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Fingerprint Enhancement Revisited and the Effects of Blood Enhancement Chemicals on Subsequent *Profiler Plus*™ Fluorescent Short Tandem Repeat DNA Analysis of Fresh and Aged Bloody Fingerprints

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ABSTRACT: This study was aimed at determining the effect of seven blood enhancement reagents on the subsequent *Profiler Plus*™ fluorescent STR DNA analysis of fresh or aged bloody fingerprints deposited on various porous and nonporous surfaces. Amido Black, Crowle's Double Stain, 1,8-diazafluoren-9-one (DFO), Hungarian Red, leucomalachite green, luminol and ninhydrin were tested on linoleum, glass, metal, wood (pine, painted white), clothing (85% polyester/15% cotton, 65% polyester/35% cotton, and blue denim) and paper (Scott® 2-ply and Xerox-grade). Preliminary experiments were designed to determine the optimal blood dilutions to use to ensure a DNA typing result following chemical enhancement. A 1:200 blood dilution deposited on linoleum and enhanced with Crowle's Double Stain generated enough DNA for one to two rounds of *Profiler Plus*™ PCR amplification. A comparative study of the DNA yields before and after treatment indicated that the quantity of DNA recovered from bloody fingerprints following enhancement was reduced by a factor of 2 to 12. Such a reduction in the DNA yields could potentially compromise DNA typing analysis in the case of small stains. The blood enhancement chemicals selected were also evaluated for their capability to reveal bloodmarks on the various porous and nonporous surfaces chosen in this study. Luminol, Amido Black and Crowle's Double Stain showed the highest sensitivity of all seven chemicals tested and revealed highly diluted (1:200) bloody fingerprints. Both luminol and Amido Black produced excellent results on both porous and nonporous surfaces, but Crowle's Double Stain failed to produce any results on porous substrates. Hungarian Red, DFO, leucomalachite green and ninhydrin showed lower sensitivities. Enhancement of bloodmarks using any of the chemicals selected, and short-term exposure to these same chemicals (i.e., less than 54 days), had no adverse effects on the PCR amplification of the nine STR systems surveyed (D3S1358, HumvWA, HumFGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820) or of the gender determination marker Amelogenin. The intensity of the fluorescent signals was very similar and the allele size measurements remained constant and identical to those of untreated bloody fingerprints. No additional background fluorescence was noted. Continuous exposure (for 54 days) to two of the seven enhancement chemicals selected (i.e., Crowle's Double Stain and Hungarian Red) slightly reduced the amplification efficiency of the longer STR loci in profiles of fresh and 7 to 14-day-old bloodprints. This suggests that long-term exposure to these chemicals possibly affects the integrity of the DNA molecules. This study indicates that significant evidence can be obtained from fresh or aged bloody fingerprints applied to a variety of absorbent and nonabsorbent surfaces which are exposed to different enhancement chemicals for short or long periods of time. It also reaffirms that PCR STR DNA typing procedures are robust and provide excellent results when used in concert with fluorescence-based detection assays after fingerprint identification has taken place.

KEYWORDS: forensic science, fingermark, fingerprint, blood enhancement chemicals, short tandem repeat (STR), multiplex, fluorescence, polymerase chain reaction (PCR), sequencer, DNA analysis, presumptive test reagents, Amido Black, Crowle's Double Stain, 1,8-diazafluoren-9-one (DFO), Hungarian Red, leucomalachite green, luminol, ninhydrin

Fingerprint detection and analysis for individual identification has undergone tremendous changes since its introduction to the investigation of crimes in 1892. In the beginning, latent fingerprints were revealed by dusting with powder such as amorphous carbon, fuming with iodine vapor, or using silver nitrate (1,2). Later on, chemicals such as ninhydrin, capable of developing latent fingerprints on paper, were identified (3). More research culminated in the characterization and development of many different methods to chemically reveal and further enhance latent fingerprints on a variety of porous and nonporous surfaces. The use of ninhydrin analogues (4), a combination of ninhydrin and trypsin (5), ninhydrin and metal salts (6,7), glues containing cyanoacrylate ester (8,9), different types of lasers (10,11) in combination with fluorescent dyes, luminescent dusting powders or conventional latent print enhancing chemicals (9,12–15) are some of the more recent developments in latent fingerprint identification. In parallel to these advancements, progress was recorded in the field of serology where a number of effective reagents were identified and used to detect the possible presence of blood on a variety of substrates at a crime scene or in the laboratory. Interestingly, luminol, which was first utilized in 1939 as a screening test for blood (16), remains one of the most popular compounds for blood detection along with phenolphthalein, leucomalachite green, and tetramethylbenzidine (17–26). Other protein stains such as Amido Black, leucocrystal violet, Coomassie Brilliant Blue R250, Crowle's Double Stain, DFO

