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EVALUATION OF DIFFERENT SWAB WETTING CHEMICALS AFFECTING THE YIELD OF DNA OBTAINED FROM BIOLOGICAL EVIDENCE ON CARTRIDGE CASINGS

Fatma Ebru YÜKSEK[1], Eda Nur AY[1], Yakup GÜLEKÇİ[1]*

[1]*Kütahya Health Sciences University, Faculty of Engineering and Natural Sciences, Department of Forensic Sciences, 43100, Kütahya, Türkiye*

Abstract: Cartridge casings made from transition metals can be examined ballistically and also serve as significant evidence by containing touch DNA. However, the success rate of profiles obtained from this type of evidence is generally low. To enhance the success of DNA profiling from suspects' biological evidence, using swabs moistened with chemicals can be beneficial. Typically, swabs are moistened with water, whose hypotonic nature disrupts cell integrity, causing the release of DNA. However, water is not the only agent used for moistening swabs; various buffer solutions are also utilized. The ability of swabs to transfer touch DNA depends on the type of buffer solution used. Sodium dodecyl sulfate (SDS), a strong anionic detergent, denatures non-covalently linked secondary and tertiary structures increasing the release of bound DNA. Another buffer solution used for swab moistening is the Te+4 buffer, which contains EDTA and Tris. EDTA chelates metal ions, inactivating enzymes that could potentially damage DNA, while Tris adjusts the pH to an optimal level. This study aims to compare the effectiveness of microfiber and cotton swabs moistened with SDS, Te+4 buffer, and water in recovering genetic material from blood and epithelial cells deposited on brass cartridge casings. The study also evaluates the impact of firing on the quality of DNA profiles by analyzing the RFU difference obtained on cartridge case and cartridges. Although the number of complete profiles obtained from water- and SDS-wetted swabs are equal, the average RFU value of SDS-wetted swabs is approximately twice that of water-wetted swabs. The minimum number of complete profiles belongs to swabs wetted with Te^{+4} buffer. SDS is particularly advantageous over water when used on casings with epithelial cells. Microfiber swabs are more effective in eliminating degradative factors caused by firing, thus enhancing profiling success. Comparisons of the RFU values indicate that casings yield lower values compared to cartridges, supporting the negative impact of the high heat, pressure, and residues generated during firearm discharge.

Keywords: Ballistics, Swab wetting chemical, DNA profiling, Identification, Touch DNA

***Corresponding author:** Kütahya Health Sciences University, Faculty of Engineering and Natural Sciences, Department of Forensic Sciences, 43100, Kütahya, Türkiye
E mail: yakup.gulekci@ksbu.edu.tr (Y. GÜLEKÇİ)
Fatma Ebru YÜKSEK https://orcid.org/0000-0002-1666-5416
Eda Nur AY https://orcid.org/0000-0001-9070-253X
Yakup GÜLEKÇİ https://orcid.org/0000-0001-9643-6850

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1. Introduction

Touch DNA, which plays a crucial role in forensic investigations due to its polymorphic nature, is obtained by analyzing cells deposited when a suspect or victim contacts or holds target surfaces at a crime scene (Aditya et al., 2011). The exact origin of the limited amount of DNA resulting from such contact remains unclear. Numerous studies suggest that touch DNA may consist of shed corneocytes (Burrill et al., 2019). It can also include endogenous or transferred nucleated epithelial cells (Lacerenza et al., 2016), fragmented cells and nuclei (Zoppis et al., 2014), and cell-free DNA (Wang et al., 2017; Kanokwongnuwut et al., 2018).

Obtaining an adequate number of cells for identification from touch DNA is more challenging than obtaining DNA found in bodily fluids such as blood (Aditya et al., 2011). Factors such as the individual's cell transfer efficiency, gender, pre-contact activities, and duration of contact also influence the amount of DNA collected (Goray et al., 2016; Kanokwongnuwut et al., 2018; Sessa et al., 2019; van Oorschot et al., 2019).

