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# Effect of Dactyloscopic Powders on DNA Profiling From Enhanced Fingerprints

## *Results From an Experimental Study*

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**Abstract:** We conducted a study on the effect of fingerprint enhancement methods on subsequent short tandem repeat profiling. First, we performed a study typing blood traces deposited on 5 different surfaces, treated with 8 types of dactyloscopic powders. Three different DNA extraction methods were used. Subsequently, we analyzed latent fingerprints on the same 5 surfaces enhanced with the 8 different powders used in the first part of the study.

This study has demonstrated that DNA profiling can be performed on fingerprints left on different substrates, and the substrate will affect the amount of DNA that can be recovered for DNA typing. In the first phase of the study, a profile was obtained in 92% of the 120 samples analyzed; in the second part, in 55% of the 80 samples analyzed, we obtained a profile complete in 32.5% of the cases. From the results obtained, it seems that the powders used in latent fingerprints enhancement, rather than having a direct inhibitory effect on extraction and amplification of DNA, may cause partial degradation of DNA, reducing the efficiency of amplification reaction. It should not be forgotten that these results were obtained under laboratory conditions, and in real caseworks, there may still be different problems involved.

**Key Words:** latent fingerprints, DNA typing, dactyloscopy, low copy number

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Fingerprint technology and DNA analysis are routinely used in the management of crime scenes during criminal investigations. The fingerprint technique relies on the mechanical application of fingerprint powders to moisture and oily components in the skin ridge deposits. Because the powder is normally colored, the ridge pattern becomes visible, and the latent print is said to have developed. Powder dusting is the simplest, oldest, and most commonly used procedure for developing latent fingerprints[1] because it is cost-effective, mainly safe, and the detection may be carried out directly at the scene of crime, giving immediate results.

With modern polymerase chain reaction (PCR)–based technologies, it is possible to type even very small amounts of DNA.[2–4] Different studies have proved that skin contact can transfer enough DNA for successful short tandem repeat (STR) typing.[5,6] The success rate in obtaining a genetic profile (partial or complete) from a latent fingerprint will depend on the individual handler, on the hand which has been used, on the activities of the individual before touching the object, and on the handled substrate. Porous substrates adhere to sloughed epithelial cells more readily than nonporous substrates. Low copy number (LCN) typing, particularly for current STR typing, refers to the analysis of any sample that contains less than 200 pg of template DNA. Generally, LCN typing can be simply defined as the analysis of any DNA sample where the results are below the stochastic threshold for reliable interpretation by standard methods. There are a number of methodologies to increase sensitivity of detection to enable LCN typing. These approaches encompass modifications during the PCR and/or post-PCR manipulations. Regardless of the manipulations, when processing a small number of starting templates during the PCR, exaggerated stochastic sampling effects will occur. The result is that several phenomena can occur; these are as follows: a substantial imbalance of 2 alleles at a given heterozygous locus, allelic dropout, allelic drop-in, or increased stutter.[7,8] To avoid the risk of these stochastic effects, different enhancement techniques are applied, and the most widely used of these techniques is based on additional PCR cycles (from 28 to 34) to increase amplification and hence compensate for the low starting template. Recently, numerous laboratory and validation protocols and reviews have been published on LCN typing or low template DNA typing. The approach most widely used for the designation of an allele in a low template DNA sample requires the division of the sample into 2 or more aliquots and reporting only the alleles that are common in at least 2 replicates.[9–11]

In the last 20 years, in obtaining reliable STR profiling from small amounts of DNA on touched objects, great attention has been paid to the possible interactions between dactyloscopic enhancement methods or bloodstain enhancement methods and DNA typing techniques.[12–18] In criminal caseworks, it may be that latent fingerprints are not useful for dactyloscopic purposes because, for example, curve and loop patterns are blurred; latent fingerprints are not commonly used for DNA typing even if they could be considered as useful DNA sources. Furthermore, investigators are sometimes confronted with evidence of fingerprints in combination with biological material, and it could be difficult to decide whether dactyloscopic analysis or DNA profiling are to be performed on the evidence. In other cases, biological stains (eg, blood) found on samples may be subjected to latent fingerprint analysis reagents. The main problem in these cases is whether dactyloscopic methods could be used on a surface without interfering with the ability to perform DNA profiling from latent fingerprints or from other biological evidences obtained on the same surface.

