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Systematic Study on STR Profiling on Blood and Saliva Traces after Visualization of Fingerprint Marks

ABSTRACT: This paper describes a systematic study of the influence of optical, physical, and chemical methods used for fingerprint enhancement on subsequent DNA analysis of biological stains. Latent fingerprints as well as fingerprints in contact with blood and saliva on different surfaces were treated with dactyloscopic methods. As a general finding, subsequent STR profiling of the blood/saliva traces led to good results after all the enhancement methods included in this study. Concerning blood enhancement procedures, the airbrush technique showed deleterious effects on subsequent STR analysis in some cases. We therefore recommend the implementation of the layer technique, as it brings advantages for fingerprint enhancement as well. It could also be shown that, as can be necessary in practical casework, two enhancement methods can be performed on a single stain without having influence on STR profiling. In terms of methodological variety, this paper reflects a comprehensive study performed on STR profiling after fingerprint enhancement methods, including rare methods and variations of techniques, which can be a useful alternative in certain case scenarios.

KEYWORDS: forensic science, DNA typing, D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01, FGA, amelogenine, blood, saliva, latent fingerprint, finger mark, blood enhancement chemicals, dactyloscopic methods

The search for dactyloscopic marks stands in the forefront of criminal investigations, especially in crime-scene casework. Investigators are sometimes confronted with evidence of fingerprints in combination with biological material. It is the crime-scene officer's responsibility to decide whether dactyloscopic analysis or DNA profiling are to be performed on the evidence. In some cases, both methods may be applied; in cases where the amount of material is limited, a consecutive order of investigations has to be defined.

An actual case prompted us to start our systematic study. In a homicide the crime-scene officer secured a transfer voucher as evidence as bloody fingerprints were visible on the voucher. The question of the executive was whether they could use dactyloscopic methods on this piece of paper without interfering with the ability to perform DNA profiling of the blood traces. Further discussion revealed that this is a common problem, because the individual departments use dactyloscopic methods on the evidence in their routine work and then transmit it to a laboratory for further molecular analysis. In most of these cases, blood or saliva traces are involved. To investigate the effect of fingerprint-enhancement methods on subsequent STR profiling, we therefore performed a systematic study with a great variety of enhancement methods, involving not only commonly used techniques, but also rare variants, which can be a useful alternative to traditional dactyloscopic methods in some situations. Both defined amounts of blood and saliva were deposited with fingerprint marks on different surfaces and latent fingerprints were used. The samples were treated first with the dactyloscopic methods, and the biological traces were subsequently swabbed or cut and subjected to STR profiling.

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Material and Methods

Samples

Various porous and nonporous surfaces, respectively substrates, which are frequent in crime scenes, were used to investigate biological stains consisting of blood or saliva.

We used blood and saliva samples from three persons, two females and one male. Each proband donated a complete sample, i.e., a latent fingerprint and biological material.

Blood drops on glass slides (nonporous surface): 3 μL of blood were aliquoted onto glass slides (51 by 76 mm) precleaned with absolute alcohol and dried. Fingerprints were made close to the stain.

Blood drops on denim (porous surface): 3 μL of blood were pipetted on different parts of a pair of jeans.

Bloody fingerprints on glass slides, a can, silver paper, and the nonsticky side of adhesive tape (nonporous surfaces) and on the sticky side of adhesive tape, stamps and envelopes (porous surfaces): 3 μL of blood were aliquoted onto a finger prior to depositing a fingerprint on the different surfaces.

Saliva on stamps and envelopes (porous surfaces): Stamps and flaps of envelopes, which had been licked, were used. Additionally fingerprints were produced on the papers.

Saliva fingerprints on glass slides (nonporous surface) and envelopes (porous surfaces): A finger wetted with saliva was used to produce fingerprints on precleaned glass slides and on flaps of envelopes.

All samples were stored at room temperature for a minimum of two days to a maximum of 30 days before treatment.

Enhancement of the Fingerprint Marks

The fingerprint marks were visualized with the methods used in the routine lab of the Crime Scene Unit, Department of Crime, Gendarmerie Tyrol, Austria, according to handbooks from U. Amerkamp, Federal Police of Germany, BKA (1), P. Margot and C. Lennard, Universite de Lausanne, Institut de police scientifique et de criminologie (2), and the Processing Guide for Developing Latent Prints of the FBI (3).