Another parameter influencing the success of DNA profiling from touch DNA collected from a surface is the surface type itself. Certain materials interact with cells, affecting the efficiency of DNA collection. For example, transition metals and alloys can alter DNA's double helix structure, complicating the amplification of desired strands (Bonsu et al., 2020). Metal ions can form ion-metal bonds with various molecules within the DNA, hindering its collection from surfaces. Additionally, metals as polymerase chain reaction (PCR) inhibitors result in low STR amplification yields (Horsman-Hall et al., 2009).

Cartridge casings made from transition metals like copper, brass, and nickel disperse at the crime scene when a firearm is discharged (Combs et al., 2015; Bonsu et al., 2020). These casings can be examined ballistically and can also serve as significant evidence containing

touch DNA (Sterling, 2017; Nandi et al., 2021). However, factors such as the high heat and pressure generated during firing, and ion-metal interactions from the metals, which act as PCR inhibitors, make DNA recovery from casings challenging (Horsman-Hall et al., 2009; Thanakiatkrai and Rerkamnuaychoke, 2019; Bonsu et al., 2020). Despite these challenges, DNA evidence remains highly important due to its reliability and definitive results in personal identification, leading to the development of various methods to enhance DNA yield. These methods include swabbing (Thomasma and Foran, 2013), tape lifting (Milnthorp et al., 2015), soaking (Prasad et al., 2022), and vacuuming (Prasad et al., 2022). Swabs moistened with a solution can improve the profiling success of biological evidence transfer from suspects (Schulte et al., 2023). Wetting swabs to collect trace biological stains has become a standard practice, as it facilitates the rehydration of the stain and the transfer of cells onto the swab, maximizing the amount of biological material collected (Bonsu et al., 2020). Water is typically used for moistening swabs (Lee et al., 1998). However, the hypotonic nature of water can compromise cell integrity, potentially leading to the release of DNA that may become trapped within the swab fibers, thereby reducing recovery efficiency (Martin et al., 2006; Benschop et al., 2010).

Sampling from metal surfaces using swabs moistened with water can cause oxidative damage to the DNA template during collection (Holland et al., 2019; Kuffel et al., 2021). Additionally, copper found in brass cartridges can degrade DNA upon contact with water (MacDonald et al., 2015).

Various buffer solutions are used as wetting agents instead of water to mitigate these issues. Buffer solutions chemically facilitate the dissolution of nucleic acids from surfaces, enhance absorption onto the swab, and minimize the degradation potential of DNA by binding to metal cations released from surfaces (Bonsu et al., 2020). The effectiveness of buffers in extracting touch DNA from surfaces varies depending on the type of buffer solution used (Thomasma and Foran, 2013; Adamowicz et al., 2014). These solutions typically contain detergents (such as Triton X-100, sodium dodecyl sulfate (SDS)), a chelating agent (like EDTA), or phosphate-buffered saline (PBS) (Bonsu et al., 2020).

Detergents are molecules that contain both hydrophobic and hydrophilic parts, allowing them to disperse easily in water and interact with cell membranes, altering their properties. A three-stage model has been developed to describe how detergents dissolve cell membranes. In the initial stage, detergent molecules distribute between aqueous and lipid components. Interaction with the lipid compartment leads to the development of vesicle bilayers. The detergent-saturated vesicle bilayers coexist with lipid-detergent mixed aggregates, increasing the lipid-detergent ratio. As the ratio increases, detergent-enriched bilayer fragments are forced to transform into a smaller structure where unimers with hydrophilic and hydrophobic segments are combined (Kedar et al., 2010). Although this provides a general understanding of how detergents function, each detergent may act uniquely (Ahyayauch et al., 2010).

