Extraction and Profiling of Wearer DNA from Different Types of Glove Surfaces in Bank Robbery

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DOI: https://doi.org/10.51244/IJRSI.2025.120700015

Received: 21 June 2025; Accepted: 27 June 2025; Published: 31 July 2025

ABSTRACT

DNA fingerprinting and profiling are emerging as important and authentic tools for criminal investigations. The “Touch DNA” or “Trace DNA” is the manifestation of this at the miniature level. In burglary-related crimes, multiple tools have been used; therefore, profiling and exact matching with the accused have become problematic. In addition, the surface of the tool is a deciding factor for DNA accumulation. Moreover, it is difficult to obtain DNA from sweat cells because of the lower amount of DNA in sweat cells than in other body fluids. In this article, we used three pairs of gloves made up of different materials (latex and denim cloths). The amount of DNA obtained from gloves A and B (latex) were 1.877 ng/µl and 0.91 ng/µl, respectively. However, in the case of glove C, we demonstrated that the extraction of DNA from the denim cloth was difficult; therefore, we chose a single fiber thread for this purpose. For a single thread, the quantity obtained was 0.06 ng/µl even after 520 days of incidence. These results show that the type of surface plays a critical role in DNA extraction. The non-absorbing and smooth surface are preferred over absorbing and rough surface. However, while working with absorbing and rough surface like cloth evidence, use of a single thread for DNA extraction is desirable.

Keywords: Wearer DNA, Soft and rough surface, Bank robbery, Single-fiber DNA.

INTRODUCTION

Since the initial use of DNA (deoxyribonucleic acid) in criminal cases in 1986 (Jeffreys *et al.* 1985; Jeffreys *et al.* 1985*)* science and technology have opened up new avenues in criminal and civil proceedings. This approach not only helps investigation agencies determine the presence of offenders at a particular crime scene but also helps in paternity-related disputes. The field of forensic science that deals with DNA analysis is termed “DNA profiling” or “DNA fingerprinting”. Historically, various methods, including restriction fragment length polymorphism (RFLP), polymerase chain reaction (PCR) of amplified fragment length polymorphisms (AFLPs) and, most recently, PCR of short tandem repeats (STRs), have been developed for DNA analysis (McDonald and Lehman 2012: Jobling and Gill 2004). However, the STR method outperforms the other methods in terms of requiring smaller quantities as well as partially degraded samples of DNA and multiplexing.

Various materials of human, animal and plant origin were used for DNA analysis. Most of the time, blood, saliva, hair, bones, nails, various body tissues, teeth, etc., are expected to be used for DNA profiling. On the

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue VII July 2025

other hand, sweat and grease substances (SGS), which are constantly secreted by the human body, are less likely to be useful in DNA analysis (Faleeva *et al.* 2018; Ahmad and Soekry 2013). In recent years, with scientific advancements and increased sensitivity in collection, extraction and amplification have led to the demand for obtaining more informative DNA profiles from trace samples. "Touch DNA" can be defined as DNA transferred from a person to an object via contact with the object itself (Dash *et al.* 2020; Meakin and Jamieson 2013). In the literature, this DNA transfer is sometimes referred to as "contact DNA", "trace DNA", or "transfer DNA". The first such report published in 1997 described the recovery of DNA from handled objects (Oorschot *et al.*1997). Since then, various research groups have reported the extraction of DNA from different handled items, such as handbags, clothing, jewellery and steering wheels (Findlay*et al.* 1997; Schulz and Reichert 2000; Barbaro and Cormaci 2006; Petricevic *et al.* 2006; Franke *et al.* 2008; Sewell *et al.* 2008 and Aditya *et al.* 2011). However, in burglary-related crimes, different tools, including mock and personal tools, are considered to be useful materials for possible trace DNA analysis (Pfeifer and Wiegand 2017). Several factors, including the type of surface on which DNA is deposited, the time frame between deposition and recovery, the efficiency of extraction and environmental factors, are responsible for the amount of DNA deposited on the tool (Alketbi 2018). In general, rough and porous surfaces are capable of absorbing more DNA than smooth and nonporous surfaces (Wickenheiser 2002).

