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Assessing Methods for Enhanced Recovery of Touch DNA from Fingerprints: A Pilot Study

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ABSTRACT

The rapid advancement of science and technology, particularly in forensic science, has significantly enhanced crime investigation methodologies. One such advancement is the utilization of Scientific Crime Investigation methods, specifically the analysis of touch DNA from fingerprints. This research investigates the efficiency of fingerprint powders and swabbing agents in improving the quality and quantity of touch DNA for forensic applications. Touch DNA, derived from cellular materials like sweat and skin cells, presents a valuable source of genetic material for identification purposes. The study involved experimental analyses using Regular Silk Black Fingerprint Powder and Magnetic Dual-Purpose Powder, coupled with non-ionic detergent surfactants as swabbing agents. DNA samples were collected from volunteers with varying DNA shedding levels, processed, and analyzed using quantitative PCR and capillary electrophoresis. Results indicated that fingerprint powders significantly reduce the quantity and quality of recovered DNA due to DNA damage caused by the powders' chemical composition. Conversely, using non-ionic surfactants like Triton™ X-100 in swabbing improved DNA recovery and stability, leading to more complete DNA profiles. This study underscores the importance of optimizing fingerprint powder formulations and DNA sampling techniques to enhance forensic DNA analysis. The findings advocate for the development of less damaging fingerprint powders and improved DNA extraction protocols to preserve the integrity of touch DNA evidence in forensic investigations.

1. Introduction

Genetic materials left on items handled by individuals are called touch DNA. Touch DNA comes from bodily materials such as sweat and skin cells, and it is hypothesized that most touch DNA is composed of cell-free DNA (Quinones and Daniel 2012; Yudianto and Margaret 2017; Yudianto *et al.* 2017, 2020). Touch DNA can be very beneficial in the absence of DNA-containing biomaterials, such as blood, saliva, semen, vaginal fluid, and sweat. The use of touch DNA first

comes from a landmark article showing the possibility of extracting DNA from fingerprints (Daly *et al.* 2012). Since its discovery and subsequent development, touch DNA has been used for forensic purposes by law enforcement forces worldwide for over a decade (Barash *et al.* 2010). Moreover, touch DNA may be collected during fingerprint collection (Jansson *et al.* 2022). By integrating the data from fingerprints and touch DNA, the identification process has become more specific.

While it is undeniable that the existence of fingerprints is invaluable, this does not mean that the use of fingerprints for forensic purposes is without challenges. External factors are at play in causing this problem: differing environmental conditions and the surface onto

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which the fingerprints are deposited can complicate the detection of fingerprints. This is because properties and adsorption characteristics are highly variable between surface materials (Daly *et al.* 2012; Bonsu *et al.* 2020; Schulze Johann *et al.* 2022). To aid with fingerprint detection, fingerprint powder was developed and commercially available in the 19th century, with the first powders being made from chalk and mercury. Since then, the formulations of fingerprint powders have changed to address these concerns (Templeton *et al.* 2013; Cavanaugh and Bathrick 2018). Fingerprint powders come in many formulations that can be grouped into six types: granular, magnetic, fluorescent, metallic, nano, and infrared powders. The research on the effect of fingerprint powders on the quantity and quality of recovered touch DNA has been inconclusive, with most studies agreeing that the effect is insignificant (Linacre *et al.* 2010; Gino and Omedei 2011; Al Oleiwi *et al.* 2017).

Several methods are used to sample touch DNA, with the tape lifting and swabbing method being the most common as they are the least destructive to the exhibit, cheap, and the easiest to transport. Several forensic genetics studies demonstrate that the double swabbing technique yields a higher amount of DNA than the single-swabbing technique (Sweet *et al.* 1997; Hedman *et al.* 2020). The superiority of double-swabbing makes it the standard technique in DNA sampling, used by many police forces and forensic laboratories worldwide (Sessa *et al.* 2019). Despite the superiority of double-swabbing, sterile cotton swabs can be expensive in developing countries, and double-swabbing doubles the number of cotton swabs needed to sample DNA from crime scenes. This encourages an improvement in another factor in DNA sampling: the swabbing agent. Several studies have performed DNA sampling using simple detergents as swabbing solutions.

2. Materials and Methods

2.1. Research Design

The research design used in this study is a quantitative experimental design with a within-subjects approach. This research was divided into 2 (two) methods of data collection, experimental of DNA recovery from fingerprints, and experimental touch DNA sampling technique analysis. The data from this research was a pilot study that had not been published before.