Due to containing both hydrophobic and hydrophilic structures, detergents solubilize cell components such as lipids, fats, and proteins in buffer solutions (Thomasma and Foran, 2013; Salager, 2002). Detergents that assist in cell lysis can cause precipitation at higher concentrations and may interfere with commercial DNA extraction kits (van Oorschot et al., 2003; Bonsu et al., 2020). SDS, a strong anionic detergent, denatures secondary and non-disulfide-linked tertiary structures to increase the release of bound DNA (Farrell, 2010).

Another buffer solution used for swab moistening is Te+4 buffer, which consists of EDTA and Tris. EDTA binds to metal ions as a chelating agent, depleting the metals available to metal-dependent enzymes. The resulting ion depletion inactivates enzymes like deoxyribonucleases (DNases) that catalyze the hydrolytic cleavage of phosphodiester bonds, potentially damaging DNA (Farrell, 2010). Tris maintains the environmental pH at a level where DNases are inactive (Li, 2015).

The type of swab used is as crucial as the wetting agent in determining DNA profile quality. Cotton swabs are the most commonly used swab type globally for collecting biological material or stains (Aloraer, 2017). Microfiber swabs, specially designed to easily release collected materials into extraction fluids, are becoming increasingly popular (Ambers et al., 2018).

This study compares the effectiveness of microfiber and cotton swabs moistened with SDS, Te+4 buffer, and water in recovering genetic material from blood and touch DNA on brass cartridge casings. Additionally, the study evaluates the impact of firing on DNA profile quality. Thus, the wetting chemical and swab type that will maximize DNA profile quality for both biological materials will be determined.

2. Materials and Methods

To prevent contamination, all consumables, magazines, and cartridge cases were cleaned with Zefiran IM (Molteni, Switzerland) before the start of the study. Consumables were autoclaved at 121°C for 20 minutes (Labor İldam, Türkiye). A male participant over the age of 18 contaminated 9x19 mm MKE brand brass cartridge cases with epithelial cells and blood. The participant washed his hands with soap and water one hour before applying epithelial cells to the cartridge cases. The epithelial cell contamination on the cartridge cases was achieved by having the participant press the cases with his thumb and index finger during routine activities, without any additional procedures apart from washing his hands (Figure 1). During the epithelial cell contamination process, care was taken to ensure that finger contact with the cartridge case did not exceed 10 seconds. Under sterile conditions, 10 µL of blood collected from the participant was evenly distributed on

the outer wall of the cartridge cases using a micropipette (Figure 2). The cartridge cases, loaded into the weapon by the participant, were fired by a professional shooter using a Sarsılmaz Kılınç Mega 2000 model firearm at a designated shooting range with obtained permissions. The tarpaulin spread over the area where the cartridge cases fell was changed after each shot. A researcher wearing double gloves collected the cartridge cases, changing gloves after picking up each one. The cartridge cases, placed in paper evidence bags, were then transported to the laboratory. The collected cartridge cases were subjected to DNA analysis on the same day. Each cartridge case was stored under sterile conditions in the laboratory until analysis.

Figure 1. Cartridges cases contaminated with epithelial cells.

Figure 2. Cartridge cases contaminated with blood.

2.1. Preparation of Swab Wetting Chemicals

To investigate the effect of swab-wetting chemicals on DNA yield from cartridge cases, 2% SDS (Multicell, Canada), Te+4 buffer (Multicell, Canada), and distilled water (Merck, Germany) were used. The swabs included COPAN brand (Italy), FLOQ swabs, and BeyanLab brand (Türkiye) cotton swabs. For distilled water, 1 liter was placed in a sterile jar and autoclaved at 121°C for 20 minutes. To prepare 2% SDS, 2 grams of SDS powder was measured and distilled water was added to reach a total volume of 100 mL. A magnetic stirrer (Velp Scientifica-Arex) was used to dissolve the SDS. The Te+4 buffer (10mM Tris – 1mM EDTA) was used as received in solution form and required no further preparation. From each swab-wetting chemical, 400 µL was drawn using a micropipette and applied to the swab head to ensure the entire surface was moistened.

