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DNA Typing of Fingerprint Reagent Treated Biological Stains

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ABSTRACT: DNA profiling was performed on bloodstains and saliva on various supports after treatment with commonly used reagents for developing latent fingerprints. The results showed no effect of investigated dactyloscopic methods on DNA typing compared with untreated samples.

KEYWORDS: forensic science, blood, saliva, latent fingerprint, cyanoacrylate ester, ninhydrin, gentian violet, DNA typing, DNA, restriction fragment length, polymorphism, polymerase chain reaction, APOB, D1S80, FESFPS, VWA, CSF1PO, TPOX, THO 1, D5S43, D7S21.

Since the early days of fingerprint analysis for crime investigation, there has been an important progress in personal identification because of application of many modern technologies to the field of forensic science. One of the oldest methods for developing latent fingerprints is brushing very fine powder (amorphous carbon) lightly over the surface supposed to be carrying the fingerprints. Ninhydrin (2,2 dihydroxyindane-1,3-dione) has become the universal reagent for chemical development of latent fingerprints on porous surfaces such as paper (1). It reacts with excreted amino acids in perspiration and becomes a purple colored product. Sensitivity of ninhydrin developed fingerprints can be enhanced by complexation with metal salts followed by laser examination (2–4). A quite effective method to develope prints on smooth nonwhite surfaces such as metals, glass, plastic, fine leather etc. is the fuming with glues containing cyanoacrylate ester. In a fuming chamber, the cyanoacrylate ester of the glue evaporates and polymerizes on the ridges of a fingerprint to form a white product (5). Glue-treated prints can also be efficiently combined with laser examination (6). Gentian violet is used for development of latent fingerprints on adhesive surfaces of postage stamps, envelopes, parcel tape, and for contrasting cyanoacrylate ester developed fingerprints.

The most important development in forensic science probably was the introduction of a technique known by the popular term "genetic fingerprinting." Two basic DNA typing methods are available for characterizing biological evidence: (a) Typing of DNA for variable number of tandem repeat (VNTR) loci by restriction fragment length polymorphism (RFLP) analysis (7), (b) Typing of DNA by increasing the number of copies of a target DNA sequence by amplification using the polymerase chain reaction (PCR) (8).

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In comparison with RFLP analysis, technology based on PCR offers the possibility of typing very small amounts of DNA and degraded DNA respectively so that this technology is an extremly useful tool for analyzing biological material found in a crime scene. Because materials subjected to latent fingerprint analysis also may show biological stains like blood or saliva, it is necessary to know the influence of cyanoacrylate ester fuming and solution dye staining on DNA typing. Shipp et al. (9) reported on successful RFLP-typing on cyanoacrylate and laser treated bloodstains on white cotton clothes. This study was supported by PCR based typing of cyanoacrylate, solution dye (Rhodamine 6G and Ardrox™), alternate source light and argon laser treated bloodstains on razor blade and plastic foil (10).

The present study proposed to examine whether cyanoacrylate ester fuming, dye staining of fingerprints, and biological materials such as blood and saliva on various supports would affect DNA typing results.

Materials and Methods

Samples

Latent fingerprint samples and bloodstains (2 to 20 μL blood) of three individuals were collected on the surface of razor blades and plastic foils for cyanoacrylate fuming, on glass slides for treatment with amorphous carbon, on the sticky surface of an adhesive tape for gentian violet treatment and on groundwood-free white paper for ninhydrin treatment. Saliva samples of two individuals were collected on the back of postage stamps for ninhydrin treatment. Samples were kept at room temperature. One part of the samples serves as untreated controls, the other part serves for developing latent fingerprints at intervals of 1, 14, and 56 days (glass slides 3 days).

Carbon Treatment

Amorphous carbon (Fa. Tilge, Hamburg, Germany) was lightly dusted with a very fine feather brush over the surface of the samples (glass slides).

Cyanoacrylate Treatment

Samples (razor blade = metal, plastic foil) were treated with one component cyanoacrylate adhesive SICOMET (Sichel Werke GmbH, Hannover, Germany) for 35 min at 50°C in a fuming chamber.