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and Hungarian Red can also be included in the list of chemicals currently employed as good indicators for the possible presence of blood (26–31). Benzidine and ortho-tolidine, routinely used in the early days of fingerprint analysis, have been banned by many laboratories because of their potential carcinogenic properties. Since fingerprints in blood were frequently encountered at crime scenes, it became pertinent to determine the effects of enhancing chemicals or latent fingerprint detection procedures on subsequent serological tests to be performed on the exhibits collected. Earlier reports demonstrated that the direct treatment of dried bloodstains with several presumptive test reagents or with fingerprint enhancing chemicals could have significant detrimental and destructive effects on subsequent serological tests using ABO typing or polymorphic enzymes as genetic markers (2,32–34). More recent investigations using modern technologies to analyze body fluid stains, such as the restriction fragment length polymorphism (RFLP) variable number of tandem repeat (VNTR) analysis (35–37), have indicated that some presumptive test reagents (e.g., silver nitrate, benzidine dissolved in glacial acetic acid, leucomalachite green, *o*-tolidine) could have an adverse effect on the recovery of high molecular weight DNA which could compromise typing results (32,38). In contrast, successful RFLP DNA typing was achieved from bloodstains on white cotton cloth exposed to laser light, alternate light sources (i.e., nonlaser high-energy light sources such as Omniprint 1000), cyanoacrylate ester ("Super Glue") fuming, acetone, iodine fumes, Rhodamine 6G and crystal violet (32,39). Hochmeister et al. (38) also reported successful RFLP typing from blood deposited on glass and 100% cotton substrates treated with luminol, benzidine dissolved in ethanol, and phenolphthalein. A study by Stein et al. (40) indicated that exposure to cyanoacrylate ester fuming, ninhydrin, and gentian violet for up to 14 days did not have any deleterious effect on the suitability of bloodstains on metal, paper and adhesive tape for typing using the RFLP procedure. The advent of the polymerase chain reaction (PCR; 41) targetting smaller size range VNTR loci such as the short tandem repeats (STRs; 42–44) offers new and more sensitive strategies for the analysis of challenging samples found at crime scenes. Smaller size samples can be used for typing and DNA profiles have been generated from highly degraded material (45–51).

One of the latest developments in PCR STR DNA typing technology has been the simultaneous PCR amplification of multiple STR loci in a single reaction tube (i.e., multiplex reaction), further reducing the quantity of genetic material required for a DNA analysis (52–55). This major advancement combined with the highly sensitive four-color fluorescence-based detection technology, has enabled reliable identifications from challenging forensic specimens presenting minute amounts of genetic material (56–59). Three independent research groups have investigated the effects of enhancement reagents on subsequent PCR-based typing of treated bloodstains. Hochmeister et al. (60) reported successful PCR-based typing of bloodstains on razor blades and plastic foil that had been enhanced using cyanoacrylate, Rhodamine 6G and Ardrox™, alternate light source and argon laser. Stein et al. (40) tested amorphous carbon on glass slides, cyanoacrylate on razor blades and plastic foils, gentian violet on sticky surfaces of adhesive tapes, and ninhydrin on white paper; no deleterious effects on the subsequent analysis of STRs were noted even 56 days post-treatment. A study by Andersen and Bramble (61) focused on the effects of fingermark enhancement light sources on PCR STR DNA analysis of fresh blood smears. These authors found that four of the five light sources had no appreciable effect on the PCR analysis. However, exposure of the bloodstains to shortwave UV light for more than 30 seconds had an adverse effect on the recovery of DNA which was clearly evident from the lack of significant PCR typing results.