2.2. Conformity Criteria

Inclusion Criteria (participants must meet these conditions): Healthy adults capable of giving informed

consent. Living in a household setting was selected through probability sampling. Willing and able to follow pre-experiment instructions (e.g., handwashing, avoiding food contact, not wearing gloves, etc.). Available to provide fingerprint samples (on object glass), buccal swabs, smartphone touch DNA samples, and no recent use of hand sanitizers or lotions prior to sample deposition (implied by controlled handwashing procedure).

Non-Inclusion Criteria (participants who would not be considered at all): Individuals outside the household sampling frame (i.e., not part of the selected population unit). Children or minors, as the study likely targeted adults due to ethical constraints. Individuals with known skin conditions or excessive skin shedding could influence DNA deposition.

Exclusion Criteria (participants who were considered but had to be excluded during or after recruitment):

Volunteers who did not comply with the pre-deposition instructions (e.g., touched food or other participants). Those who contaminated the sample during or after fingerprint deposition (e.g., touched the surface post-deposition). Individuals who experienced insufficient DNA yield in buccal swabs (possibly indicating underlying health or sampling issues). Any indication of DNA contamination in controls during PCR or electrophoresis processing. Participants who failed to complete all phases of sample collection (fingerprint, buccal, and phone swabs).

2.3. Research Facilities

All of the research processes were obtained from the Forensic Laboratory Center, Criminal Investigation Agency, Indonesian National Police, Indonesia, in 2024.

2.4. Research Duration

This research was conducted for 2 years from 2022 to 2024.

2.5. Medical Procedure Description

The sample for touch DNA fingerprint analysis came from five individuals. A probability sampling with a household analysis unit was conducted. An object glass was used as a fingerprinting surface in this experiment. Before fingerprint deposition, the object glass underwent sterilization using DNAZap™ PCR DNA Degradation Solutions (Invitrogen, USA).

After the fingerprinting surface was prepared, all volunteers deposited their fingerprints onto the surface for 5 seconds. Before deposition, all volunteers washed their hands, dried them with sterile tissue, and were

exposed to various activities for 15 minutes. Volunteers were prohibited from touching each other, touching food, and wearing gloves. Each volunteer deposited three fingerprints, resulting in a total of fifteen fingerprints.

After the fingerprints were deposited, one fingerprint from each volunteer was left untreated, one fingerprint from each volunteer was powdered using Regular Silk Black Fingerprint Powder (Sirchie, USA), and one fingerprint from each volunteer was powdered with Magnetic Dual-Purpose Powder (BVDA, the Netherlands). Then, the fingerprints powdered with Regular Silk Black Fingerprint Powder were brushed with a brush that was sterilized with DNAZap™ PCR DNA Degradation Solutions (Invitrogen, USA), followed by a 30-minute UV light exposure in a laminar air flow chamber. Afterwards, all fingerprints were swabbed with 4N6FLOQSwabs® for Crime Scene (Copan, Italy).

Buccal swabs were used as comparators in this experiment. Three buccal swabs were taken from each volunteer using a dry single-swabbing method with 4N6FLOQSwabs® for Crime Scene (Copan, Italy). Then, the buccal swabs were spread on a sterilized object glass. One swab was untreated, one swab was treated with Regular Silk Black Fingerprint Powder (Sirchie, USA), and one swab was treated with Magnetic Dual-Purpose Powder (BVDA, the Netherlands). The resulting treatment can be seen in Figure 1. Afterwards, all samples were swabbed with 4N6FLOQSwabs® for Crime Scene (Copan, Italy). A wet-and-dry double-swabbing technique was again used with nuclease-free water as the swabbing agent.

The volunteers' fingerprint and buccal swab samples were processed with PrepFiler™ BTA Forensic DNA Extraction Kit (Applied Biosystems, USA). Afterwards, 29-cycle quantitative PCR was done on the samples with a 7500 PCR System thermal cycler (Applied Biosystems, USA) and Quantifiler™ Trio DNA Quantification Kit (Applied Biosystems, USA). Subsequently, amplification was done for 29 cycles using a ProFlex™ PCR System thermal cycler (Applied Biosystems, USA) and GlobalFiler™ PCR Amplification Kit (Applied Biosystems, USA) with a reaction volume of 25 μ L.