The preparation of the reagents and the components of the solutions are shown in Table 1. The following methods were applied:

TABLE 1—*Chemicals and their preparation.*

ENHANCEMENT CHEMICALS		RECIPE
Manoxol Molybdenum Method	Concentrate:	solve 10 g Manoxol (dioctyl sodium sulfosuccinate) in 1000 mL distilled water
	Reagent:	solve 40 mL of concentrate and 5 g molybdenum sulfite in 1000 mL distilled water
Iodine and chemical fixing with 7.8-Benzoflavone	Solution A:	0.3 g benzoflavone (æ-naphtoflavone) 10 mL methylene chloride 90 mL Trichlortrifluorethan (Arklon™)
	Solution B:	0.1 g Iodine crystals 100 mL Trichlortrifluorethan (Arklon™)
	Reagent:	mix 2.5 mL of Solution B with 50 mL of Solution A
RAM (Rhodamine 6G + Ardrox™ + MBD)	Concentrate:	solve 0.01 g Rhodamine 6G, 10 mL Ardrox™ and 0.05 g MBD in 100 mL methanol and 100 mL 2-butanone (methyl ethyl ketone)
	Reagent:	dilute 10 mL of concentrate to 100 mL with petroleum ether
Safranin	Concentrate:	solve 0.5 g safranin in 1000 mL methanol
	Reagent:	dilute 5 mL of concentrate to 500 mL with methanol
Silver Nitrate		solve 5 g silver nitrate in 100 mL distilled water
Ninhydrin		solve 4 g ninhydrin in 20 mL methanol; add 10 mL glacial acetic acid and 70 mL ethyl acetate; dilute to 1000 mL with petroleum ether
DFO (1,8-Diazafluoren-9-one)		solve 0.2 g DFO in 50 mL methylene chloride; add 50 mL methanol and 20 mL glacial acetic acid; dilute to 1000 mL with petroleum ether
RTX (Rutheniumtetroxide)	Solution A:	solve 100 mg Ruthenium(III)-chloride in 100 mL distilled water
	Solution B:	solve 11.3 g Ammoniumcer(IV)-nitrate in 100 mL distilled water
	Reagent:	mix Solution A and Solution B
Gentian Violet (Parabene K)	Solution A:	solve 150 mL ethanol and 12 mL Parabene K in 800 mL distilled water at 80°C
	Solution B:	solve 50 g Gentian violet in 50 mL warm ethanol
	Reagent;	mix Solution A and Solution B
Gentian Violet (Phenol)	Concentrate:	solve 5 g Gentian violet and 10 g phenol in 50 mL ethanol
	Reagent:	4 mL of concentrate and 100 mL distilled water
Sticky-Side Powder		solve 0.5 g Lightning Sticky Side in 90 mL distilled water and 10 mL Photo-Flo™ 600 solution

BLOOD ENHANCEMENT CHEMICALS		RECIPE
Fixative		2% solution of sulfosalicylic acid
Luminol	Solution A:	solve 1.6 g sodium hydroxide in 100 mL distilled water
	Solution B:	solve 2 mL hydrogen peroxide 30% in 100 mL distilled water
	Solution C:	solve 0.08 g Luminol and 0.2 g sodium hydroxide in 100 mL distilled water
	Reagent:	mix Solutions A, B, and C and 700 mL distilled water
Amido Black (Methanol Base)	Staining:	solve 0.2 g Amido black in 90 mL methanol and 10 mL glacial acetic acid
	Destaining:	(a) methanol, glacial acetic acid (9:1/v:v) (b) distilled water, glacial acetic acid (95:5/v:v) (c) distilled water
Coomassie Blue	Staining:	solve 0.2 g Coomassie blue in 90 mL methanol and 10 mL glacial acetic acid
	Destaining:	(a) methanol, glacial acetic acid (9:1/v:v) (b) distilled water, glacial acetic acid (95:5/v:v) (c) distilled water
TMB (Tetramethylbenzidine)	Solution A:	solve 0.25 g tetramethylbenzidine in 2.5 mL glacial acetic acid and 12.5 mL ethanol
	Solution B:	15 mL hydrogen peroxide 3%
	Reagent:	mix Solutions A and B
LCV (Leucocrystal Violet)/ LMG (Leucomalachite Green)	Solution A:	solve 1 g Leucocrystal violet/ Leuco-malachite green in 100 mL glacial acetic acid and 150 mL distilled water
	Solution B:	50 mL hydrogen peroxide 3%
	Reagent:	mix Solutions A and B
Hungarian Red	Staining:	Hungarian red solution
	Destaining:	distilled water

Optical method:
- UV-Light 350 nm (PoliLight PL10, Rofin, Australia)

Physical methods:
- Dust with powder: Argentorat (Fingerprint powder silver special B-320, BVDA International bv.), Carbon Black (Fingerprint powder black special B-340, BVDA International bv.)
- Flame-Soot-Method
- Manoxol-Molybdenum-Method

Physico-chemical methods:
- Iodine and Chemical Fixing with 7.8-Benzoflavone
- Cyanacrylate Fuming and Cyan-Coloring Methods: RAM (Rhodamine 6G + Ardrox™ + MBD) and Safranin

Chemical methods:
- Silver Nitrate
- Ninhydrin
- DFO (1,8-Diazafluoren-9-One)
- RTX (Rutheniumtetroxide)

Special methods for adhesive tapes:
- Gentian Violet
- Sticky-Side Powder
- Cyanacrylate Fuming and Safranin

Special methods for blood traces:
- Luminol
- Amido Black
- Coomassie Blue
- TMB (Tetramethylbenzidine)
- LCV (Leucocrystal Violet)
- LMG (Leucomalachite Green)
- Hungarian Red

DNA Extraction and Quantitation

For every method investigated, a portion of the stain was separated and used as an untreated control for amplification. Furthermore, extraction blanks and PCR negative and positive controls were carried through the entire process. All blood or saliva marks were either swabbed or cut out of the substrate. DNA was extracted using the Phenol/chloroform method and quantified by fluorescent measurement with Hoechst Dye on a Hoefer Dyna Quant 200 Fluorimeter as described in (4). When no, weak, or partial STR results were obtained, the DNA extracts were purified using the QIA Quick PCR Purification Kit (Qiagen) according to the manufacturer's recommendations.