To complement these studies and expand the spectrum of substrates and blood enhancement reagents examined in previous investigations, as well as take advantage of the latest developments in the field of DNA profiling, seven enhancement chemicals (Amido Black, Crowle's Double Stain, DFO, Hungarian Red, leucomalachite green, luminol and ninhydrin) were tested on bloodstains and blood drops applied to nonporous (linoleum, glass and metal) and porous substrates [wood (pine, painted white), clothing (85% polyester/15% cotton; 65% polyester/35% cotton; blue denim) and paper (Scott® 2-ply; Xerox-grade)]. The first phase of the study established the optimal blood dilutions required to obtain sufficient genetic material to ensure typing results in the subsequent phases of the study. In addition, Phase 1 evaluated the effect of one preselected chemical enhancement agent (e.g., Crowle's Double Stain) on the DNA yield from bloodstains deposited on a nonporous surface such as linoleum. The second phase of the study defined the limit of blood detection of the seven enhancement chemicals applied to various porous and nonporous surfaces. The short-term and long-term exposure effects of enhancement on the subsequent *Profiler Plus*™ fluorescent STR DNA analysis of fresh and aged bloody fingerprints were determined in the third and fourth phases of the study, respectively. This recently developed commercial multiplex amplification system (62) surveys nine STR loci simultaneously consisting of D3S1358, HumvWA, HumFGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820, as well as the gender determination marker, Amelogenin.

Materials and Methods

Substrates—The selection of substrates for this study was based on those most commonly encountered at crime scenes. This included linoleum, glass and metal as the nonporous surfaces and wood (pine, painted white), clothing (85% polyester/15% cotton; 65% polyester/35% cotton; blue denim) and paper (Scott® 2-ply, Xerox-grade) as the porous substrates. In Phase 1, linoleum was selected from all surfaces because of its nonporous nature which, in combination with Crowle's Double Stain, represented one of the worst scenarios for recovering DNA from bloody fingerprints. In Phase 2 of the study, seven of the nine substrates were evaluated; the 65% polyester/35% cotton blend fabric and the Xerox-grade paper were not used. Phases 3 and 4 tested five of the nine selected substrates: linoleum, glass, wood (pine, painted white), clothing (65% polyester/35% cotton) and paper (Xerox-grade).

All selected substrates were cut in a 15 × 8 cm size and each surface was cleaned, whenever possible, using ethanol prior to the application of blood.

Blood Samples—Blood samples from two individuals (one female, one male) were collected in 7 mL Vacutainers™ (containing the anticoagulant EDTA). The blood from the female individual is referred to as blood A and the blood from the male individual as blood B. Blood was applied to the various surfaces as drops or fingerprints; the aliquots used in each phase of the project are listed in Table 1. Dilutions of whole blood were prepared using filtered, autoclaved and deionized (FAD) water. Using an Eppendorf pipet tip, the bloodprints were made by placing the undiluted or diluted blood of donors A or B on a fingertip, cleaned with ethanol, making an effort to spread the blood over the surface of the fingertip. Then by applying some pressure on the fingertip the blood was

TABLE 1—*Quantities and dilutions of blood used in this study.*

Phase of Study	Quantities of Blood Used for Making Drops or Fingerprints	Tested Dilutions
1 A	20 μL; blood drops	undiluted, 1:2, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200
1 B	5 μL, 10 μL, 15 μL 20 μL; bloodprints	undiluted, 1:10, 1:50
1 C	20 μL; bloodprints	undiluted, 1:2, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200
2 A	20 μL; blood drops	undiluted, 1:2, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200
2 B	20 μL; bloodprints	undiluted, 1:2, 1:5, 1:10, 1:20, 1:50, 1:100, 1:200
3	10 μL; bloodprints	undiluted, 1:20
4	10 μL; bloodprints	undiluted

TABLE 2—*Substrates and blood enhancement regimen used in each phase of the project.*

Phase	Substrate	Blood Enhancement Chemical
1	Linoleum Clear glass Metal White painted wood Blue denim 85% polyester/15% cotton Paper towel (Scott® 2-ply)	Crowle's Double Stain
2	Linoleum Clear glass Metal White painted wood Blue denim 85% polyester/15% cotton Paper towel (Scott® 2-ply)	Amido Black, Crowle's Double Stain, Hungarian Red, leucomalachite green, luminol
	Blue denim 85% polyester/15% cotton Paper towel (Scott® 2-ply)	1,8-diazafluoren-9-one, ninhydrin
3 & 4	Linoleum Clear glass White painted wood	Amido Black, Crowle's Double Stain, Hungarian Red, leucomalachite green, luminol
	65% polyester/35% cotton	luminol
	White paper (Xerox-grade)	1,8-diazafluoren-9-one, ninhydrin

transferred onto each different surface. The bloodprints were dried overnight at room temperature, in a laminar flow hood, then were immediately subjected to enhancement procedures or were left at room temperature on a bench or in the laminar flow hood for further periods of time before enhancement (see Phase 4).