Moving from the low number of experimental studies published in last 3 years, we performed this study to investigate the effect of fingerprint enhancement powder methods on subsequent STR profiling from bloodstains and latent fingerprints. The research was conducted in 2 phases. The aim of the first one was to establish whether 8 different types of fingerprint powders can interfere with the analysis for genetic profile determination

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and in what way. In the second part of the study, latent fingerprints, visualized with the same 8 powders, were tested for their usefulness for STR typing. The aim of this study was to provide preliminary findings, under a controlled laboratory condition, with the purpose to analyze simultaneously and compare the effect of 8 different powders on the DNA typing of fingerprints left on 5 different substrates, which was not previously done in the referred literature.

## MATERIALS AND METHODS

### Sample Preparation

Five different porous and nonporous substrates (a glass plate, a sheet of paper, a piece of plexiglass, a ceramic plate, and a piece of metal) were used to investigate biological stains consisting of blood (first phase) and latent fingerprints (second phase). All the surfaces were precleaned with absolute alcohol, dried and then treated with DNA Remover.

Four different female donors (namely 1, 2, 3, and 4), extraneous to the laboratory and unrelated to its personnel, were involved as donors of blood samples for bloodstains and as depositors for latent fingerprints. Saliva samples from the donors were used as positive control.

With regard to the first phase of the study, 8 aliquots of 50 μL of blood were deposited on each surface (with 40 bloodstains in total). All bloodstains were dried at room temperature and then dusted (after 24 hours) with 8 different dactyloscopic powders routinely used by the police; these are as follows: metal white, metal black, metal grey, magnetic black, magnetic grey, fluorescent pink, fluorescent yellow, and fluorescent orange. On each surface, every single bloodstain was treated with 1 powder method, leaving 1 bloodstain from each donor untreated as control for DNA extraction, with a total of 20 untreated bloodstains as untreated controls. The brushes were cleaned before treating a new stain, and all powders were applied with gentle strokes to avoid contamination and overpowdering. Each dusted bloodstain was sampled with 3 different sterile cotton swabs, moistened with sterile water, which were stored at +4°C until the DNA extraction.

In the second part of the study, fingerprints were placed by the same 4 donors 30 minutes after washing their hands. When placing the fingerprints, the pressure was subjectively firm and was exerted for 60 seconds. No preference for dominant hand versus nondominant hand was made. We obtained 16 fingerprints for each surface, with a total of 40 latent fingerprints. On each substrate, fingerprints were enhanced with the same 8 powders used in the first phase (2 fingerprints with the same powder), and 4 fingerprints for each substrate (1 fingerprint belonging to each donor) were used as untreated control for DNA extraction, with a total of 20 untreated controls.

Each visualized fingerprint was sampled with a sterile cotton swab moistened with sterile water and then stored at +4°C until the DNA extraction.

In both phases, sampling with the cotton swab was undertaken within 60 minutes from the powder dusting.

### DNA Extraction

DNA was extracted from bloodstains using 3 different methods for each stain; these are as follows: the Chelex method,[19] the ChargeSwitch Forensic DNA Purification Kit (Invitrogen), and the QIAamp DNA Micro Kit (Qiagen).

In the second part of the study, DNA extraction from enhanced fingerprints was performed with ChargeSwitch Forensic DNA Purification Kit and with QIAamp DNA Micro Kit, with a final eluted volume of 20 μL.

### DNA Amplification

Amplification was performed using the AmpFℓSTR Identifiler Plus PCR Amplification Kit (Life Technologies). The typed STRs were D8S1179, D21S11, D7S820, CSF1P0, D3S1358, TH01, D13S317, D16S539, D2S1338, D19S433, vWA, TPOX, D18S51, D5S818, and FGA. All PCR reactions were carried out in a GeneAmp PCR System 9700, 96-Well Gold-Plated (Life Technologies). The PCR-negative and PCR-positive controls were carried through the entire process.

Polymerase chain reaction was performed following the manufacturer's recommendations, modified by different technical enhancement—reduction of the final amplification volume to 12.5 μL (in the first part of the study) and to 13.5 μL (in the second part of the study), the adding of 1 μL of bovine serum albumin (1 mg/mL), and additional PCR cycles (34) in the second part of the study.

In the second part of the study, the amplification was repeated 3 times for each sample.