STR Amplification and Typing

Amplification was performed using the AmpFℓSTR SGM plus systems kit (Applied Biosystems) according to the manufacturer's recommendations.

All PCR reactions were carried out in a Perkin Elmer 9600 thermal cycler, amplification products were electrophoresed on a CE310 Genetic Analyzer (Applied Biosystems) using default conditions (24 min at 15 kV, POP 4). The previously known profiles of the three probands were always compared to the results.

Results and Discussion

Latent fingerprints as well as fingerprints in contact with blood and saliva were investigated on different surfaces as listed in Table 2. As a general finding, STR profiling of the blood/saliva traces led to good results using all the enhancement methods in this study. This is in concordance with previously published studies as far as common methods are concerned. In terms of methodological variety, however, this paper reflects a comprehensive study performed on fingerprint enhancement methods, including rare methods and variations of techniques, which can be a useful alternative in certain case scenarios.

Optical Method

Bloody fingerprints on glass were irradiated with UV light of 350 nm. This wavelength is routinely used for fingerprint enhancement of untreated evidence. There were no noticeable effects on subsequent DNA profiling. In all five variants (see Table 2 for details) complete DNA profiles with high signals similar to the untreated control were achieved. These findings complete the studies of Shipp et al. (5) and Lee et al. (6), who obtained results by RFLP analysis after using fingerprint enhancement light sources on bloodstains. Anderson et al. (7) investigated the effect of different light sources using wavelengths ranging from 255 to 514 nm on subsequent DNA typing of small bloodstains with STR quadruplex analysis. They found that only exposure of the bloodstains to shortwave UV light of 255 nm for more than 30 s affected PCR testing negatively.

Physical Methods

Sodhi et al. (8) gave a review about the powder method for detecting latent fingerprints as one of the oldest, simplest, and most commonly used procedures performed by fingerprint experts. Argentorat and carbon black were used in our study. There were no detrimental effects on PCR amplification, which confirms and completes the findings of Stein et al. (9), who used carbon treatment on glass slides.

Additionally, we investigated the Flame-Soot method using camphor crystals and an acetylene-welding torch to produce the soot on the samples. We also transferred fingerprints on a silicon layer, which is routinely used for documentation. In all the variations, complete STR profiles were amplified. Even the fingerprint, which was transferred on a silicon layer, was typed successfully (Fig. 1).

To complete the physical methods, the Manoxol-Molybdenum-Method was tested on different samples (see Table 2 for details). There was again no negative effect on STR profiling.

Physico-Chemical Methods

Iodine and chemical fixing with 7.8-benzoflavone showed no detrimental effect on STR analysis.

The effect of cyanoacrylate treatment on blood and saliva was tested in two variations: cyanacrylate was vaporized in a fuming chamber and by vacuum. Additionally, the fluorescent dyes RAM and safranin were applied. In all these variants complete SGM Plus profiles with signal intensities similar to the untreated control were obtained. These results conclude the studies of Hochmeister et al. (10), Shipp et al. (5), and Stein et al. (9), who reported successful RFLP analysis and PCR based AMPFLP, VNTR, and STR typing after cyanacrylate fuming. In a recently published paper, Wurmb et al. (11) conclude that cyanacrylate ester hampers amplification from small saliva stains after chelex extraction. Phenol/chloroform extraction in our study gave amplification results similar to those of the untreated controls, even when cyanacrylate fuming was used on saliva fingerprints.

Chemical Methods

Neither the presence of silver nitrate nor of ninhydrin hampered DNA amplification from saliva or blood in any of the cases (see Table 2 for details). These results confirm and complete the paper of Stein et al. (9), who used ninhydrin staining on blood and saliva samples and performed subsequent RFLP analysis as well as PCR based typing of AMPFLP and STR loci.