After quantification and amplification, all DNA samples were processed under capillary electrophoresis using a 3500 Series Genetic Analyzer (Applied Biosystems, USA). DNA profiles were generated using GeneMapper™ v4.1 (Applied Biosystems, USA).

Five people, identical to those before, were chosen as volunteers, and their smartphones were used as sampling exhibits due to routine contact with the skin, assuring

significant deposition of touch DNA. Two swabbing agents were used: Triton™ X-100 (Promega Corporation) and nuclease-free water (Promega Corporation). Nylon swabs (4N6FLOQSwabs® for Crime Scene) were used for the experiment.

A wet single-swabbing technique was used. The wet single-swabbing technique involves dipping the swab tip into one of the swabbing agents and then applying pressure to the volunteer's smartphone. The swab tips were cut approximately 1 millimeter and put into PCR tubes. A buccal swab from each volunteer served as a positive control for the experiment. Extracted DNA samples were then quantified and typed for autosomal 21 STR loci using methods described earlier.

2.6. Ethical Review

Ethical approval was obtained from the ethics committee of the Faculty of Dentistry, Universitas Airlangga (Number:191/HRECC.FODM/II/2023).

2.7. Statistical Analysis

To observe if the use of fingerprint powders alters the quantity and quality of recovered DNA, statistical analysis was done using SPSS® Statistics 26 (IBM Corporation, USA). Subsequently, Friedman's two-way analysis of variance by ranks was performed.

Then, two independent samples tests were conducted: Levene's test for equality of variances and the t-test for equality of means. Meanwhile, one-way ANOVA was performed to see if shedder status impacts DNA profile completeness.

3. Results

3.1. DNA Recovery from Lifted Fingerprints

The quantity of recovered DNA was assessed by measuring its concentration in each sample. We found that our data on DNA concentration is not normally distributed. Then, we found that the use of fingerprint powder(s) significantly reduces the concentration and, thus, the quantity of DNA recovered from our samples ($P<0.005$). Our results confirm several similar studies that have been published 25, 27, 28. The average DNA concentration from each treatment and sample type is listed in Table 1.

The measure of recovered DNA quality in our study was the number of detected alleles, degradation index (DI), and internal positive control of the cycle threshold (IPC-CT). We found that our data on the number of detected alleles is not normally distributed.

Table 1. Analysis of comparison result between fingerprints and buccal swabs on touch DNA fingerprints methods

Parameters (average)	Fingerprints			Buccal swabs		
	Untreated	Granular	Magnetic	Untreated	Granular	Magnetic
DNA Concentration	0.01688	0.00503	0.00666	0.53270	0.74718	0.62506
No. of detected alleles	30.6	24.2	23.4	42.0	42.0	42.0
DI	1.173	1.943	0.956	0.915	0.948	0.868
IPC-CT	27.55556	27.70594	27.71492	28.03486	28.25148	28.04158

Meanwhile, our data on the DI is normally distributed except for samples treated with granular powder. Then, we found that our data on IPC-CT is normally distributed ($P>0.05$). We found that the use of fingerprint powder(s) significantly reduces the number of detected alleles and IPC-CT from our samples ($P = 0.001$), but such a reduction was absent in the DI ($P>0.05$). The average of detected alleles, DI, and IPC-CT from each treatment and sample type is listed in Table 1.

3.2. Touch DNA

Despite using the Applied Biosystems Global Filer PCR amplification kit, two loci were not included for analysis because the volunteers were of mixed sex: Y-indel and DYS391. The exclusion of Y-indel and DYS391 leaves twenty-two loci that can be analysed.

The least detectable alleles come from swabs that are then run through PCR for twenty-nine cycles. The most detectable alleles come from swabs that were swabbed with Triton™ X-100 and then run through PCR for thirty-four cycles. Even though there were expectations that exhibits that were run through PCR for thirty-four cycles would generate a more complete profile, exhibits that were swabbed with nuclease-free water and run through PCR for thirty-four cycles generated a less complete profile than exhibits that were swabbed with Triton™ X-100 and then run through PCR for twenty-nine cycles.

The use of Triton™ X-100 significantly improved the completeness of DNA profiles taken from the volunteers compared to nuclease-free water ($P<0.005$). Running the PCR for 34 instead of 29 cycles improved the completeness of DNA profiles taken from the volunteers ($P<0.005$). Meanwhile, shedder status did not significantly affect DNA profile completeness ($P<0.005$). The detectable alleles can be shown in Table 2.