In this case study, we focused on the analysis of trace DNA obtained from the three different pairs of hand gloves used in bank robbery. Two pairs of gloves were used: surgical hand gloves (A and B), which had smooth and nonporous surfaces, and denim cloth hand gloves (C), which had rough and porous surfaces. To the best of our knowledge, this is the first such case study in which two different kinds of glove material were used in a single criminal event and trace DNA analysis was carried out.

Case History

Investigating agencies reported a bank robbery case with the DNA Division of Regional Forensic Science Laboratory (R.F.S.L), Amravati, Maharashtra, India. As informed by the police officer, the bank was robbed of approximately 2.1 million INR by the group of thieves (5-8 persons). The thieves used gas cutter machine to break in into bank premises as well as into strong rooms and locker rooms. Subsequently, other members of the gang destroyed the security alarm system, electric supplies equipment and CCTV cameras to avoid any traces of evidence remaining. During the crime scene investigation, the investigating officer seized a pair of surgical hand gloves from the backyard of the bank premise and two more pairs from one of the suspects. Of these two pairs, one was surgical hand gloves, and the other was denim cloth gloves. As provided by the police officers, the thieves used surgical hand gloves to prevent any type of fingerprint at the crime scene. The person wearing the gas cutter machine used pairs of hand gloves made of denim to prevent burning from hot flames. Overall, three pairs of gloves were submitted to the DNA division, as well as a control blood sample from five suspect individuals.

MATERIALS AND METHODS

Instruments: For DNA extraction, Prefiler™ with Automate Express (Thermo Fisher Scientific, UK) and EZ1® with EZ1 advance (QIAGEN, Germany) were used. DNA was quantified using a Quantifiler™ Human DNA Quantification Kit and an Investigator® Quantiplex Kit on a 7500 RT–PCR System (Thermo Fisher Scientific, UK). For amplification, an AmpFl STR™ Identifiler™ Kit (Thermo Fisher Scientific, UK) and an Investigator® IDplex plus Kit (QIAGEN, Germany) were used on a Veriti PCR system (Thermo Fisher Scientific, UK). For profiling, a 3500 Genetic Analyzer (Thermo Fisher Scientific, UK) was used.

DNA sampling:

Before sampling, the working platform, working hand gloves and equipment were decontaminated by wiping with 70% ethanol-soaked tissue paper. After each sampling, the hand gloves and working surface were cleaned with 70% ethanol. First, we sampled surgical gloves (A and B), which were made up of polymer latex material. This material is non-absorbing and soft, so the wearer's sweat dissolves cells adhering to the inner side of the glove. Therefore, we used cotton buds moistened with nuclease-free water (NFW) to wipe both pairs of surgical gloves and collected cotton bud samples in 2 ml Eppendorf tubes. In the case of denim cloth

ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue VII July 2025

gloves (C), the gloves were cut into small pieces, and the threads were separated and collected in a sampling tube.

Extraction:

By Automate Express: Moistened swabs from A and B, cut pieces of C, were taken in different sampling tubes, followed by the addition of PreFiler™ Lysis Buffer and Dithiothreitol (1.0 M). The samples were incubated at 70°C and 750 rpm for 40 minutes using a thermoshaker.

Using EZ1, DNA was extracted from the swabs of A and B, and cut pieces and threads derived from C were cut out using Buffer G2 and Proteinase K in the EZ1® DNA investigator kit. The samples were incubated at 56°C and 900 rpm for 15 minutes.

After incubation, the samples were subjected to centrifugation at 10000 rpm for 1 minute. The supernatant cell-lysate solution was removed by pipetting and used further.

DNA quantification:

Sample extracted by Automate Express: The extracted DNA from A, B and C (2 µl each) was mixed with a master mix of Quantifiler™ [primer mix (10.5 µl) and reaction mix (12.5 µl)]. The resultant solutions of A, B and C were run in parallel with eight standard samples of control DNA provided in the kit along with a single blank sample of nuclease-free water.