Ninhydrin Treatment

Samples (paper, postage stamps) were dipped in ninhydrin dye solution (0, 5% ninhydrin, 4% ethanol, and 96% petrol) for 5 and 10 s respectively.

Gentian Violet Treatment

Samples (sticky surface of adhesive tape) were dipped in dye solution (0, 9% gentian violet, 2% phenole, and 9% ethanol) and rinsed with water.

DNA Extraction

According to the nonorganic method of Grimberg et al. (11), DNA was extracted from whole blood samples serving as controls. DNA from treated, untreated bloodstains, and saliva was extracted by using organic standard method with centricon 100 purification according to Comey et al. (12). An estimate of quality and quantity of recovered DNA was made by agarose gel electrophoresis/ethidiumhromide staining and hybridization to the human alphoid probe D17Z1 by using a slot blot method described by Waye et al. (13).

DNA Typing

For RFLP analysis, 200 to 500 ng DNA were digested overnight with Hinf I (5 U/μg DNA) at 37°C; the completeness of digestion was determined by gelelectrophoresis. Electrophoresis of restricted DNA was carried out in a 0, 7% agarosegel in 1 by TBE at 50 V for 20 h. DNA was transferred to Hybond-N membran (Amersham Buchler GmbH & CoKG, Germany) and hybridized with SLS NICE™ MS 8 and NICE MS 31 (Zeneca Bioproducts, ICI Corp.). 1 to 5 ng DNA were amplified alternatively at loci D1S80 (14), Apo B (15), HumFESFPS (16), HumVWA (17), HumCSF1PO, HumTPOX, and HumTHO 1 (18). Amplifications and typing were performed as described by manufacturer's recommendations (19, 20), for Apo B according to Wiegand et al. (21) and for HumVWA as described by Möller et al. (22).

Results

DNA Extraction

The potential effects of brushing amorphous carbon, cyanoacrylate ester fuming, ninhydrin and gentian violet staining on DNA quantity of bloodstains and saliva respectively on various supports were examined 1, 14, and 56 days after sample preparation (amorphous carbon—three days after sample preparation). Concerning DNA isolation, there were no significant differences in relative amounts of DNA between treated and untreated samples (data not shown). DNA quality was assessed of bloodstains treated with amorphous carbon (on glass), with cyanoacrylate (on metal and plastic), with ninhydrin 5 and 10 s (on paper), with gentian violet (on adhesive tape) by agarose gel electrophoresis. In all cases, high molecular weight DNA could be isolated.

Figure 1 shows an example of DNA quality after treatment of blood with cyanoacrylate, ninhydrin, and gentian violet. As only relative small amounts of DNA could be isolated from saliva on stamps (50 to 80 ng DNA/stamp), we renounced testing DNA quality of these samples by agarose gel electrophoresis.

RFLP Analysis of DNA Exposed to Cyanoacrylate Ester, Ninhydrin and Gentian Violet

RFLP analysis was effected by using 200 to 500 ng Hinf I digested DNA of bloodstains on metal, paper, and adhesive tape treated 14 days after sample preparation with cyanoacrylate ester, ninhydrin, and gentian violet, respectively. Results obtained by hybridization using polymorphic probes MS 8 (D5S43; Fig. 2) and MS 31 (D7S21; data not shown) did not show any apparent