TABLE 2—*Experimental designs and results.*

Method	Sample	DNA Typing
OPTICAL METHOD:		
UV light (350 nm, working distance about 10 cm)		
for 15 s	Bloody fingerprint on glass slide	c.p.
for 30 s	Bloody fingerprint on glass slide	c.p.
for 1 min	Bloody fingerprint on glass slide	c.p.
for 5 min	Bloody fingerprint on glass slide	c.p.
for 15 min	Bloody fingerprint on glass slide	c.p.
PHYSICAL METHODS:		
Dust with powder:		
Argentorat	Blood drop on glass slide	c.p.
Carbon black	Blood drop on glass slide	c.p.
Argentorat	Saliva fingerprint on glass slide	c.p.
Carbon black	Saliva fingerprint on glass slide	c.p.
Flame-Soot-Method:		
Camphor crystals	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
Acetylene welding torch	Bloody fingerprint on can	c.p.
and transfer on a silicon layer	Bloody fingerprint on can	c.p.
	Bloody fingerprint on silicon	c.p.
Manoxol Molybdenum Method	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
	Saliva on stamp	c.p.
	Saliva on envelope	c.p.
PHYSICO-CHEMICAL METHODS:		
Iodine and chemical fixing with 7.8-Benzoflavone	Blood drop on glass slide	c.p.
	Saliva on stamp	c.p.
	Saliva on envelope	c.p.
Cyanacrylate fuming and cyan coloring methods		
fuming chamber	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
fuming chamber and RAM	Blood drop on glass slide	c.p.
fuming chamber and safranin	Saliva fingerprint on glass slide	c.p.
vacuum	Bloody fingerprints on silver paper	c.p.
vacuum and RAM	Bloody fingerprints on silver paper	c.p.
CHEMICAL METHODS:		
Silver nitrate	Saliva on stamp	c.p.
	Saliva on envelope	c.p.
Ninhydrin	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
	Saliva on stamp	c.p.
	Saliva on envelope	c.p.
DFO and 100°C for 15 min	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
	Saliva on stamp	w.p.
after purification*		c.p.
replicates†	Saliva on stamp	c.p.
	Saliva on envelope	c.p.
DFO and 60°C for 60 min	Bloody fingerprint on stamp	c.p.
	Bloody fingerprint on envelope	c.p.
	Saliva fingerprint on stamp	c.p.
	Saliva fingerprint on envelope	p.p.
after purification*		p.p.
replicates†	Saliva fingerprint on envelope	p.p./n.p.
after purification*		c.p.
RTX	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
	Saliva on stamp	c.p.
	Saliva on envelope	c.p.
SPECIAL METHODS FOR ADHESIVE TAPES:		
Gentian violet and phenol	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
Gentian violet and Parabene K	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.

TABLE 2—*Continued.*

Method	Sample	DNA Typing
Gentian violet and Parabene K/80°C (after cyanacrylate/Gentian violet)	Bloody fingerprint on the nonsticky side of adhesive tape	c.p.
Sticky-side powder	Bloody fingerprint on the sticky side of adhesive tape	c.p.
Cyanacrylate and safranin	Bloody fingerprint on the nonsticky side of adhesive tape	c.p.
	Bloody fingerprint on the sticky side of adhesive tape	c.p.
SPECIAL METHODS FOR BLOOD TRACES:		
Luminol	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
Amido black	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
	Saliva on stamp	c.p.
	Saliva on envelope	c.p.
Coomassie blue	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
TMB and air brush technique	Blood drop on glass slide	c.p.
	Bloody fingerprint on glass slide	c.p.
	Saliva fingerprint on glass slide	n.p.
after purification*		w.p.
replicates†	Saliva fingerprint on glass slide	w.p., n.p.
after purification*		w.p., n.p.
TMB and layer technique	Bloody fingerprint on glass slide	c.p.
	Plastic film	n.p.
after purification*		n.p.
LCV and air brush technique	Blood drop on glass slide	p.p.
after purification*		c.p.
replicates†	Blood drop on glass slide	c.p.
	Bloody fingerprint on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
	Saliva on stamp	n.p.
after purification*		p.p.
replicates†	Saliva on stamp	c.p.
	Saliva on envelope	w.p.
after purification*		w.p.
replicates†	Saliva on envelope	c.p.
LCV and layer technique	Bloody fingerprint on glass slide	c.p.
	Plastic film	n.p.
after purification*		n.p.
LMG and air brush technique	Blood drop on glass slide	n.p.
after purification*		p.p.
replicates†	Blood drop on glass slide	c.p.
	Bloody fingerprint on glass slide	w.p., n.p.
after purification*		w.p., n.p.
	Saliva fingerprint on glass slide	c.p.
LMG and layer technique	Bloody fingerprint on glass slide	c.p.
	Plastic film	n.p.
after purification*		n.p.
Hungarian red and air brush technique	Blood drop on glass slide	c.p.
	Saliva fingerprint on glass slide	c.p.
Hungarian red and layer technique	Bloody fingerprint on glass slide	c.p.
	Plastic film	n.p.
after purification*		n.p.
SIMULATION OF CASEWORK CONDITIONS		
UV light, DFO and 60°C, LCV and layer technique;	Blood drop on denim	n.p.
after purification*		c.p.
	Plastic film	n.p.
after purification*		n.p.
UV light, DFO and 100°C, LCV and layer technique;	Blood drop on denim	n.p.
after purification*		c.p.
	Plastic film	n.p.
after purification*		n.p.

c.p. = complete STR profile
p.p. = partial STR profile (concerning high molecular weight markers, mainly D2S1338 and D18S51)
w.p. = weak STR profile
n.p. = no STR profile
* means that the DNA extract was purified using the QIA Quick PCR Purification Kit.
† are repeated experiments. Both (*, †) were performed when no, weak, or partial STR results were obtained at first attempt. Illustrative examples are given in Figs. 1-3.

