked evidence mechanism at crime scenes by perpetrators. Through a multifaceted evaluation of these findings, a wealth of information about the incident can be uncovered. The study observed the variability of DNA yields on fired casings based on biological sample type and time. Additionally, different types

of swabs used in the collection of biological material were compared.

Blood-contaminated cartridges had higher DNA quantitation than epithelium-contaminated cartridges. In a study where eccrine, sebaceous, and bloody fingerprints were applied to different types of cartridges, only the bloody fingerprints yielded full or partial DNA profiles after the cartridges were fired, while no DNA profiles were obtained from cartridges with other biological samples, regardless of whether they were fired or not (19). Since blood is more easily visible to the naked eye than touch DNA, more blood cells may have been collected with the swab.

The time elapsed between touching the cartridge case and conducting the analysis has no significant effect on DNA quantity ( $p = 0.076$ ). This finding is supported by most studies in the literature. McElhoe et al. (20) attributed the lack of time-related effects to the formation of a layer on the metallic surface, which constitutes the main material of the cartridge case. This layer protects the underlying layers from degradation. In the aforementioned study, the temporal variation of mtDNA accumulation on copper bullet projectiles was examined. No significant differences in quantity were observed between cartridges collected immediately after cellular material was deposited and those stored for 3, 5, 7, 10, 39, 60, and 70 days before extraction (20).

In a study where DNA was collected from participants' fingerprints on an aluminum magazine using swabbing and stored at room temperature for 1 and 2 months, no statistically significant difference was found between the two-time intervals (21).

However, Winnepeninckx et al. (22) found a difference in DNA quantity over time. Natural fingerprints on 9x19 mm brass cartridges were rinsed with BSA and Gly-Gly-His, and then samples were collected from the surfaces using swabs. Cartridges stored for 24 hours had more DNA than those compared with those stored for one week. The difference in the impact of time on DNA quantity compared to other studies might be due to the use of chemicals for rinsing before swabbing.

The degradation amount on the cartridge cases over time was statistically significant ( $p = 0.005$ ). According to a 2022 study, oxidation caused by metal ions, rather than heat or time, is the main cause of degradation (23). A contrasting view suggests that the high temperatures generated by firing accelerate chemical reactions and increase corrosion (24). Additionally, metal ions complicate the collection of DNA from surfaces by affecting the oxygen atoms in the phosphate backbone and the hydrogen bonds in certain regions of the nitrogenous bases (25-27). When the cartridge is exposed to high temperatures, such as during firing, chemical reactions are accelerated, and corrosion becomes more pronounced, increasing the amount of free metal ions along the papilla.

The degradation amount observed in control samples was lower than in samples swabbed after a certain period following firing. However, external factors that cause degradation, such as time or heat, are not present in control samples. This suggests that while the heat generated by firing and the elapsed time contribute to degradation, oxidation caused by metal ions also plays a role.

When swabbing the surface, PCR inhibitors such as gunshot residue are collected along with DNA molecules (28). During the purification stage, the final step of DNA isolation, these inhibitors are removed from the environment, but the total DNA quantity decreases as a result (29,30).

There is no difference in DNA quantity and degradation value between the two types of swabs could potentially be attributed to the initially limited accumulation of DNA on the cartridge case upon contact, the restricted sample size, and the difficulty in releasing DNA from the swab fibers. Additionally, the results may be influenced by the fact that the microfiber swab is broken off at a specific point and placed whole in the tube, while the cotton swab is cut into smaller pieces to increase surface area and allow the isolation chemicals to penetrate the fibers more effectively (31).

Nylon, which constitutes the fibers of microfiber swabs, contains N-H groups that form hydrogen bonds with nucleic acids, causing the nucleic acids to bind tightly to the fibers. While this binding is advantageous during the swabbing process, it may create challenges when releasing the samples into the extraction solution (32,33).

In a 2020 study, using microfiber swabs instead of cotton swabs to collect DNA from saliva-contaminated, fired cartridge cases resulted in  $2.8 \pm 1.4$  times more DNA. Regardless of whether the m/39B brass cartridges were fired, more DNA was obtained with microfiber swabs. However, the high DNA yield from microfiber swabs could not be fully utilized due to a significant increase in complex STR profiles, which were too intricate for comparison (34). In contrast, the tightly wound fibers of cotton swabs around the shaft make it more difficult to release cells into the extraction solution compared to the free fiber structure of microfiber swabs (35).

## CONCLUSION

Cartridge cases are among the most likely evidence to be encountered at a crime scene involving a firearm. Ballistic evidence, such as determining which weapon was used, can be derived from cartridge cases, as well as information about the shooter's identity. Cartridge cases bear the cells containing the shooter's identifying characteristics during the loading process and fall near the shooter when the weapon is fired. The shooter's actions prior to loading the cartridge into the weapon affect the amount of DNA that can be obtained. According to the study, blood-contaminated cartridge cases contain more

DNA than epithelial cell-contaminated ones. Even when there is a time delay between the shooting and the analysis, potentially as long as three months, sufficient DNA can still be obtained to yield a high-quality profile. Therefore, every cartridge case that arrives at the laboratory should be analyzed, regardless of the time elapsed since the event.

In this study, different swabs were used to collect samples in order to enhance DNA yield. The microfiber swab, considered an alternative to the routinely used cotton swab, did not demonstrate superiority over the cotton swab. The number of studies comparing the changes in DNA quantity due to different biological materials on cartridge cases post-firing is quite limited. Furthermore, during the preparation of this article, no systematic study was found that examined DNA quantity on fired cartridge cases over such an extended time period. Future research could design experiments to simulate various environmental conditions to which cartridge cases might be exposed before analysis. Additionally, the quality of the study could be improved by testing different swab chemicals to reduce degradation in the cartridge cases.

## ETHICS

**Ethics Committee Approval:** The research permission for our study was given by the Kütahya University of Health Sciences Rectorate Clinical Research Ethics Committee with the decision dated 06.09.2023 and numbered 2023/10-14.

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## FOOTNOTES

### Authorship Contributions

Concept: Y.G., H.Ş., Design: H.Ş., Data Collection or Processing: Y.G., F.E.Y., Y.T., Analysis or Interpretation: E.N.A., Y.G., H.Ş., Y.T., Literature Search: E.N.A., F.E.Y., Writing: E.N.A., Y.G.

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