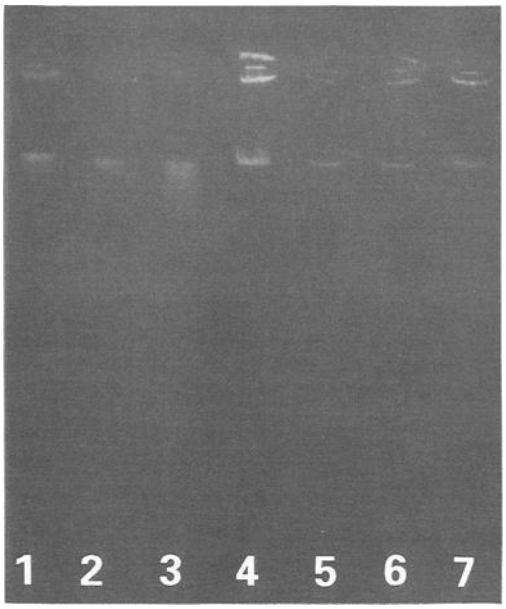

FIG. 1—*Test of DNA quality after treatment of bloodstains with cyanoacrylate ester, ninhydrin, and gentian violet 14 days after sample preparation. Lanes 1 and 7 contain genomic DNA extracted from blood. Lanes 2 and 3 contain DNA of cyanoacrylate ester treated bloodstains on metal and plastic, respectively. In lanes 4 and 5 DNA bloodstains from 5 and 10 s ninhydrin stained paper is shown, and lane 6 contains DNA after exposure to gentian violet.*

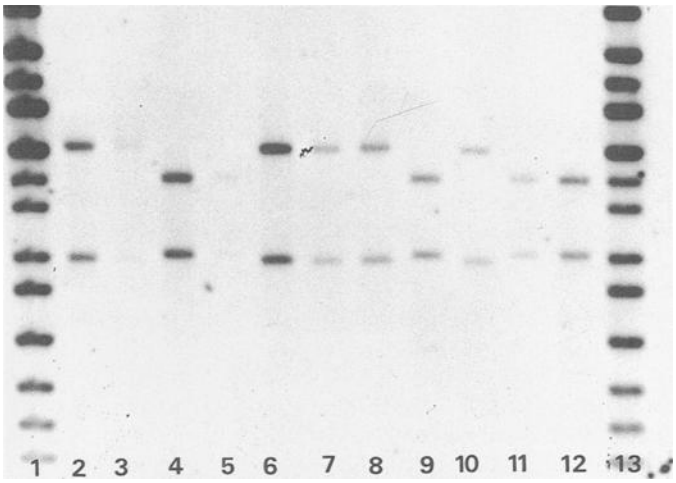

FIG. 2—*RFLP analysis of dactyloscopic treated bloodstains. DNA was extracted from samples exposed to cyanoacrylate ester, ninhydrin, and gentian violet 14 days after sample preparation. Lanes 1 and 13–molecular weight standard GIBCO BRL NICE™ analysis ladder; lanes 2 to 8 and 11 to 12 contain DNA from bloodstains on untreated metal (lane 2), on cyanoacrylate ester treated metal (lane 3), on untreated plastic (lane 4), on cyanoacrylate ester treated plastic (lane 5), on untreated paper (lane 6), on 5 s ninhydrin stained paper (lane 7), on 10 s ninhydrin stained paper (lane 8), on the untreated sticky surface of adhesive tape (lane 11), and on gentian violet treated adhesive tape (lane 12). Lane 9 contains DNA isolated from blood of individual 1 (5,91/4,73 kb), and lane 10 DNA of individual 2 (6,46/4,67 kb).*

differences in allelic patterns between treated and untreated samples or rather genomic DNA extracted from blood.

PCR Based Typing of Bloodstains Treated with Amorphous Carbon

DNA of blood on glass slides brushed with amorphous carbon was amplified at loci D1S80, Apo B, CSF1PO, TPOX, THO 1, VWA, and FES. Typing results showed no effect of amorphous carbon on analysis with STR systems (Fig. 3, VWA, FES data not shown) AMPFLP (Figs. 4c, 5a).