### Capillary Electrophoresis

Profiles were generated using a 3130 Genetic Analyzer (Life Technologies). The sample solution was 15.5 μL (15 μL formamide + 0.5 μL internal standard) and 1 μL amplified DNA. Analysis was undertaken using GeneMapper ID Software Version 3.2 (Life Technologies).

The previously known profiles of the 4 donors have been compared with the results in both phases.

### Quality of DNA

At the end of the second phase of this study, we performed agarose gel electrophoresis on samples that did not show any results, with the purpose to find out whether null results were due to an extraction failure or an amplification failure.

Electrophoresis of 5 μL of PCR was performed in 1.0% agarose gel in 1X Tris-borate-ethylenediaminetetraacetic acid buffer with 0.5 μg/mL of ethidium bromide to directly visualize amplicons under ultraviolet. We used 1 Kb Plus DNA Ladder (Invitrogen) as molecular weight marker.

## RESULTS

The results, both for bloodstains and for fingerprints, were divided into 4 categories; these are as follows:

- Full profile (FP): all 15 polymorphisms successfully typed;
- Partial profile (PP): from 6 to 14 successfully typed loci;
- Poor profile (POP): lesser than 5 successfully typed polymorphisms;
- Null profile (NP): no results at all.

All negative controls, both for bloodstains and for fingerprints, did not give any results, confirming that there was no contamination.

### Bloodstains

Among the 120 powder-treated blood specimens, 110 were successfully typed (92%) and 72 showed complete genetic profiles (60%). All obtained alleles conformed to data obtained from the saliva of the respective subjects. The QIAamp DNA Micro Kit has proved to be the most efficient method in this phase, with 40 typed profiles, among which 26 were complete (Fig. 1).

The ChargeSwitch Forensic DNA Purification Kit showed good results as well, leading to 40 profiled samples, with 24 full profiles. For bloodstains treated with thin powders, such as magnetic or metallic grey/black, this extraction kit gave a high number of partial profiles.

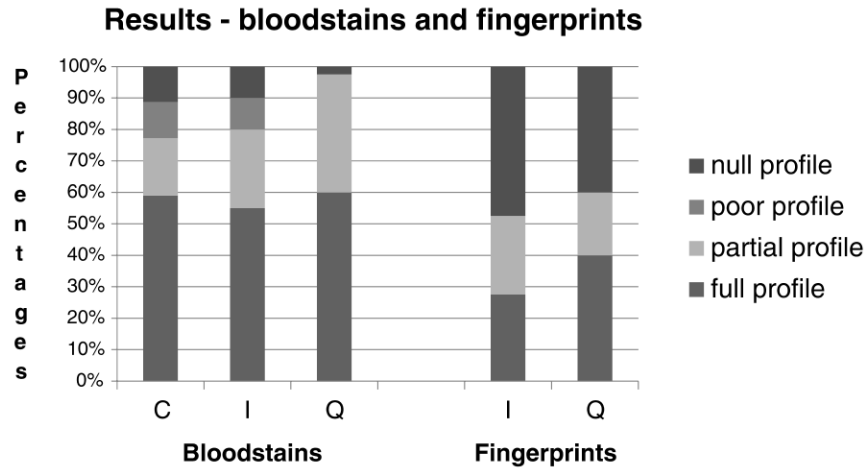


**FIGURE 1.** Results obtained on bloodstains and fingerprints, considering the extraction methods and the number of successfully typed STR loci. The results were divided into 4 categories as follows: FP, all 15 polymorphisms successfully typed; PP, from 6 to 14 successfully typed loci; POP, lesser than 5 successfully typed polymorphisms; NP, no results at all. (C indicates Chelex method; I, ChargeSwitch Forensic DNA Purification Kit; Q, QIAamp DNA Micro Kit). With fingerprints samples, we did not use Chelex extraction methods, and we did not obtain POP profiles.

Regarding the type of powders used, when using magnetic powders, the success rates of each type of powder expressed as numbers of successfully typed were as follows: 26 samples for magnetic, 41 samples for metallic powders, and 33 samples with fluorescent powders. These results are graphically represented in Figure 2.

The Chelex extraction method led to 30 typed samples with 22 complete profiles. For bloodstains, full profile typing seems to be low, at only 60%, especially considering that 50 μL of blood is quite a large stain. This low success rate could be explained by the fact that during the Chelex method extraction, we did not obtain a good purification from powders. As a result, this method led to the worst results.