2.2. Control Sample

A control sample was obtained by contaminating an identical cartridge case with blood, similar to those used in the experiment. This cartridge case was not fired, and the day after the sample was prepared, a cotton swab was used to collect the sample for DNA analysis. Blood, which is rich in DNA, was chosen as a positive control to verify the accuracy of the analyses that the kit worked correctly and was able to produce the expected results.

2.3. DNA Isolation

DNA analyses were conducted on 12 cartridge cases (6 contaminated with blood and 6 with epithelial cells) that had been fired, and on 6 cartridges (3 contaminated with blood and 3 with epithelial cells) that had not been fired (Table 1).

Table 1. Types of swabs used for sampling from cartridge cases and cartridges

Types of Swabs Used for Sampling from Cartridge Cases		
Biological Material Type	Swab Wetting Chemical (400 µL)	Swab Type
Blood	SDS	Cotton Swab
		Microfiber Swab
	Te^{+4} Buffer	Cotton Swab
		Microfiber Swab
	Distilled Water	Cotton Swab
		Microfiber Swab
Epithelial Cells	SDS	Cotton Swab
		Microfiber Swab
	Te^{+4} Buffer	Cotton Swab
		Microfiber Swab
	Distilled Water	Cotton Swab
		Microfiber Swab
Types of Swabs Used for Sampling from Cartridges		
Biological Material Type	Swab Wetting Chemical (400 µL)	Swab Type
Blood	SDS	Cotton swab
	Te^{+4} Buffer	
	Distilled Water	
Epithelial Cells	SDS	Microfiber swab
	Te^{+4} Buffer	
	Distilled Water	

Due to the low number of cells transferred to the cartridges through touch, the organic isolation method (phenol-chloroform), preferred for isolating trace amounts of samples as high amounts of DNA were obtained, was selected (Eychner et al., 2016; Semizoğlu, 2013). While isolating DNA using the organic isolation method (Semizoğlu, 2013), modifications were made by adding 10 µL of proteinase-K to the samples moistened with SDS, Te+4 Buffer, and distilled water, and 3 µL for the isolation negative control. The isolation negative control is a mixture where all steps required for organic isolation are followed, but no DNA-containing cells are added to the tube. After evaporating the alcohol from the microcentrifuge tubes, 30 µL of Te+4 buffer was added, and the tubes were spun briefly (approximately 5 seconds) to ensure that the Te+4 buffer containing DNA was not left on the sidewalls or the inner lid. The resulting isolates were stored at -80 °C until the STR amplification stage.

2.4. Amplification of STRs

The amplification of STR regions was performed using the GlobalFiler™ PCR Amplification Kit with 24 loci (Applied Biosystems, Waltham, USA). For each sample, 7.5 µL of the reaction mix (Applied Biosystems, Waltham, USA), 2.5 µL of the primer mix (Applied Biosystems, Waltham, USA), and 15 µL of DNA isolate were used. A total of 25 µL of the mixture was prepared, and the samples were placed into a GeneAmp® 9700 PCR system (Applied Biosystems, Waltham, USA). Samples were kept in the initial incubation phase at 95 °C for one minute. This was followed by ten seconds of denaturation at 94 °C followed by 30 cycles of elongation at 59 °C for ninety seconds. Finally, after a final extension at 60 °C for ten minutes, PCR was completed. The STR amplification parameters were set according to the manufacturer's recommendations. Since electrophoresis was performed on the same day, the samples were stored at +4°C after amplification.

2.5. Electrophoresis Analysis

For each sample analyzed on the ABI 3500 Genetic Analyzer (Applied Biosystems, Waltham, USA), a mixture was prepared consisting of 9.6 µL Hi-Di™ formamide (Applied Biosystems, Waltham, USA), 0.4 µL GeneScan™ 600 LIZ™ Size Standard v2.0 (Applied Biosystems, Waltham, USA), and 1 µL of PCR product (Applied Biosystems, Waltham, USA). A total of 10 µL of the formamide/size standard mixture was placed into the ABI PRISM 3500 instrument (Applied Biosystems, Waltham, USA), and 1 µL of the PCR product was added on top.