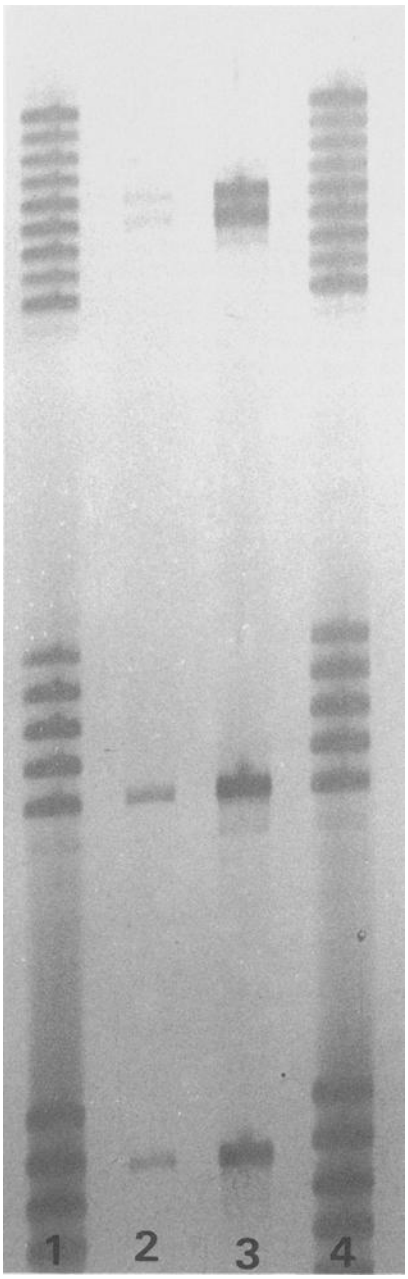

FIG. 3—*Effect of amorphous carbon on DNA typing. DNA of bloodstains on untreated glass and glass treated with amorphous carbon was amplified and typed at STR-loci CSF1PO, TPOX, and THO 1. Lanes 1 and 4 contain allelic ladders, lane 2 DNA of bloodstains on untreated glass, lane 3 DNA on glass treated with amorphous carbon (CSF1PO type 11/12; TPOX type 8/8; THO 1 type 6/9.3).*

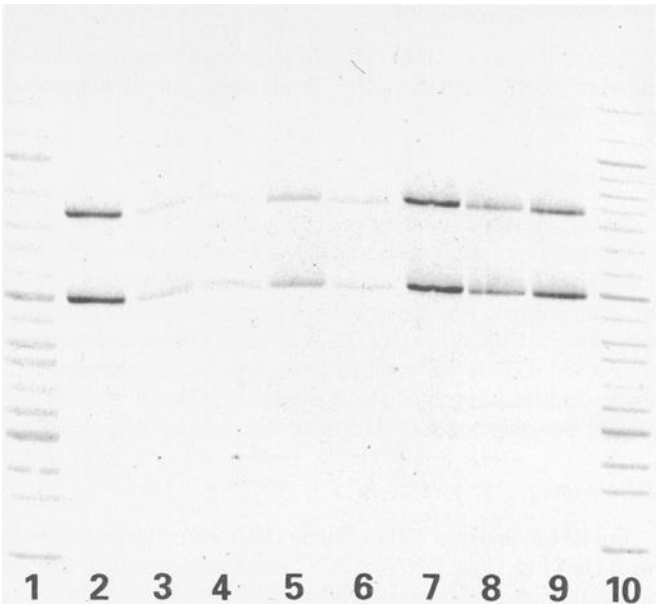

FIG. 4—*Effects of amorphous carbon, cyanoacrylate ester, ninhydrin, and gentian violet on DNA typing of bloodstains and saliva. DNA was amplified and typed at the locus D1S80. Figure 4a—Lanes 1 and 10–D1S80 allelic ladder, lane 2–genomic DNA extracted from blood (type 24/28), lane 3–DNA of bloodstains on untreated plastic, lanes 4, 5, and 6–DNA of bloodstains on cyanoacrylate ester treated plastic (treatment 1 day, 14 days, and 56 days after sample preparation), lane 7–DNA of bloodstains on untreated metal, lanes 8 and 9–DNA of bloodstains on cyanoacrylate ester treated metal (14 and 56 days after sample preparation).*