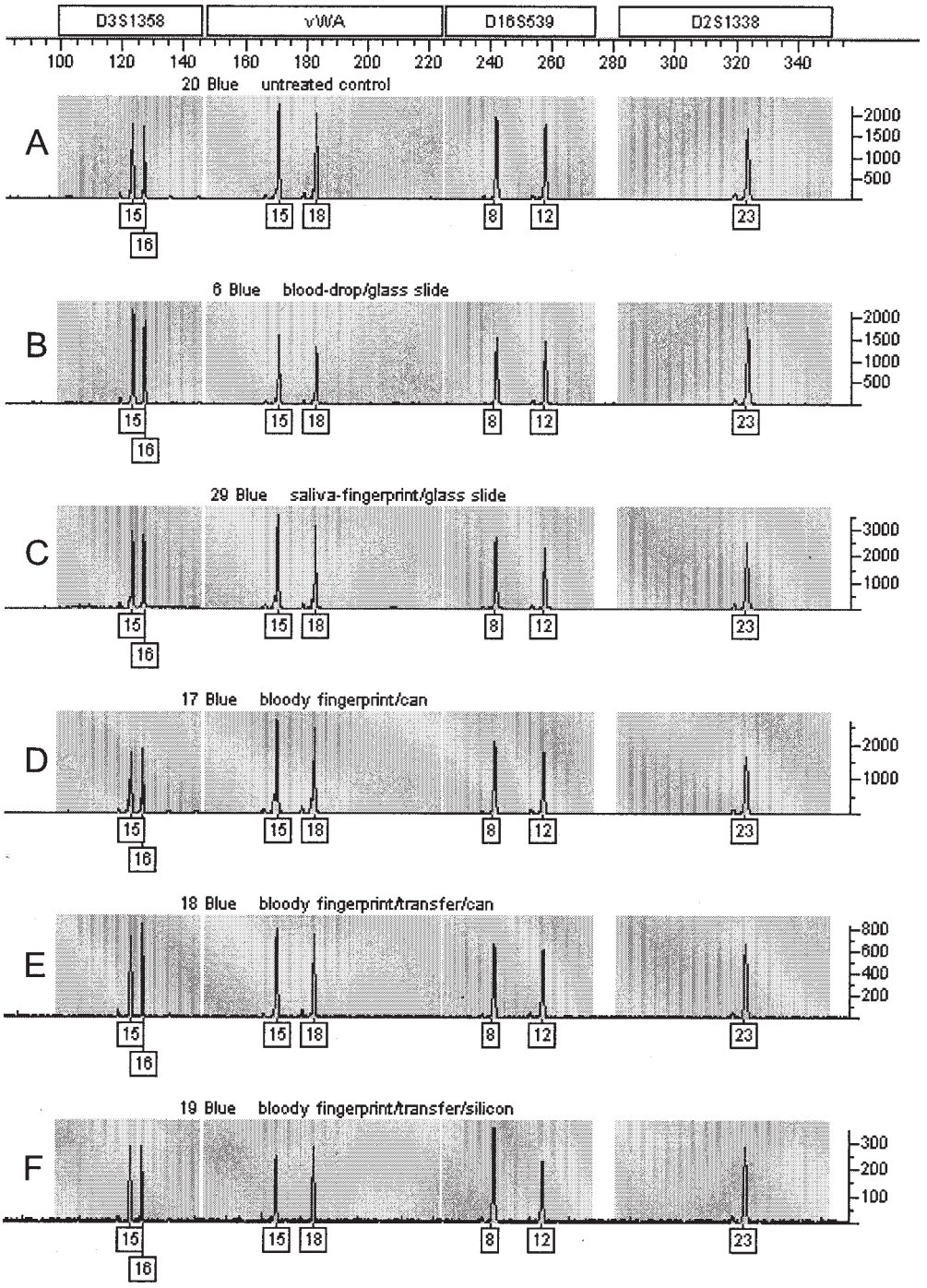

FIG. 1—*Physical methods/flame-soot method: examples of SGM plus (ABI) electrophoretograms, blue panels (FAM-labeled profiles only); the relative fluorescent intensity (RFU) of each sample is depicted at the right margin, all samples from Individual I*: (A) *untreated control, no enhancement, complete profile*; (B) *blood drop on glass slide, camphor crystals, complete profile*; (C) *saliva fingerprint on glass slide, camphor crystals, complete profile*; (D) *bloody fingerprint on can, acetylene welding torch, complete profile*; (E) *bloody fingerprint on can, acetylene welding torch and transfer on a silicon layer, complete profile*; (F) *bloody fingerprint on a silicon layer (after acetylene welding torch and transfer on a silicon layer), complete profile.*