Sample extracted by EZ1: The extracted DNA from A, B and C (2 µl each) was mixed with master mix solution from the Investigator®Quantiplex Kit [Reaction Mix YQ (11.5 µl) + Primer Mix IC FQ (11.5 µl)]. The resultant solutions of A, B and C were run in parallel with six standard samples of control DNA provided in the kit along with a single blank sample of nuclease-free water.

DNA amplification:

After quantification, DNA was amplified for samples extracted using automated expression with Identifiler™ [AmpFlSTR™ PCR Mix (10.5 µl) + AmpliTaq Gold™ DNA Polymerase 0.5 µl+ AmpFlSTR™ Identifiler™ Primer Set (5.5 µl)] from 15 µl mixed with 10 µl of DNA samples from A, B and C. Thus,a 25 µl volume was used for the amplification process. The initial incubation was at 95°C for 11 min, followed by 28 thermal cycles, which included denaturation at 94°C, annealing at 59°C, and extension at 72°C for 1 min each. and a final extension at 60°C for 60 minutes.

Similarly, samples extracted from EZ1 were amplified by using an Investigator®IDplex plus kit [Fast Reaction Mixture (7.5 µl) and Primer mix (2.5 µl)] and 15 µl of DNA from sample C. The initial incubation was 95°C for 5 min, followed by 30 thermal cycles at 96°C for 10 seconds and 61°C for 2 minutes each.

DNA profiling:

For Identifiler™, a mixture of Liz600 (0.5 µl), HIDI formamide (9.5 µl) and the amplified products of A, B and C (1 µl) was used for profiling via the use of a ruler allelic ladder containing 15 STR loci and a sex-specific amelogenin locus.

For the Investigator®IDplex, a mixture of SST-BTO 550 (0.5 µl), HIDI formamide (12 µl) and the amplified products A, B and C (1 µl) was used for profiling via the use of a ruler allelic ladder of 15 STR loci and a sex-specific amelogenin locus.

RESULTS

After A, B and C were sampled, the DNA was extracted, followed by quantification, amplification and profiling. The profile obtained thus matched with the suspects. The detailed procedure is shown in (Scheme 1). First, the DNA extracted by PreFiler™ was used for quantification, and the quantity of DNA in pair A was