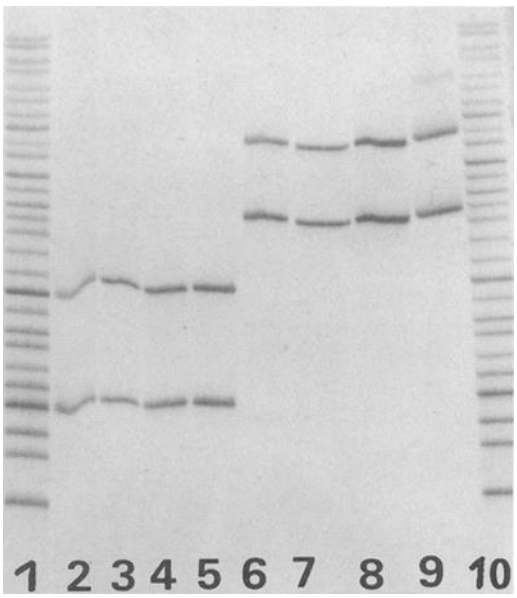

FIG. 4b—*Lane 1 and 10-D1S80 allelic ladder, lane 2-genomic DNA isolated from blood (type 18/24), lane 3-DNA extracted from saliva on untreated postage stamp, lanes 4 and 5-DNA from saliva on ninhydrin stained postage stamps (5 and 10 s staining 14 days after sample preparation), lane 6–genomic DNA isolated from blood (type 28/33), lane 7–DNA of bloodstains on untreated paper, lanes 8 and 9–DNA of bloodstains on ninhydrin stained paper (5 and 10 s staining 14 days after sample preparation).*

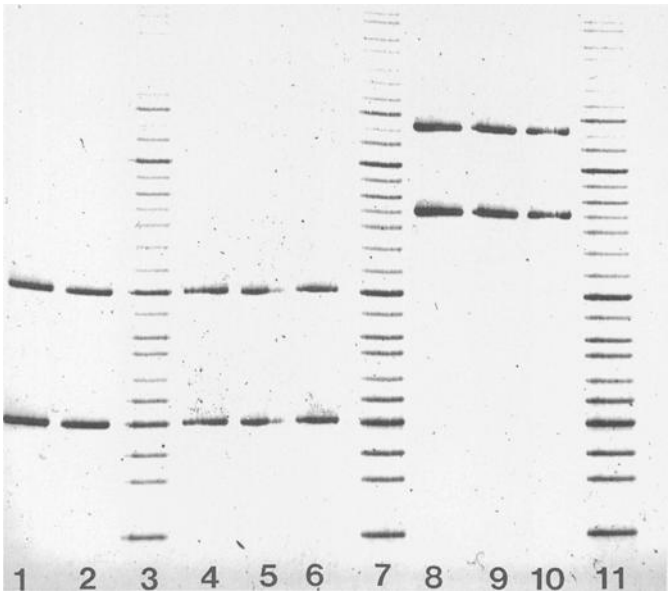

FIG. 4c—*Lanes 3, 7, and 11–D1S80 allelic ladder, lane 1–genomic DNA isolated from blood (type 18/24), lane 2–DNA of bloodstains on untreated adhesive tape (56 days after sample preparation), lanes 4, 5, and 6–bloodstains on gentian violet treated adhesive tape (1, 14, and 56 days after sample preparation), lane 8–genomic DNA from blood (type 28/33), lanes 9 and 10–DNA of bloodstains on untreated glass and glass brushed with amorphous carbon.*

PCR Based Typing of DNA After Exposure to Cyanoacrylate Ester, Ninhydrin and Gentian Violet

Two AMPFLP systems (D1S80, Apo B) and three STR systems (THO 1, FESFPS, VWA) were used for studying potential effects of cyanoacrylate ester fuming on bloodstains applicated to metal, plastic, and ninhydrin staining (5 and 10 s, respectively) of bloodstains on paper and saliva on the back of postage stamps, as well as gentian violet staining of bloodstains on sticky surfaces of adhesive tapes. Figures 4a–c and Fig(s). 5b–d show identical typing results of dactyloscopic treated samples compared with untreated samples and genomic DNA from blood at loci D1S80 and Apo B. The same results were obtained in the STR systems (data not shown). Even storage of samples at room temperature for 1, 14, and 56 days before dactyloscopic analysis had no effect on DNA typing.

The apparent decrease of amplification products in Fig. 5c (saliva, ninhydrin treatment, and Apo B) could neither be confirmed in other Apo B gels nor in the AMPFLP D1S80 and the STR systems. A summary of results is shown in Table 1.