DFO, a frequently used chemical enhancement procedure, was applied to various samples following two different protocols: incubation of the DFO-treated samples at 100°C for 15 min had no negative effect on STR analysis. Amplification after a period of 60 min at 60°C resulted in full STR profiles, except for the saliva fingerprint sample on the envelope. Repeated experiments in replicates resulted in no or partial STR profiles only, whereas full profiles were obtained after DNA purification (see Fig. 2). The varying amplification success with DFO-treated samples may be attributed to the longer incubation rather than the absolute temperature, as the STR results were weaker performing 60-min incubation at 60°C. Zamir et al. (12) obtained full six loci STR profiles after treatment with DFO at 80°C for 30 min in two cases of threatening letters.

To complete the chemical methods RTX was tested as well, which showed no appreciable effect on the results obtained from subsequent DNA profiling.

Special Methods for Adhesive Tapes

First, Gentian violet solutions containing phenol and alternatively Parabene K were applied to blood and saliva samples besides latent fingerprints. All latent fingerprints were enhanced. Thus, Parabene K can be regarded as an adequate surrogate for the toxic phenol. Parabene K was also used to enhance a bloody fingerprint on the nonsticky side of an adhesive tape, which had already been treated with cyanacrylate and Gentian violet, but did not show enough contrast. For this purpose, the solution was heated to 80°C in order to enhance the contrast. None of these treatments had any negative effect on STR profiling.

Sticky-side powder was used for the enhancement of a bloody fingerprint on the sticky side of an adhesive tape. No influence on DNA profiling was observed.

Bloody fingerprints were also treated with cyanacrylate on the nonsticky and on the sticky sides of an adhesive tape and subsequently visualized with the luminescent dye safranin. Again, no influence on DNA profiling was observed.