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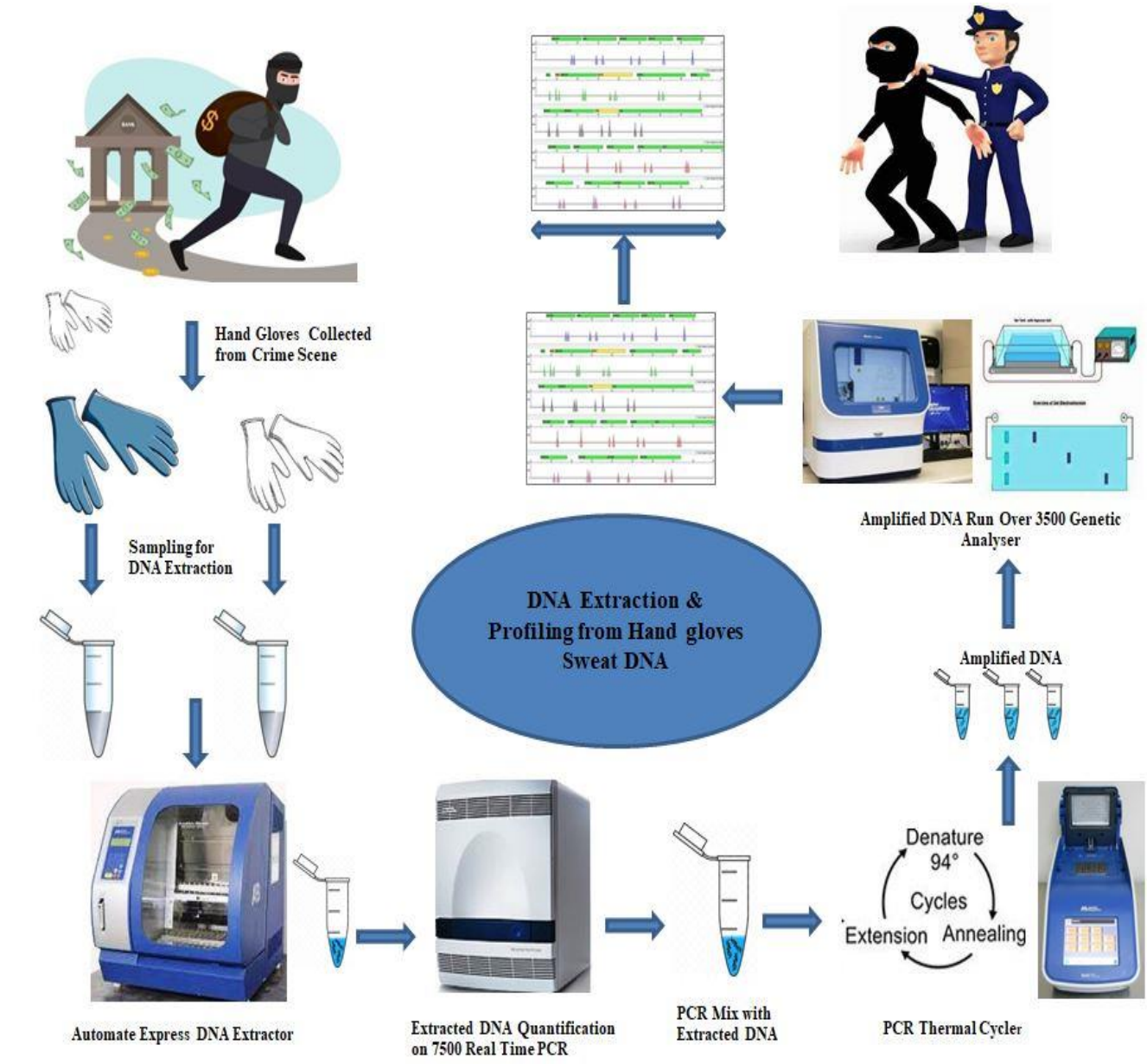
0.123 ng/µl and 1.877 ng/µl; in pair B, it was 0.062 ng/µl and 0.056 ng/µl, whereas, in pair C, it was 0.00 ng/µl and 0.065 ng/µl. The details of the quantities obtained are provided in Table 1. The decent quantity of DNA obtained from glove A is consistent with the fact that one of the suspects used two pairs of gloves one above the other. Pair A was in direct contact with the suspect, whereas pair B was used above pair A. Therefore, the amount of DNA obtained from pair B was less than that obtained from glove A. The amount of DNA obtained in glove A is sufficient to obtain the DNA profile, whereas that in glove



Scheme 1. Procedure flow for DNA profiling.

Table 1. Details of quantity obtained from different pair of gloves.

Method	Prefiler / Quantifiler (quantity in ng/µl)			EZ1/Quantiplex (quantity in ng/µl)		
Pair of Gloves	**A**	**B**	**C**	**A**	**B**	**C**
Sample 1	0.123	0.062	0.00	0.26	**0.91**	0.01
Sample 2	**1.877**	0.056	0.065	-	0.01	0.00
Profile Obtained	**Yes**	No	No	**Yes**	**Yes**	No

B is inadequate. In the case of glove C, the dye present in denim cloth interferes with the extraction process and therefore we were not be able to obtain the desired amount of DNA or profile. Moreover, denim cloths have a thick, rough surface and greater absorption capacity for sweat, which might reduce cell size.