Furthermore, it was noted that among the 10 samples that were not typed, all were extracted using the Chelex method; 9 were treated with fluorescent powders and 1 with black metallic powder. In particular, fluorescent powder shows interference with capillary electrophoresis, enhancing the signal with an overflow in the scale.

All 20 untreated bloodstains were successfully typed with full profile results (Fig. 3).

In accordance with what has previously been reported in literature,[5,12,13,15] these results showed that it is possible to obtain complete profiles from bloodstains treated with powder-based fingerprint enhancement methods, with no evidence of negative effect of powders on DNA extraction and amplification.

## Fingerprints

In this phase, different technical assessments were performed to enhance the efficiency of analysis.

During the extraction phase, we did not use the Chelex method because in the first phase of the study it gave the lowest number of profiles, and we reduced the DNA-eluted volume to 20 μL. In the amplification step, the following technical assessments were performed: increased number of PCR cycles from 28 to 34 and reduction of the final amplification volume to 13.5 μL, including bovine serum albumin (1 mg/mL) addition.

Each DNA sample was amplified 3 times to obtain a consensus profile, and only those alleles that were present in at least 2 of the 3 replicates were considered.

All obtained alleles conformed to data obtained from the saliva of the respective subjects.

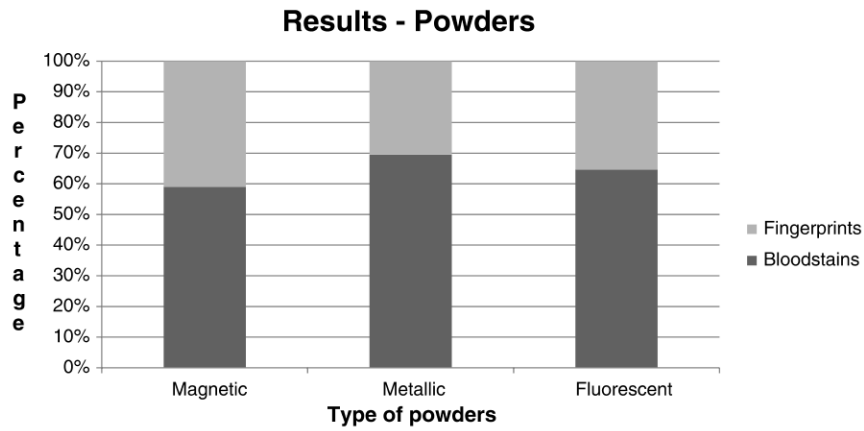


**FIGURE 2.** Results obtained on bloodstains and fingerprints, considering the powders used (magnetic, metallic, and fluorescent) in relation to the percentage of obtained profiles.

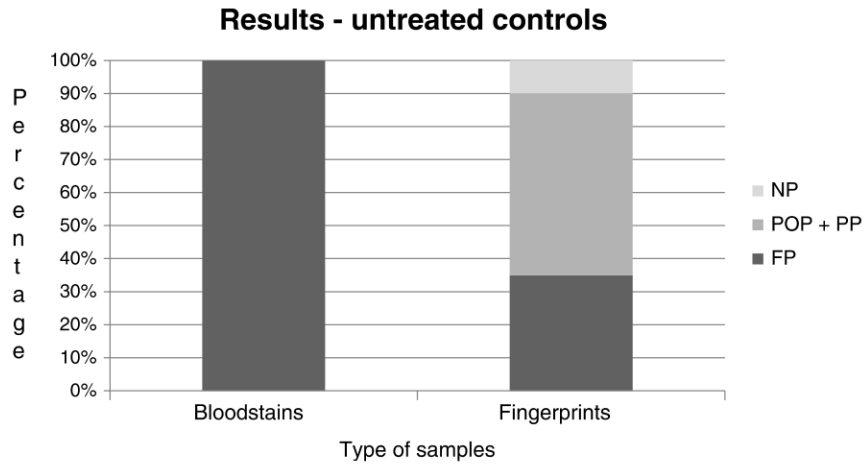


**FIGURE 3.** Results obtained on bloodstains and fingerprints, considering the quality of DNA profiles obtained from untreated controls. FP, all 15 polymorphisms successfully typed; PP, from 6 to 14 successfully typed loci; POP, lesser than 5 successfully typed polymorphisms; NP, no results at all.

Among the 80 fingerprints analyzed, 44 were successfully typed (55%) and 26 showed complete genetic profiles (32.5%) (Fig. 1).