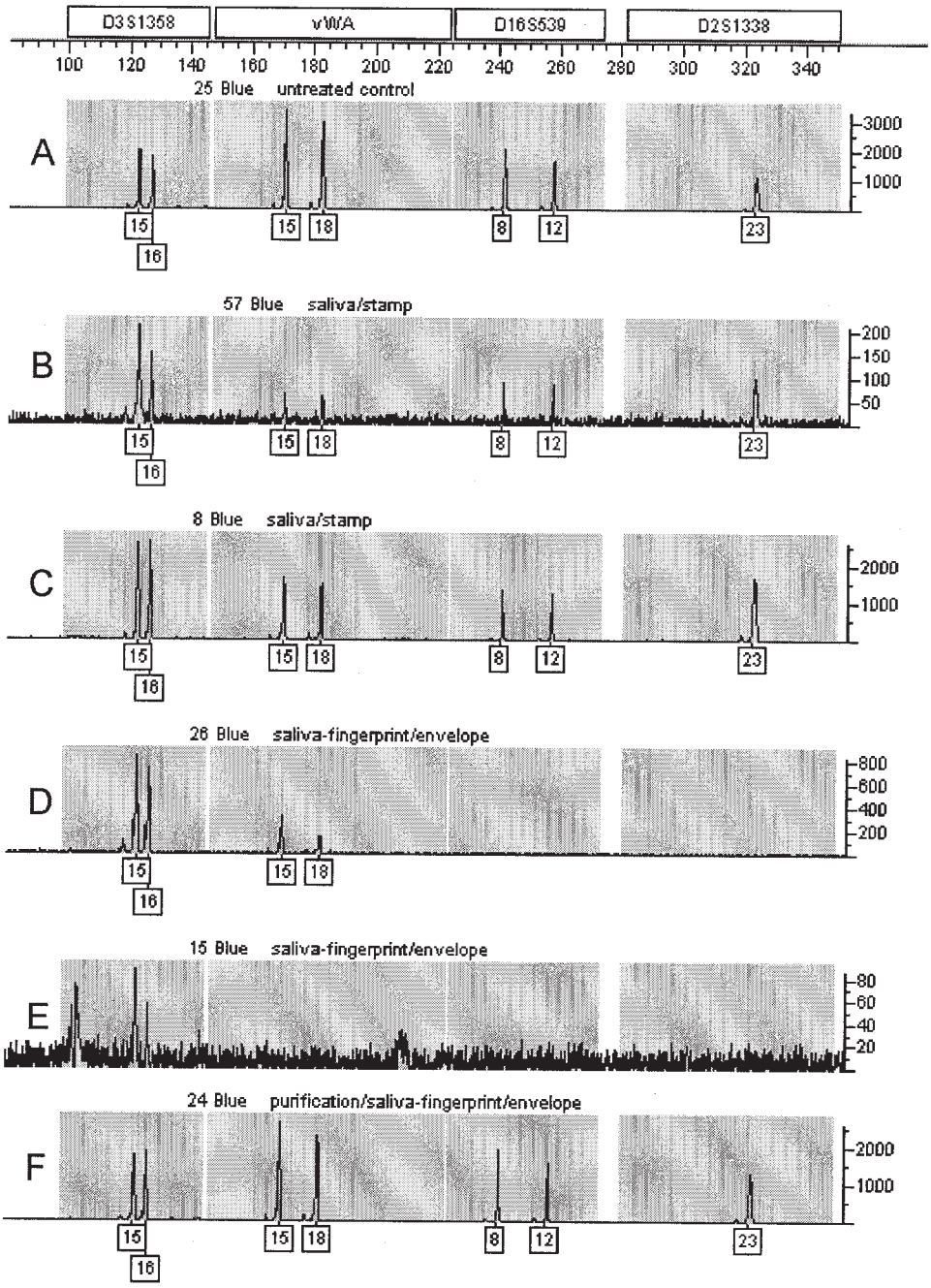

FIG. 2—*Chemical methods/DFO: Examples of SGM plus (ABI) electrophoretograms, blue panels (FAM-labeled profiles only); the relative fluorescent intensity (RFU) of each sample is depicted at the right margin, all samples from Individual I*: (A) *untreated control, no enhancement, complete profile*; (B) *saliva on stamp, DFO and 100°C for 15 min, weak profile*; (C) *saliva on stamp, DFO and 100°C for 15 min, purification, complete profile*; (D) *saliva fingerprint on envelope, DFO and 60°C for 60 min, partial profile (concerning high molecular weight markers)*; (E) *saliva fingerprint on envelope, DFO and 60°C for 60 min, no profile*; (F) *saliva fingerprint on envelope, DFO and 60°C for 60 min, purification, complete profile.*

Special Methods for Blood Traces

In 1989, Lee et al. (6) investigated the effects of different presumptive tests on dried bloodstains on subsequent serological identification and DNA testing. Gross et al. (13) showed that Luminol did not adversely effect PCR testing. Frégeau et al. (14) prepared bloody fingerprints on various surfaces and showed that none of the seven tested chemical enhancement procedures had detrimental effects on PCR amplification using Profiler Plus™ STRs.

To complete these findings, blood drops and blood marks were prepared on glass slides, and saliva fingerprints on glass and on paper were used (see Table 2 for details). Apart from ninhydrin, DFO, and RTX, the following methods were tested on these samples: Luminol, Amido black, Coomassie blue, TMB, LCV, LMG, and Hungarian red. None showed detrimental effects on STR profiling, which is in agreement with Frégeau et al. (14).

Both airbrush and layer technique with a transparent gelatine gel were used in conjunction with TMB, LCV, LMG, and Hungarian red. The airbrush technique showed deleterious effects on subsequent STR analysis in some cases, i.e., when it was used in conjunction with LMG on bloody fingerprints on glass (see Fig. 3). TMB on saliva fingerprints on glass and also LCV on saliva on stamps and envelopes did not work every time (see Table 2 for details). Concerning TMB and saliva traces, we could achieve as well complete but weak profiles as no results. LCV and saliva traces worked better—here it was sometimes necessary to purify the DNA extract; more often we got complete profiles at first attempt. But both TMB and LCV showed no problems when we used them on their main subject blood traces.

When performing the layer technique, full profiles were obtained every time. In this case, the plastic films were swabbed, too. No amplification products were obtained from these films,