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Due to the incompatibility of method 1 (PreFiler™/Quantifiler™/Identifiler™), we moved toward method 2 (EZ1/Investigator®Quantiplex/Investigator®IDplex). The details of the amount of extracted DNA are shown in Table 1. As expected, the quantity of DNA in glove A was sufficient for profiling. However, the amount of DNA obtained by glove B was greater than that obtained by method 1. By this method, we were able to obtain a clear profile from pairs of gloves B. Despite changing Method 1 to Method 2, there was no positive improvement in the extraction of DNA from pairs of gloves C (Figure 1c). Dye interference in pairs of gloves C heavily hampered our efforts toward obtaining a clear DNA profile. Therefore, we must look for a method that can provide a desirable amount of DNA. There are few reports that explain the sampling procedure for a single fibre derived from cloth material (Blackie*et al.* 2016). For this purpose, we derived five fibres from different areas opposite the site of the palm. The quantities obtained are shown in Table 2. The quantity obtained was 0.06 ng/µl, which was sufficient to obtain a clear profile. The single-threading experiment was also carried out after 520 days of incidence, yetwe obtained the desired quantity of DNA from the pair of gloves C (Figure 1d). This finding indicates that rough and porous cloth surfaces are good candidates for preserving cells and keeping nuclear information intact. Thus, we proposed the use of a single thread of cloth Fibers in similar criminal investigations.

Table 2. Single Fibre thread quantification from the pair of gloves C

Method	**EZ1/Quantiplex (quantity in ng/µl)**				
Sample Number	**1**	**2**	**3**	**4**	**5**
Quantity obtained	0.00	0.01	**0.06**	0.02	0.03
Profile obtained	No	No	**Yes**	No	No

DISCUSSION

As previously discussed, the “Trace DNA” widely used for extracting DNA form various objects but it poses serious challenges regarding extraction process for obtaining DNA from coloured or dyed objects. In case of fabrics, two standard approaches have been utilised; first one is to cut out the desired cloth piece followed by extraction using commercially available techniques and isolation of DNA whereas second approach is to use adhesive tape to remove traces of cellular material (Linacre *et al* .2010). The first approach is well suited for undyed fabrics whereas the later approach is more convenient for the smooth surface material. Fre´geau and Moors[23] showed that the use of positively charged Promega DNA IQ™ paramagnetic beads are used for the DNA binding. The process of lysis breaks the cell wall and releases proteins and DNA from the cell. After that, Protinase-K enzyme separates proteins by bonding with itself thus leaving behind free DNA in the lysate. The DNA in lysate gets attached to the beads and released for further amplification. However, in case of denim fabrics, the basic nature of dyes/chemicals from denim fabric interact directly with paramagnetic beads and therefore the DNA in lysate face competition from the dyes/chemicals and therefore lesser amount of DNA gets attached to the beads which leaves behind unbound in lysate[23]. One way to overcome that problems is by reducing dye concentration in the lysate. In this regard, we thought of using single fibre for the DNA extraction. As the single fibre will have lesser amount of dye as compared to cut out cloth piece hence there is lesser competition for masking of magnetic beads for DNA binding. Therefore, while dealing with denim cloth, utilisation of single fibres strategy is feasible approach with the material having low source of DNA.

After acquiring the DNA profile from gloves, A, B and C, (Figure 1a, 1b and 1d) we moved toward profiling the suspect. The blood collected from five different suspect individuals (S1, S2, S3, S4 and S5) for DNA profiling and matching. When the DNA profiles of gloves A, B and C were compared with the DNA profiles of five different suspect, 15 STR loci and sex determining Amelogeninloci were found to match those of gloves A and B with suspect 3 (Figure 1e). However, the profile of glove C matches that ofsuspect 2 (Figure 1f). The detailed interpretation results are shown in Table 3. The interpretation of the DNA profiles acquired from A and B and their matching with the suspect 3 (S3) confirm that glove A might be in direct contact with the skin during the whole process of robbing, resulting in excess secretion of sweat and adsorption of dissolved cells on the inner soft surface of the gloves.