Enhancement of Blood Drops or Bloody Fingerprints—Detection and enhancement of blood drops and fingerprints mixed with blood were performed using seven different chemicals. Amido Black (methanol based), Crowle's Double Stain, Hungarian Red (fuchsin acid), leucomalachite green and luminol were tested on seven of the nine surfaces selected (Table 2); the 65% polyester/35% cotton clothing and Xerox-grade paper were not treated with these blood reagents. Ninhydrin and 1,8-diazafluoren-9-one were used solely on the porous surfaces (i.e., blue denim, 85% polyester/15% cotton clothing, and paper [Scott® 2-ply and Xerox-grade]).

The preparation of each reagent and components of the staining and destaining (washing) solutions are detailed in Table 3. Experiments were designed to mimic procedures adopted by the Royal Canadian Mounted Police (RCMP) forensic identification officers and, as none of these protocols use fixative solutions in the field, the bloodmarks were not fixed to the substrate prior to enhancement (63). The blood drops or bloody fingerprints were treated for 1 to 5 min with the staining solution (depending on the surface/reagent combination), using a dropper for application in the case of Amido Black, Crowle's Double Stain and Hungarian Red, a spray bottle for leucomalachite green and luminol, or immersing the substrate in solution in a beaker for DFO and ninhydrin. In the case of Amido Black, Crowle's Double Stain and Hungarian Red, an identical dropper was used to apply a destaining solution to eliminate the background staining and better reveal the bloodmarks.

In Phases 1, 2 and 3 of the project, treatment of the blood drops or bloody fingerprints with the reagents was performed immediately after they were allowed to dry. In Phase 4, the bloodprints were allowed to dry for different periods of time (overnight, 7 days and 14 days) prior to their chemical treatment. All enhanced blood drops or bloody fingerprints were left to dry at room temperature in a laminar flow hood for a few minutes up to 18 hours prior to being photographed. Bloodprints were then visually inspected to assess the limit of detection of each chemical reagent (Phase 2) or were directly processed for DNA extraction (Phases 1, 3 and 4).

DNA Extraction—Dried bloodprints (3 × 1.5 cm) on linoleum, glass, metal or painted wood were swabbed with 1 cm^2 pieces of VWR 238 blotting paper (VWR Scientific, Ville Mont-Royal, Québec; manufactured by Ahlström Filtration Inc., Mt. Holly Springs, PA) moistened with FAD water. For clothing, denim or paper, bloodprints were cut into 1 cm^2 pieces of material. The entire bloodprints were used up in the process. All samples were subjected to a one-step organic DNA extraction protocol (64). Essentially, the swabs or the cuttings (up to three pieces per bloodprint in certain cases) were incubated at 56°C for a minimum of 6 h and a maximum of 18 h in the presence of stain extraction buffer (10 mM Tris, pH 8.0, 10 mM EDTA, 100 mM NaCl, 40 mM DTT, 2% SDS) and proteinase K solution (20 mg/mL). Following centrifugation, the stain extract was mixed with equal volumes of phenol and chloroform/isoamyl alcohol 24:1 (v/v). The aqueous phase containing the DNA was then transferred to a membrane-based microconcentrator device (Microcon®-100, cut off at 100 basepairs or 300 bases for DNA/RNA; Amicon® Inc., Beverly, MA) for further purification and concentration following recommendations by the manufacturer. DNA extracts in a final volume of 50 μL were stored at 4°C until required.

DNA Quantitation—Quantitation of human genomic DNA extracted from blood drops or bloodprints was determined using a chemiluminescence-based detection slot blot hybridization procedure (65). A biotinylated primate-specific D17Z1 α-satellite probe was used to hybridize to the "unknown" and reference samples (i.e., twofold serial dilutions of the control cell line GM9947A, (66; NIST Standard Reference Material #2391 PCR-based DNA Profiling Kit), immobilized on the membrane.