The percentage of allelic dropout from the experiment is shown in Figure 1. Allelic dropout indicated a failure to detect one of the two alleles at a specific locus, which can lead to misleading conclusions about genetic variation within a population.

4. Discussion

This study aimed to provide a broader understanding of touch DNA analysis as a DNA fingerprinting method, assessing the extensive application of DNA technology to assist authorities in solving forensic cases by applying scientific principles to criminal investigations in Indonesia (Ruspita *et al.* 2022). The use of touch DNA in criminal cases involves a shift from traditional fingerprint methods towards more sophisticated sampling techniques. Touch DNA analysis provides a higher level of detail by examining DNA traces left on objects or surfaces that the perpetrator has touched. In this research, methods and techniques were explored to obtain touch DNA results by maintaining the concentration of touch DNA so that it could be analyzed genetically using PCR (Quinones and Daniel 2012; Vandewoestyne *et al.* 2012; Ehrhardt *et al.* 2015). Touch DNA is highly fragile and prone to degradation, which significantly impacts the amount of DNA from an exhibit. Proper sampling techniques are crucial in mitigating these challenges (Alcaide *et al.* 2020; Mungreiphy *et al.* 2011). Kapoor and Sodhi (2022) found that the multipurpose fluorescent powder composition can detect latent fingerprints (Kapoor *et al.* 2022; Lavrukova and Antipov 2025). This finding underscores the importance of sampling methods: the extraction process, while necessary for some types of DNA analysis, may introduce additional steps that can lead to further degradation or loss of the already fragile DNA. Direct PCR, although advantageous in preserving the DNA present on the exhibit, comes with its limitations. Specifically, it consumes the exhibit in the process, making re-examination nearly impossible if further analysis is required. This highlights the need for careful and efficient sampling techniques to maximize DNA recovery and ensure the integrity of the evidence throughout the analytical process. Proper handling and sampling are therefore critical to optimizing the outcomes of touch DNA analysis, given its susceptibility to degradation and the limitations of

Table 2. Number of detectable alleles from the experiment, the abbreviation A stands for amylogenic, and NFW for nuclease-free water

Volunteer	Sample	Number of detectable alleles				Positive control	Shedder level		
		29 cycles		34 cycles					
		NFW	Triton™ X-100	NFW	Triton™ X-100				
A	1	5	27	31 + A	38 + A	42 + A	Low		
	2	5	25 + A	33 + A	42 + A	42 + A			
	3	5	27 + A	31 + A	38 + A	42 + A			
B	1	23 + A	34 + A	22 + A	41 + A	42 + A	Intermediate		
	2	20 + A	34 + A	24 + A	42 + A	42 + A			
	3	33	34 + A	22 + A	40 + A	42 + A			
C	1	4	14 + A	13	33 + A	42 + A	Low		
	2	4	23 + A	16	38 + A	42 + A			
	3	4	20	15	32 + A	42 + A			
D	1	8 + A	27 + A	27 + A	40 + A	42 + A	High		
	2	8 + A	27 + A	27 + A	40 + A	42 + A			
	3	8 + A	27 + A	27 + A	42 + A	42 + A			
E	1	20 + A	42 + A	28 + A	42 + A	42 + A	High		
	2	22 + A	42 + A	26 + A	42 + A	42 + A			
	3	22 + A	42 + A	38 + A	42 + A	42 + A			