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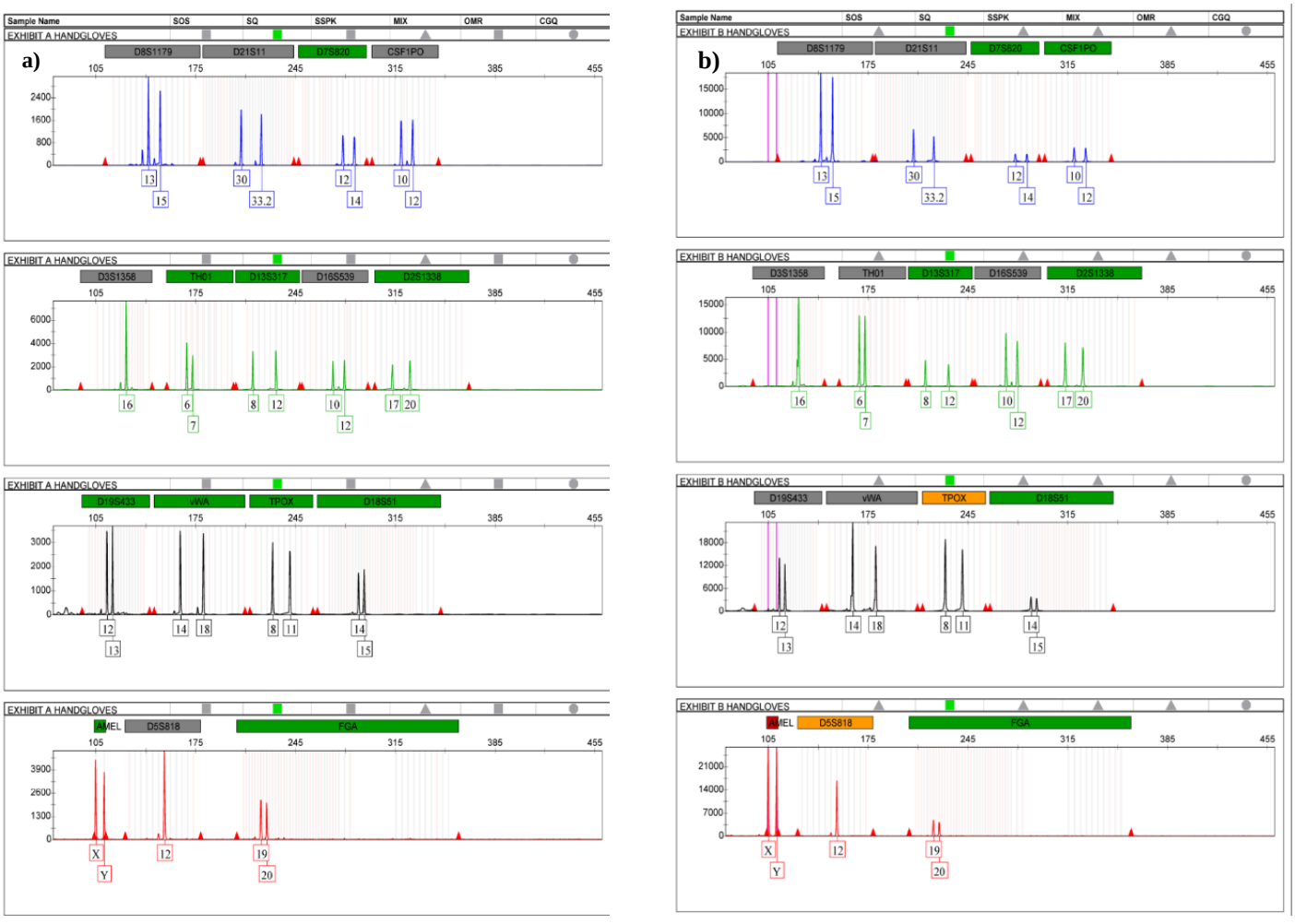
Table 3. Matching of the STR loci profiles of the A, B and C gloves with five different suspect gloves.