The profile of untreated controls was typed in 18 of 20 samples, with 35% of complete profiles (7 fingerprints), 55% of PPs or POPs (11 fingerprints), and 10% of NPs (2 fingerprints) (Fig. 3).

We obtained the best results when using the QIAamp DNA Micro Kit, and this kit seems to be the more suitable for typing latent fingerprints enhanced with dactyloscopic powders. The higher number of complete profiles were obtained in glass and metal plates, probably because these surfaces are smoother than the others and thus less powder adheres to them (Fig. 4).

Regarding the type of powders used, when using magnetic powders, the success rates of each type of powder expressed as numbers of successfully typed were as follows: 13 samples for magnetic, 13 samples for metallic powders, and 18 samples with fluorescent powders (Fig. 2).

Among the 36 samples that were not typed, 24 were treated with magnetic or metallic powders and 10 with fluorescent powders. Also in this case, fluorescent powder shows interference with capillary electrophoresis, enhancing the signal with an overflow in the scale.

At the end of the second phase of this study, we performed agarose gel electrophoresis on samples that did not show any results, with the purpose of finding out whether null results were dependent on extraction failure or on amplification failure due to the presence of dactyloscopic powders. All samples showed the presence of partially degraded DNA, suggesting that when dealing with low quantity of DNA, as is the case for

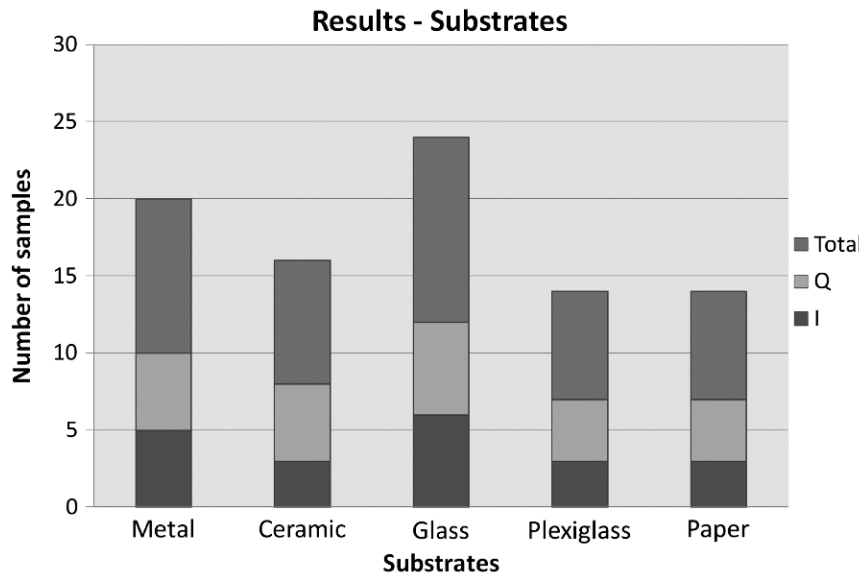


**FIGURE 4.** Results obtained on fingerprints, considering the extraction methods and the substrates. (I indicates ChargeSwitch Forensic DNA Purification Kit; Q, QIAamp DNA Micro Kit).

single fingerprints, dactyloscopic powders could have a direct degrading effect on DNA rather than inhibiting extraction or amplification.

## DISCUSSION

This study proved that it is possible to obtain and type DNA from single latent fingerprints, enhanced with powder-based dactyloscopic methods, in accordance with previous studies.

From the results obtained, it seems that the powders used in latent fingerprint enhancement, rather than having a direct inhibitory effect on extraction and amplification of DNA, may cause partial degradation of DNA, thereby reducing the efficiency of amplification reaction.

The higher number of complete profiles obtained with glass and metal surfaces suggested that their characteristics facilitate the recovery of DNA.

In conclusion, despite these results, we think that it should not be forgotten that they were obtained under laboratory conditions; however, in real caseworks, there may be still different scenarios that might be more complex than the controlled laboratory condition followed in this study, as for example, the time from the stain production and their collection, the amount of biological material left with touching, and the cleaning condition of the substrates on which we could find evidence. Despite this limitation, the aim of this study was to provide preliminary findings under standard condition, with the purpose to analyze simultaneously and compare the effect of 8 different powders on the DNA typing of fingerprints left on 5 different substrates, which was not previously done in the cited literature.

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