The samples were run using 36 cm capillaries with POP-4 polymer, with an injection time of 15 seconds at 1.2 kV and a run time of 1550 seconds at 13 kV. The DS-36 Matrix standard and GS POP-4 (1ml) – C Filter were selected for the analysis. After the PCR products were processed under the appropriate conditions on the ABI PRISM 3500 Genetic Analyzer (Applied Biosystems, Waltham, USA), the raw data obtained were analyzed using GeneMapper IDx version 1 software (Applied Biosystems, Waltham, USA) for genotype determination. The RFU threshold was set at 200 (SWGDAM, 2021). The concept of a complete profile was defined based on the number of alleles possessed by the participant.

3. Results

The success of the swab types and wetting chemicals was evaluated by averaging the RFU values observed in the DNA profiles. The success percentages of showing full profiles for cartridge cases and cartridges were calculated based on the specific swab type and biological materials. No contamination was detected in the isolation negative control samples. The average RFU values of the profiles obtained from the positive control samples ranged between 5,000 and 10,000. A total of eight swabs showed complete profiles (Table 2).

Table 2. Samples showing complete profiles

Biological Material	Wetting Chemical	Cartridge Case*(n=12)		Cartridge* (n=6)	
		Swab Type			
		Cotton Swab	Microfiber Swab	Cotton Swab	Microfiber Swab
Blood	SDS	✓	✓	✓	
	Te+4 Buffer	X	✓	✓	
	Distilled Water	✓	✓	✓	
Epithelial Cell	SDS	X	—		—
	Te+4 Buffer	X	X		X
	Distilled Water	X	—		X

*: The ✓ symbol indicates a complete profile, while the — symbol represents that the sample showed a partial profile, X symbol represents no results were obtained from the DNA analyses.

After firing the cartridges contaminated with epithelial cells and blood, samples were collected using cotton swabs. For cartridge cases contaminated with epithelial cells, no complete profile was obtained regardless of the swab wetting chemical used. However, complete profiles were obtained from cartridge cases contaminated with blood using cotton swabs moistened with SDS and distilled water. Comparing the profiles, the average RFU value obtained with SDS was higher than that obtained with distilled water (Table 3).

Microfiber swabs were used to collect samples from cartridges contaminated with epithelial cells and blood and then fired. For cartridge cases contaminated with epithelial cells, no alleles were observed when microfiber swabs were moistened with Te+4 buffer. Partial DNA profiles were obtained from swabs moistened with distilled water and SDS, but these were unsuitable for comparison due to significant degradation of key alleles. In terms of RFU values, SDS (16001-17000) was found to be more successful than distilled water (13001-14000) (Table 3). Complete DNA profiles were obtained from all swab wetting chemicals used on cartridge cases contaminated with blood (Table 2). However, when examining RFU values, the highest amount of DNA was observed with SDS treatment (Table 3). In the DNA profiles obtained from microfiber swabs moistened with distilled water, the morphological appearance of alleles at specific loci deviated from the expected standards, disrupting the heterozygote peak balance. Therefore, after SDS, the next most successful chemical was Te+4 buffer at the stage of sampling blood cartridge case with microfiber swabs.

In the swab samples collected from cartridges contaminated with blood using cotton swabs, all wetting chemicals produced complete profiles, and no degradation was observed (Table 2). Based on RFU values, the least successful swab was the one moistened with distilled water (6001-7000). The RFU value of the swab moistened with Te+4 buffer (83001-84000) was

approximately twice that of the one moistened with SDS (17001-18000) (Table 3).

In the DNA profile of the swab moistened with SDS, N-binding (split peaks) was observed in each gene region (Figure 3).