STR Loci			Genotype					
	Pair of gloves A	**Pair of gloves B**	**Pair of gloves C**	**Suspect 1 (S1)**	**Suspect 2 (S2)**	**Suspect 3 (S3)**	**Suspect 4 (S4)**	**Suspect 5 (S5)**
D8S1179	**13, 15**	**13, 15**	**15, 15**	12, 13	**15, 15**	**13, 15**	10, 13	13, 14
D21S11	**30, 33.2**	**30, 33.2**	**29, 32.2**	28, 31.2	**29, 32.2**	**30, 33.2**	30, 32.2	28, 32.2
D7S820	**12, 14**	**12, 14**	**11, 11**	8, 12	**11, 11**	**12, 14**	8, 12	12, 12
CSF1PO	**10, 12**	**10, 12**	**12, 12**	11, 11	**12, 12**	**10, 12**	12, 12	10, 11
D3S1358	**16, 16**	**16, 16**	**15, 17**	17, 17	**15, 17**	**16, 16**	16, 18	14, 17
TH01	**6, 7**	**6, 7**	**9, 9**	6, 9	**9, 9**	**6, 7**	7, 9.3	7, 9
D13S317	**8, 12**	**8, 12**	**8, 11**	8, 9	**8, 11**	**8, 12**	9, 11	8, 8
D16S539	**10, 12**	**10, 12**	**11, 13**	8, 12	**11, 13**	**10, 12**	11, 13	11, 12
D2S1338	**17, 20**	**17, 20**	**19, 21**	17, 25	**19, 21**	**17, 20**	19, 21	17, 21
D19S433	**12, 13**	**12, 13**	**14, 14**	14, 15.2	**14, 14**	**12, 13**	13, 14	14, 14
vWA	**14, 18**	**14, 18**	**18, 18**	17, 18	**18, 18**	**14, 18**	17, 17	17, 17
TPOX	**8, 11**	**8, 11**	**8, 11**	8, 10	**8, 11**	**8, 11**	8, 11	8, 11
D18S51	**14, 15**	**14, 15**	**12, 13**	13, 14	**12, 13**	**14, 15**	13, 13	12, 13
AMELOGENIN	**X Y**	**X Y**	**X Y**	X Y	**X Y**	**X Y**	X Y	X Y
D5S818	**12, 12**	**12, 12**	**11, 12**	12, 12	**11, 12**	**12, 12**	11, 12	10, 12
FGA	**19, 20**	**19, 20**	**23, 24**	22, 22	**23, 24**	**19, 20**	20, 22	22, 22