Discussion

For development of latent fingerprints, cyanoacrylate ester fuming and staining with dyes like ninhydrin and gentian violet are widely used methods. Amorphous carbon as one of the oldest methods has lost its importance for the daily routine. In practice of criminal investigations, offence tools often show fingerprints and biological stains simultaneously. For optimal personal identification in such cases, it is important to know the influence of procedures for developing latent fingerprints on examination of biological stains. The present study proposed to examine the possibility of typing biological stains such as blood and saliva on various surfaces found in a crime scene (glass, metal, paper, adhesive tape, and plastic), after development of fingerprints with commonly used methods. The examination intervals of 1, 14, and 56 days were determined according to the fact that for most of crime cases, latent fingerprints are investigated within 14 days after the crime, and most of the remaining latent fingerprints within 56 days after the crime. The presented results of this examination showed no influence of used chemicals on DNA extraction, DNA quality, and

TABLE 1—*Dactyloscopic treatment and DNA typing of biological stains.*

Dactyloscopic treatment	Biological material	Support	Storage (days)	DNA quality	RFLP	AMPFLP*	STR†
Cyanoacrylate	blood	plastic	1	HMW‡	–	+	+
			14	HMW	+‖	+	+
			56	HMW	–	+	+
	blood	metal	1	HMW	–	+	+
			14	HMW	+	+	+
			56	HMW	–	+	+
Gentian violet	blood	adhesive tape	1	HMW	–	+	+
			14	HMW	+	+	+
			56	HMW	–	+	+
Ninhydrin 5/10 in.	blood	paper	1	HMW	–	+	+
			14	HMW	+	+	+
			56	HMW	–	+	+
	saliva	stamp	1	–	–	+	+
			14	–	–	+	+
			56	–	–	+	+
Amorphous carbon	blood	glass	3	HMW	–	+	+

*AMPFLP: DIS80, APo B.
†STR : CSF1PO, TPOK, THO 1; FBSFPS, VWA.
‡High molecular weight DNA.
§not tested.
‖+ Successful typing.

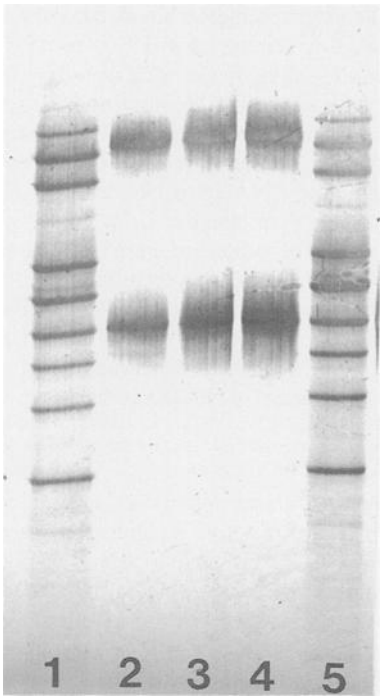

FIG. 5—*Effects of amorphous carbon, ninhydrin, and gentian violet on DNA typing at the locus Apo B. Figure 5a—Lanes 1 and 5–Apo B allelic ladder, lane 2–genomic DNA from blood (type 37/49), lanes 3 and 4–DNA of bloodstains on untreated glass and glass brushed with amorphous carbon (treatment 3 days after sample preparation).*