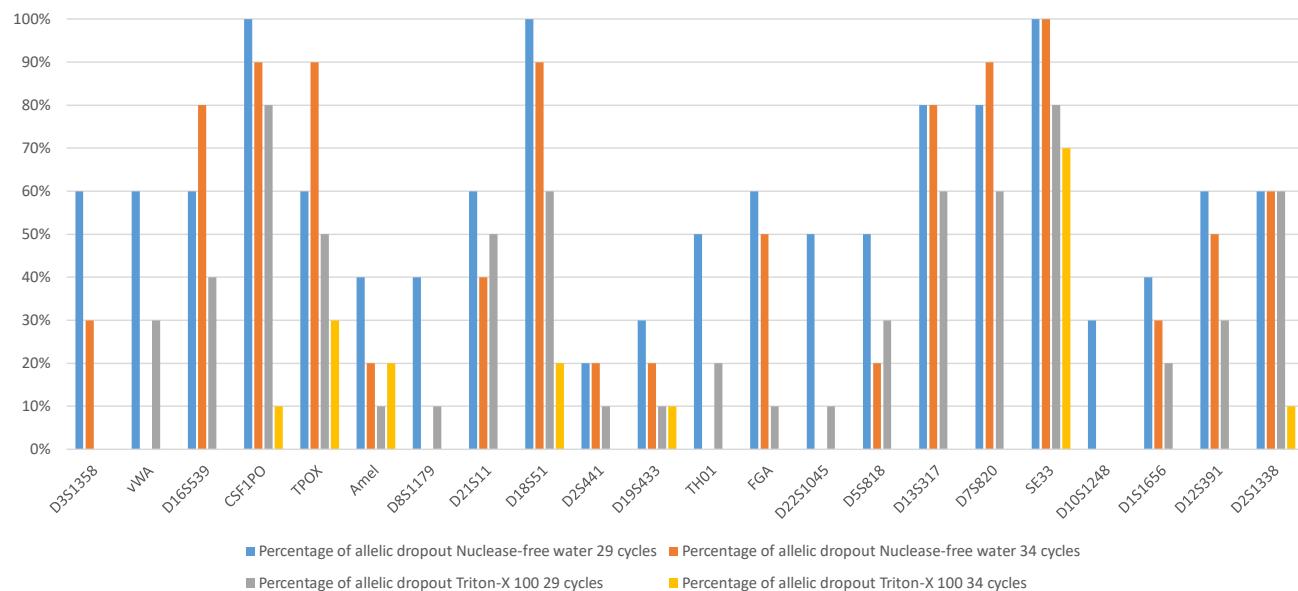


Figure 1. Percentage of allelic dropout from the experiment. Y-indel and DYS391 excluded

direct PCR methods (Cavanaugh and Bathrick 2018; Mittal and Tayal 2019; Alcaide *et al.* 2020).

Based on this research, the ingredients of fingerprint powder significantly influence the quantity and quality of recovered DNA. The granular powder we used is composed of carbon black and Lycopodium spores, while the magnetic powder we used is composed of iron powder and ferric oxide (Fe_2O_3). Several studies have confirmed that exposure to carbon black and iron oxides can cause DNA damage. Several other studies have also confirmed that the presence of metals,

especially transition metals, can cause DNA folding and damage, resulting in poorer PCR performance (Alarifi *et al.* 2014; Kyjovska *et al.* 2015; You *et al.* 2015). The result also showed that the reduction of quantity and quality of DNA recovered from fingerprints is more apparent due to touch DNA being comprised mostly of cell-free DNA (Pligin *et al.* 2022). Since cell-free DNA does not have any protective membranes (such as cell membranes), metal cations can directly bind to DNA instead of proteins, causing DNA damage. This might also explain why the quantity and quality of DNA

recovered from buccal swabs are much more abundant and complete since metal cations bind onto the proteins of the epithelial cell membrane instead of directly onto the DNA (Hosahally *et al.* 2023).

While most studies on the interaction between metals and DNA were done *in vivo*, it is reasonable to infer that similar interactions also occur *in vitro* settings. The adherence of DNA to metal surfaces could impede its retrieval by conventional means, resulting in diminished yields and inaccurate assessments. This proves that the causes of low PCR performance do not only occur during PCR but can also occur as early as DNA deposition on surfaces.

This study is not the first to seek the modification of swabbing agents to enhance DNA recovery; however, it confirms the ability of surfactants to increase DNA recovery from exhibits (Thomasma and Foran 2013; Schulze Johann *et al.* 2022). There are several ways a surfactant could help with DNA recovery from exhibits. It has been suggested that the use of amphiphilic or non-ionic surfactants (such as Triton™ X-100) helped with the rehydration of the exhibit and the dehydrated cells since amphiphilic or non-ionic surfactants improve solubility in both polar and nonpolar solvents (Norris *et al.* 2007; Quinones and Daniel 2012).

Some studies have pointed out that surfactants can cause cell lysis, which helps optimise the amount of recoverable DNA needed to generate a complete profile (Koley and Bard 2010; Nazari *et al.* 2012). Even though Triton X-100 is a non-ionic surfactant, it still could cause cell lysis when the critical micelle concentration (CMC) is reached (Koley and Bard 2010). The mechanism is thought to be micelle formation that induces curvature stress upon the cell membrane (Nazari *et al.* 2012).