ISSN No. 2321-2705 | DOI: 10.51244/IJRSI |Volume XII Issue VII July 2025

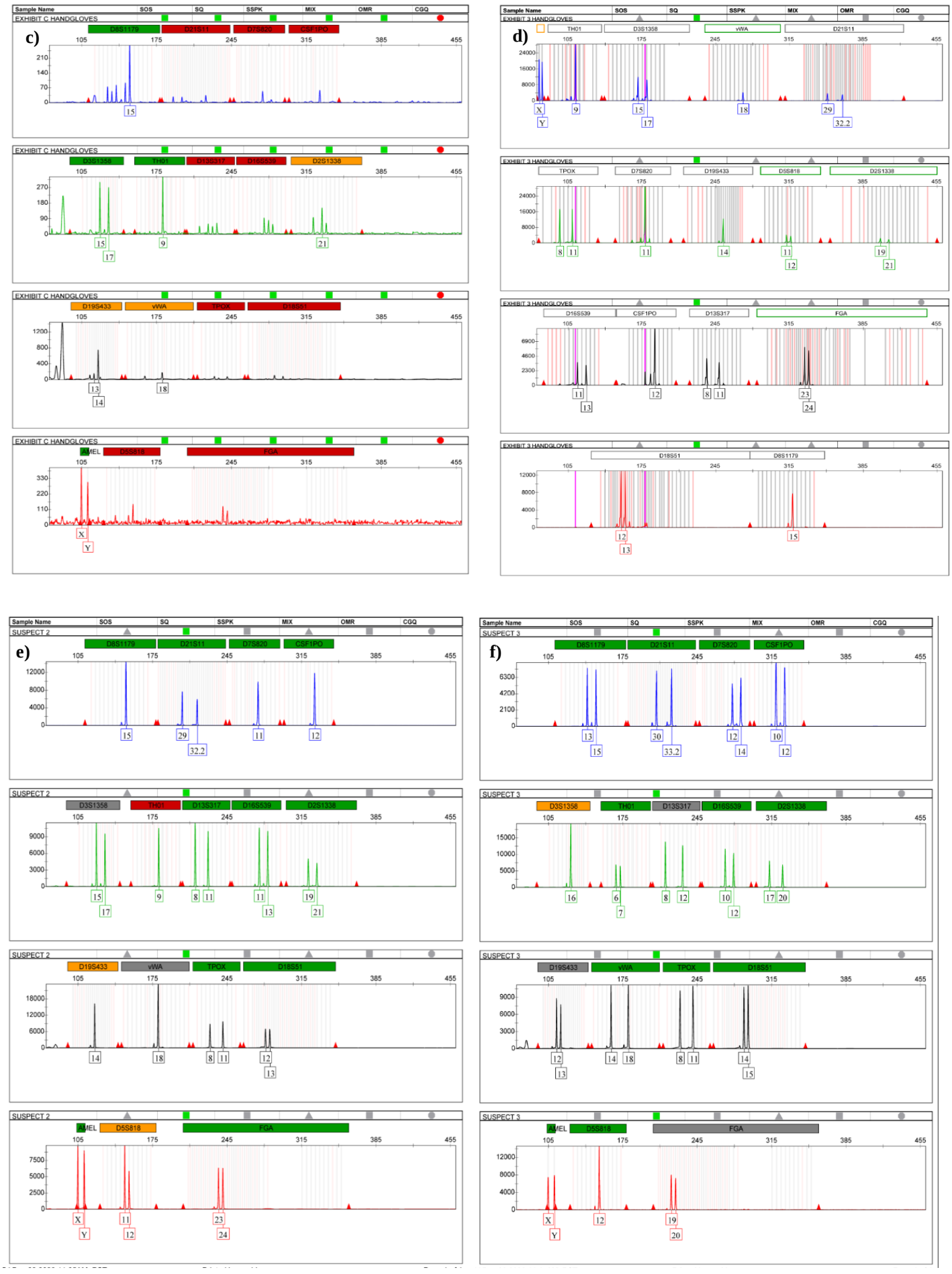


Figure 1. DNA profiles of (a) Gloves A, (b) Gloves B, (c) Denim cloth of glove C, (d) Fiber derived from denim gloves C, DNA profiles of suspect obtained from blood (e) Suspect 2 and (f) Suspect 3.

CONCLUSION

The three pairs of hand gloves, A, B and C, were the only evidence submitted by the investigating agency. It was a major challenge for us to extract DNA from soiled gloves and match it with a list of five suspects. Another challenge was to obtain DNA from the denim cloth. By taking up this challenge, we successfully extracted DNA, performed DNA profiling of both surgical and denim cloth gloves and identified two suspect persons from the list of five suspects. Additionally, we demonstrated that the surface of an object is critical for "Trace DNA" analysis. We can conclude that soft, smooth and non-absorbing surfaces, such as those of gloves A and B, are much more convenient for DNA extraction than rough and absorbing surfaces are. However, when a rough surface is damaged by interfering dyes, wearer DNA can be obtained by using single fibre thread for DNA extraction. This study will help to analyze and obtain trace amounts of DNA from rough, absorbing and porous cloth surfaces.

ACKNOWLEDGMENTS

We would like to acknowledge Mr. Sanjay Kumar Verma, IPS, Director General (Legal and Technical), Home Department, Government of Maharashtra, Mumbai, INDIA for constant encouragement and support to carry out the research.

Declaration of interest statement: The authors report there are no competing interests to declare.

Funding: No funding was received for conducting this study.

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