Table 3. Average RFU Ranges of Profiles

Biological Material	Wetting Chemical	Cartridge Case (n=12)		Cartridge (n=6)	
		Cotton Swab	Microfiber Swab	Cotton Swab	Microfiber Swab
Blood	SDS	16001-17000	29001-30000	17001-18000	
	Te+4 Buffer	0	4001-5000	83001-84000	
	Distilled Water	13001-14000	11001-12000	6001-7000	
Epithelial Cell	SDS	0	1201-1300		1101-1200
	Te+4 Buffer	0	0		0
	Distilled Water	0	0-200		0

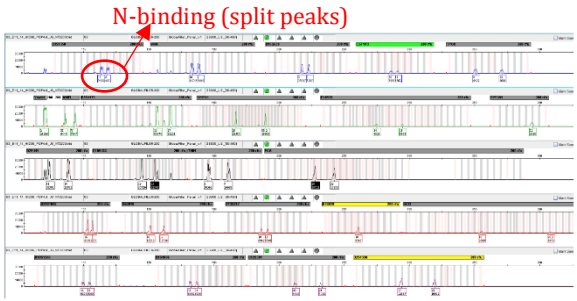

Figure 3. DNA profile image of blood collected from a cartridge using a cotton swab moistened with SDS.

Microfiber swabs were used to collect samples from cartridges contaminated with epithelial cells. A usable profile for comparison was obtained only from the swab moistened with SDS (Figure 4). However, since the profile was partial, it could not be matched to an unidentified individual.

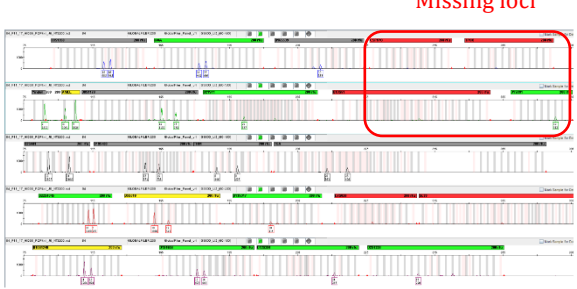

Figure 4. Partial and Degraded DNA Profile Image of Epithelial Cells Collected from a Cartridge Using a Microfiber Swab Moistened with SDS

In the phase where blood samples were collected using cotton swabs, swabs taken from cartridges showed a higher percentage of complete profiles (100%) compared to cartridge cases (67%) (Table 4). The RFU values of profiles obtained after firing were lower compared to those that were not fired (Table 3). The likelihood of obtaining a complete profile from cartridge cases contaminated with blood was higher than those contaminated with epithelial cells.

No allele loss was observed in swabs with a full profile, whereas no alleles were observed in swabs with a RFU value of zero, resulting in a loss of 45 alleles (Table 5). The 18 profiles obtained from the study is shown in supplement 1 and the detailed representation of missing alleles on the profile is shown in supplement 2.

Table 4. Percentage full profile rates by biological material and swab type

Biological Material	Wetting Chemical	Cartridge Case* (n=12)		Cartridge* (n=6)	
		Swab Type			
		Cotton Swab	Microfiber Swab	Cotton Swab	Microfiber Swab
Blood	SDS	67%	100%	100%	
	Te+4 Buffer				
	Distilled Water				
Epithelial Cell	SDS	0%	0%		0%
	Te+4 Buffer				
	Distilled Water				

Table 5. Number of missing allel by profile

Biological Material	Wetting Chemical	Cartridge Case (n=12)		Cartridge (n=6)	
		Swab Type			
		Cotton Swab	Microfiber Swab	Cotton Swab	Microfiber Swab
Blood	SDS	0	0	0	
	Te+4 Buffer	45	0	0	
	Distilled Water	0	0	0	
Epithelial Cell	SDS	45	15		9
	Te+4 Buffer	45	45		45
	Distilled Water	45	38		45

4. Discussion

Distilled water is commonly preferred as a swab wetting chemical. However, in cases where the number of cells obtained from trace or degraded samples is limited, water may be insufficient, necessitating the use of alternative chemicals. In this context, chemicals such as Te+4 buffer and SDS can support more efficient cell collection and analysis. Furthermore, it has been demonstrated in various studies that the material and design of swabs significantly affect DNA collection efficiency (Brownlow et al., 2012; Dadhania et al., 2013). Therefore, it is thought that alternative wetting chemicals and swab designs could revolutionize genetic analyses.