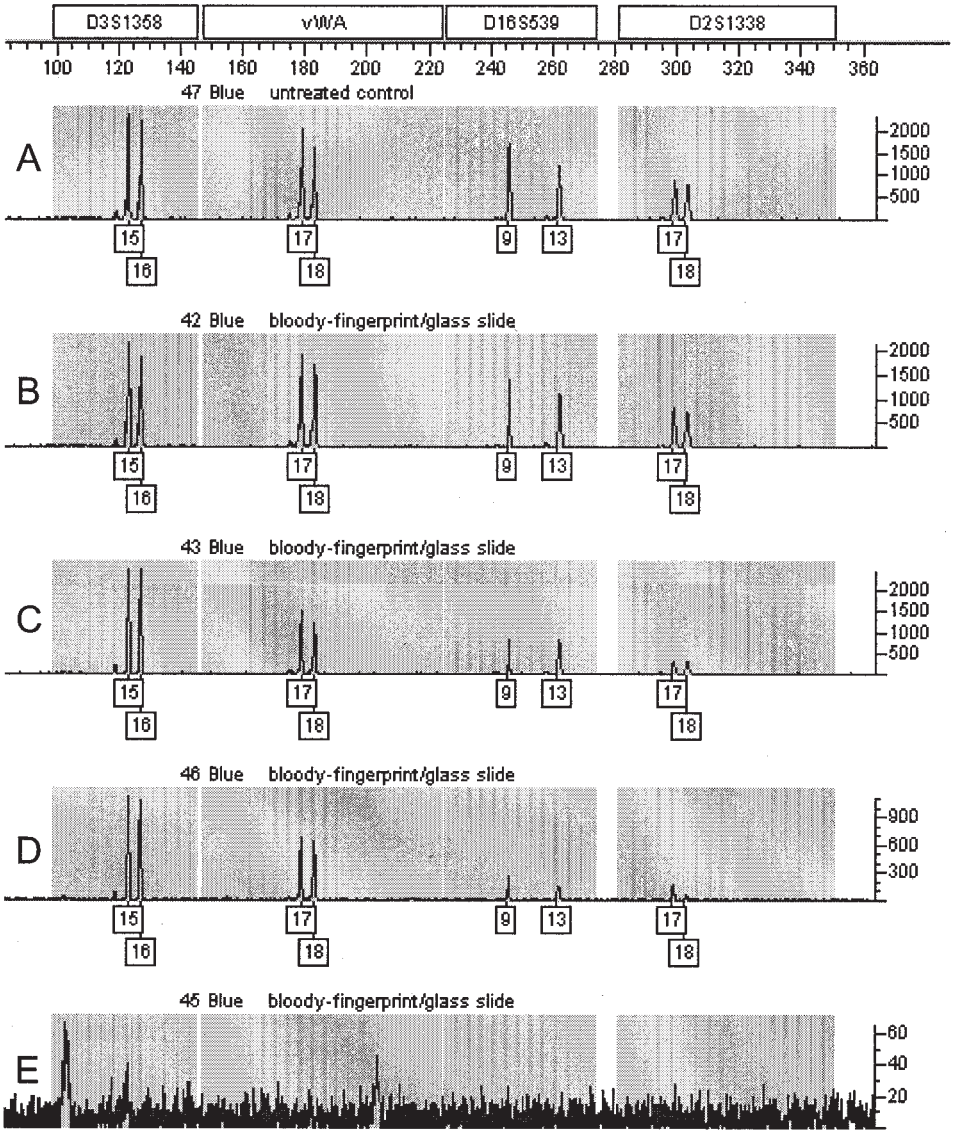

FIG. 3—*Special methods for blood traces/TMB, LCV, LMG: Examples of SGM plus (ABI) electrophoretograms, blue panels (FAM-labeled profiles only); the relative fluorescent intensity (RFU) of each sample is depicted at the right margin, all samples from Individual II*: (A) *untreated control, no enhancement, complete profile*; (B) *bloody fingerprint on glass slide, TMB and air brush technique, complete profile*; (C) *bloody fingerprint on glass slide, LCV and air brush technique, complete profile*; (D) *bloody fingerprint on glass slide, LMG and air brush technique, weak profile*; (E) *bloody fingerprint on glass slide, LMG and air brush technique, no profile.*

since the biological material remained on the investigated surface. Therefore we recommend the implementation of the layer technique, as it brings advantages for fingerprint enhancement as well.

Simulation of Casework Conditions

In a practical casework situation, it can become necessary that two enhancement methods are to be performed on a single stain. We tested bloodstains on blue denim. Visualization and documentation were performed with UV light, DFO, and Leucocrystal violet. DFO was performed using the two different protocols described above. For LCV, the layer technique was used. Both variations did not hamper subsequent STR profiling after purification of the extracted DNA.

Conclusions

In crime-scene casework investigators are sometimes confronted with evidence in which fingerprints are deposited in combination with biological material. In our study we sought to investigate the influence of a variety of dactyloscopic methods on this type of biological stain, which was used in combination with fingermarks on different surfaces. The samples were treated with the dactyloscopic methods first, and the biological traces were subsequently swabbed or cut and subjected to STR profiling. As a general finding, subsequent STR profiling of the blood/saliva traces led to good results after using all the enhancement methods included in this study.

Concerning DFO, it was sometimes necessary to purify the DNA extract using the QIA Quick PCR Purification Kit as no, weak, or partial STR results were obtained with the NaOAc/EtOH precipitation method after Phenol/chloroform extraction. In general, the STR results were weaker performing 60-min incubation at 60°C than 15 min at 100°C. The varying amplification success with DFO-treated samples may be attributed to the longer incubation rather than the absolute temperature. Illustrative examples are given in Fig. 2.

Of the blood enhancement procedures, the airbrush technique showed deleterious effects on subsequent STR analysis in some cases, especially when we used LMG on bloody fingerprints on glass (Fig. 3). TMB on saliva fingerprints on glass and also LCV on saliva on stamps and envelopes did not work every time, but both showed no problems when we used them on their main subject blood traces. When we applied blood-enhancement procedures in combination with the layer technique we could always achieve complete profiles. We therefore recommend the implementation of the layer technique as it brings advantages for the fingerprint enhancement as well.

It could also be shown (which can be necessary in practical casework), that two enhancement methods and additional UV light can be performed on a single stain without having any negative influence on STR profiling.

Comprising we could show—provided that there is a specified amount of biological material—that the crime-scene officer can use most of the enhancement methods without interfering with the ability to perform subsequent DNA profiling. In some cases, where the amount of material is limited, however, caution is needed, as enhancement may derogate the ability of DNA typing.

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