Several studies have also shown that surfactants improve DNA stability, which is essential during PCR (Doolaanea *et al.* 2015; Silanteva *et al.* 2020; Mezei and Pons 2021). A study by Natarajan *et al.* revealed that the use of surfactants (in their case, sodium dodecyl sulfate, known as SDS) reduced DNA shearing (Natarajan *et al.* 2016). DNA shearing, or fragmentation, can hamper the profiling process of samples. This effect can become significant for longer amplicons since these longer amplicons get fragmented more, causing a “ski-slope effect” that can cause allelic dropouts (Schulze Johann *et al.* 2022). This effect is noticeable in loci with longer amplicons, such as SE33 and D2S1388, which still show allelic dropout even in samples swabbed with swabs dipped in Triton™ X-100 and then run under PCR for 34 cycles.

Meanwhile, surfactants might aid in DNA stabilisation through enzyme denaturation. A study by Bryan Hanley *et al.* found that using polar surfactants might denature restriction enzymes, thus reducing the amount of DNA fragmentation or shearing. While their study revealed that the use of non-ionic surfactants such as Triton™ X-100 did not hamper DNA fragmentation, our study found that the use of the surfactant improved the results of DNA profiling, which might mean that the use of surfactant helped reduce DNA fragmentation in our samples (Hanley *et al.* 1990).

As other studies have shown, this study found that the number of cycles of a PCR run could make or break the process of DNA profiling (Kloosterman and Kersbergen 2003; Harrel *et al.* 2019). A study by Harrel *et al.* found that increasing the number of PCR cycles improved the completeness of the DNA profile, regardless of the extraction method 42. Meanwhile, Kloosterman *et al.* have shown that increasing the number of PCR cycles is helpful when no more samples can be obtained (Kloosterman and Kersbergen 2003).

Yet, increasing the number of PCR cycles carries its risks. The most adverse of these risks is the stochastic effects. Stochastic effects generally bring small risks on large samples, but these effects become more potent on touch DNA samples as very minute samples have to be replicated, sometimes to very high powers (228 to 234) (Kloosterman and Kersbergen 2003). Stochastic effects can be mitigated by using direct PCR, but if there is too much template DNA to replicate from, other effects could arise: nonspecific amplification, poor adenylation, and n+4 products (Cavanaugh and Bathrick 2018). The overload of template DNA can be mitigated by diluting the amplification product before PCR. In our study, stochastic effects were not observed since allelic dropouts were reduced as we increased the number of PCR cycles. Hence, allelic dropouts can measure the incidence of stochastic effects during direct PCR.

Nevertheless, this study has several limitations. First, this experiment design only used one powder brand for each type of fingerprint powder. It was unable to confirm if the results shown are reproducible with another brand of the same powder type. Then, the results of this study have low statistical power. This experiment's low number of volunteers and replicates made the results prone to type I errors. This suggests that other researchers should devise an experimental design that can reduce the number of potential issues,

mainly by introducing more replicates and fingerprint powder brands with different formulations.

The findings of this study suggest that care must be taken in designing DNA extraction protocols from powdered fingerprints, as the use of fingerprint powders has been shown to reduce DNA quantity and quality. It is also recommended that producers create formulations that are less damaging to DNA to preserve the quantity and quality of recovered DNA.

In conclusion, based on the result of this study, the perceived need of understand the composition of fingerprint powders was found to be beneficial for investigators considering generating DNA profiles from powdered fingerprints. As our study has confirmed, along with other studies, using fingerprint powder to detect fingerprints can inadvertently cause a reduction in DNA quantity and quality. Hopefully, fingerprint powder producers and users can choose powder formulations that are best for detecting fingerprints and DNA profile generation.

It also demonstrated the improved recovery of touch DNA and the generation of more complete profiles, which may be attributed to the surfactant's ability to increase cell rehydration, induce cell membrane rupture through micelle formation, and cause the denaturation of restriction enzymes. In essence, the multifaceted action of Triton™ X-100 not only aids in preserving the integrity of touch DNA samples but also amplifies the quality and completeness of DNA profiles, thereby advancing forensic capabilities and contributing to more accurate and reliable investigative outcomes

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