SDS was the swab-wetting chemical that yielded the highest DNA quantity in all parameters except for

collecting blood samples from cartridges using cotton swabs. A recent study involving touch DNA samples left on glass surfaces used different wetting chemicals. SDS was found to be the only chemical with significantly higher recovery than water. No significant difference was observed between 2% SDS volumes of 15, 30, 45, and 90 µL for wetting, although foaming occurred at 90 µL (Schulte et al., 2023). Since the risk of DNA precipitation is higher at high SDS concentrations, a 2% SDS solution was used in the study for DNA safety, as recommended by Thomasma and Foran (2013). No precipitation was observed during extraction.

Different cell types were placed on non-porous surfaces and the effectiveness of swab wetting chemicals was tested. While EDTA was found to be the most suitable chemical for blood, water and detergent-based chemicals were found to be more suitable for trace DNA deposits. Detergent-based chemicals were less successful in cellular DNA compared to water. However, no meaningful comparison can be made since no peak was obtained in the collection of cellular samples with cotton swabs in the study (Kuffel et al., 2024). In a 2013 study, traces formed on glass surfaces by touch were sampled first with cotton swabs moistened with 120 µL of SDS or water, followed by a dry swab. SDS produced a higher average DNA yield compared to water. Contrary to Thomasma and Foran's findings, none of the swab samples collected from surfaces by touch using cotton swabs yielded profiles in this study. Since the minifiler kit has STR primers that produce shorter amplicons than the kit used in the study, they were able to obtain a profile by touch (Prinz et al., 2007; Thomasma and Foran, 2013). In a study where the double-swab technique was preferred to collect touch DNA from magazines, 30 µL of 2% SDS was used for cotton swabs, and 60 µL for microfiber swabs (Tasker et al., 2020). The high heat from firing and the absence of a second swab for sampling may explain the insufficient DNA for profiling in contrast to Tasker's study.

In contrast to these studies, swabs moistened with both double-swab techniques using water and SDS were used to sample from various porous and non-porous surfaces. The double swab moistened with water was more successful than SDS for all surfaces (Gilmore and Glynn, 2019). In another study where saliva was deposited on non-porous surfaces, no superiority of water over SDS was observed. Unlike touch DNA, saliva is watery, so the interaction between SDS and saliva is weaker (Grosey, 2011). Additionally, the difference in materials compared to cartridge cases and no exposure high temperatures might explain the variance in results.

Complete profiles were not observed in swabs collected from cartridge cases contaminated with epithelial cells. Microfiber swabs were more successful than cotton swabs as they produced partial DNA profiles. Similarly in two different studies aimed at increasing DNA yield from cells deposited by touch, microfiber swabs were found to be more effective (Templeton et al., 2013; Jansson et al., 2020). The success of obtaining complete profiles in these studies may be due to the use of direct PCR (Templeton et al., 2013) and differences in the isolation method (Jansson et al., 2020).

Te+4 buffer is preferred in many laboratories due to its availability and low cost (Aloraer et al., 2017). Cotton and microfiber swabs moistened with 30 µL of Te+4 buffer were used to collect cells containing touch DNA from various vehicle surfaces. Cotton swabs were more successful than microfiber swabs on all surfaces (Giovanelli et al., 2022). However, contrary to Giovanelli's findings, none of the complete profiles obtained from touch DNA in this study were from swabs moistened with Te+4 buffer. The factors that can negatively impact DNA on vehicle surfaces are fewer compared to those affecting DNA on fired cartridge casings.