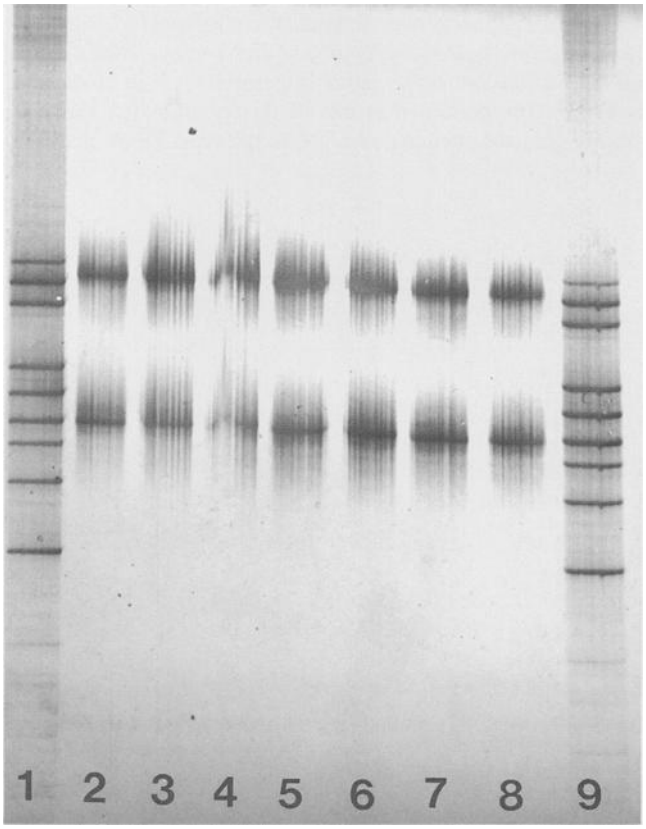

FIG. 5b—*Lanes 1 and 9–Apo B allelic ladder, lane 2–genomic DNA isolated from blood (type 37/49), lanes 3 to 8–DNA of bloodstains isolated from untreated and ninhydrin treated (10 s) paper, lanes 3 and 4–treatment 1 d after sample preparation, lanes 5 and 6–treatment 14 days after sample preparation, lanes 7 and 8–treatment 56 days after sample preparation.*

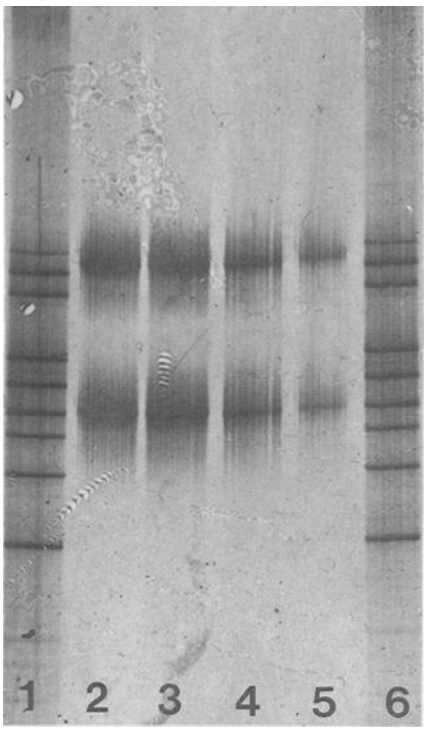

FIG. 5c—*Lanes 1 and 6–Apo B allelic ladder, lane 2–DNA isolated from saliva (type 37/49) on untreated stamp (storage 56 days), lanes 3 to 5–DNA isolated from saliva on ninhydrin treated (10 s) stamps, treatment 1, 14, and 56 days after sample preparation.*

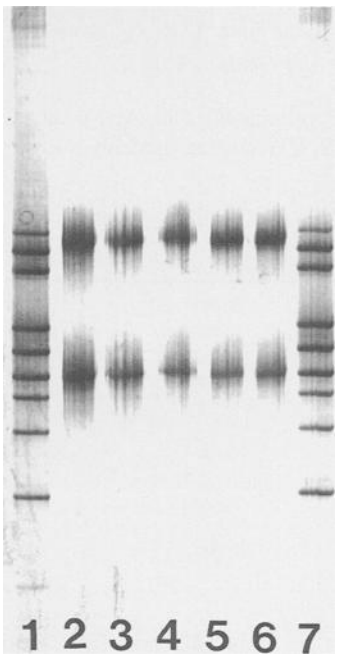

FIG. 5d—*Lanes 1 and 7–Apo B allelic ladder, lane 2–genomic DNA isolated from blood (type 37/49), lane 3–DNA of bloodstains on untreated adhesive tape (storage 56 days), lanes 4 to 6–DNA of bloodstains on gentian violet treated adhesive tape, treatment 1, 14, and 56 days after sample preparation.*

DNA typing of samples subjected to dry storage at room temperature. In comparison with untreated samples, amplified fragments of treated samples could not reveal any mobility shift. Results of this study could confirm and complete previously published data of Shipp et al. (9) and Hochmeister et al. (10) who reported on successful typing of cyanoacrylate treated and laser examined bloodstains. Consequently, the development of fingerprints as an initial criminalistics examination is practicable without any impairment of the DNA typing of biological stains.

Acknowledgments

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