In the study, water-moistened swabs were used to collect epithelial cells from cartridge cases and cartridges. However, none of the swabs produced complete DNA profiles, and the collected DNA was degraded. The water showed less degradation for epithelial cells than the Te+4 buffer. In another study conducted in 2024 with buccal cells, Te+4 buffer was more successful than water (Czado et al., 2024). DNA deposited by touch on glass, aluminum, and plastic surfaces was collected using cotton swabs moistened with either buffer (1% n-lauroylsarcosine, 10 mM Tris-HCl, 0.1 mM EDTA, 50 mM NaCl) or water. Afterwards, dry cotton swabs were also used. The buffer yielded a higher DNA concentration in all cases compared to water. When water was used, the quality of the DNA profile degraded over time and with increased temperature, whereas the detergent-based buffer stabilized the DNA for up to 24 hours and at temperatures up to 50°C (Bille et al., 2020). Elwick and colleagues explain this by the oxidation reaction caused when water contacts brass surfaces. The study investigated the DNA recovery of human epidermal keratinocyte cells from nickel and brass cartridges. Samples, including cartridge case, were sampled by rinsing and swabbing or soaking with different chemicals (Elwick et al., 2022). Holland proposes an alternative approach, suggesting that EDTA's chelation plays a role. Brass is an alloy of 70% copper and 30% zinc. Holland proposes that EDTA's chelation may reduce the impact of copper ions on DNA extraction efficiency, thus improving results from brass surfaces (Holland et al., 2019).

At the beginning of the study, it was concluded that cotton swab failed to produce a complete profile compared to microfiber swab in response to the targeted objective. If maximum yield is desired from the cartridge case regardless of the biological sample a microfiber swab moistened with SDS, which shows the highest RFU values, should be preferred.

5. Conclusion

Samples containing trace amounts of cells, such as touch DNA, must undergo maximal extraction. Achieving maximal extraction requires the use of various swab-wetting chemicals. The most commonly used chemical is often water or water-based physiological serum. SDS was chosen due to its ability to interact with the lipid components of cell membranes, and Te+4 buffer was selected to minimize oxidative damage to the DNA template (Farrell, 2010; Holland et al., 2019). The main drawback of using swab-wetting chemicals is that while water does not require prior preparation, chemicals like SDS and Te+4 buffer require preparation in advance, which can be time-consuming (Schulte et al., 2023). However, all three chemicals are commonly used in organic isolation methods and are readily available in laboratories (Semizoğlu, 2013).

SDS was the most successful chemical, irrespective of the swab type or biological material used in terms of average RFU values. In contrast, Te+4 buffer yielded the fewest complete profiles among the tested chemicals. The RFU values of profiles obtained after firing were lower than those of unfired samples. The likelihood of obtaining a complete profile from cartridge cases contaminated with blood was higher than those contaminated with epithelial cells. When situations such as firing can lead to DNA degradation, using SDS as the swab-wetting chemical and microfiber swabs can help obtain higher quality profiles, thus improving forensic analysis outcomes.

Future studies could benefit from expanding the research to include double swab techniques applied to cartridge cases made from various materials, which may yield further insights into DNA recovery efficiency.

Author Contributions

The percentages of the authors' contributions are presented below. The authors reviewed and approved the final version of the manuscript.

	F.E.Y.	E.N.A.	Y.G.
C	30	30	40
D	30	30	40
S	35	30	35
DCP	30	40	30
DAI	45	30	25
L	30	40	30
W	30	40	30
CR	30	30	40
SR	25	25	50
PM	30	30	40
FA	35	30	35

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

The study conducted at Kütahya Health Sciences University Application and Research Center was approved by the Non-Interventional Clinical Research Ethics Committee of Kütahya Health Sciences University (approval date: 12 August, 2024, protocol code of 2024/10/07). Also the study was carried out in accordance with the Helsinki criteria.

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