PCR Primers—The *Profiler Plus*™ PCR amplification multiplex recently developed by Applied Biosystems Division of Perkin Elmer and evaluated in Phases 3 and 4 of this study, is comprised of nine STR systems and the gender determination system, Amelogenin. These genetic markers are listed in Table 4 and are pre-

TABLE 3—*Enhancement chemicals and their preparation.*

Blood Enhancement Reagent	Recipe
Amido Black	Staining: 0.2 g Amido Black (Naphthalene Black 12B; BDH Chemicals) 10 mL glacial acetic acid 90 mL methanol Destaining: 90 mL methanol, 10 mL glacial acetic acid
Crowle's Double Stain	Staining: 2.5 g Crocein Scarlet 7B (Brilliant Crocein; Aldrich Chemical) 150 mg Coomassie Brilliant Blue R250 (Merck) 50 mL glacial acetic acid 30 mL trichloroacetic acid Diluted to 1 L with distilled water Destaining: 3 mL glacial acetic acid in 1 L of distilled water
DFO (1,8-diazafluoren-9-one)	Staining: 0.25 g DFO (Lumichem) 20 mL glacial acetic acid 100 mL ethanol Complete to 1 L with heptane
Hungarian Red	Staining: Commercial solution of Hungarian Red (BVDA International) Glacial acetic acid Sulfosalicylic acid Destaining: distilled water/glacial acetic acid (19:1 v/v)
Leucomalachite green	Staining: 0.2 g leucomalachite green (Aldrich Chemical Co.) 0.67 g sodium perborate 66.6 mL methanol 33.3 mL glacial acetic acid 300 mL freon (1-1-2-trichlorotrifluorethane) The reagent has no shelf life and must be prepared just prior to use.
Luminol	Staining: 0.5 g luminol (Aldrich Chemical Co.) 25 g sodium carbonate 500 mL distilled water 3.5 g sodium perborate (added immediately prior to use) The reagent has no shelf life and must be prepared just prior to use.
Ninhydrin	Staining: Ninhydrin stock solution: 25 g ninhydrin (Lumichem) 50 mL glacial acetic acid 100 mL ethanol Ninhydrin working solution: 30 mL stock solution 50 mL ethanol Complete to 1 L with heptane

TABLE 4—*Genetic markers surveyed in the study.*

Locus Designation	Chromosome Location	Common Sequence Motif	Size Range (bases)*	Dye Label†
D3S1358	3p	$TCTA(TCTG)_{1-3}(TCTA)_n$	113–144	FAM (B)
HumvWA‡	12p12-pter	$TCTA(TCTG)_{3-4}(TCTA)_n$	156–198	FAM (B)
HumFGA‡	4q28	$(TTTC)_3\ TTTTTTCT(CTTT)_nCTCC(TTCC)_2$	218–265	FAM (B)
Amelogenin	X: p22.1-p22.3	N/A	106	JOE (G)
	Y: p11.2	N/A	112	
D8S1179§	8	$(TCTR)_n$‖	126–169	JOE (G)
D21S11	21q21	$(TCTA)_n(TCTG)_n[(TCTA)_3TA(TCTA)_3TCA(TCTA)_2TCCATA](TCTA)_n$	188–245	JOE (G)
D18S51	18q21.3	$(AGAA)_n$	273–343	JOE (G)
D5S818	5q21-q31	$(AGAT)_n$	134–172	NED (Y)
D13S317	13q22-q31	$(GATA)_n$	206–236	NED (Y)
D7S820	7q	$(GATA)_n$	258–295	NED (Y)

* As defined by the AmpFℓSTR Profiler Plus™ Allelic Ladder. Includes the 3′ nucleotide addition (n + 1).

† FAM: 5-carboxyfluorescein (absorbance at 495 nm, emission at 525 nm); JOE: 2′,7′-dimethoxy-4′,5′-dichloro-6-carboxyfluorescein (525 nm, 555nm); NED: proprietary. Letters in parentheses correspond to the color of product: B = blue, G = green, Y = yellow.

‡ vWA31/A, von Willebrand factor gene; FGA (FIBRA), alpha fibrinogen.

§ In some literature references, this locus is designated as D6S502.

‖ R can represent either an A or G nucleotide.

sented according to their locus designations, chromosomal locations, repeat units, amplicon size ranges and dye labels. Due to pending patent, the primer sequences remain proprietary to the company and are unavailable for publication.

Amplification Conditions—Simultaneous amplification of the nine STR systems described in Table 4 as well as the gender determination marker, Amelogenin, was conducted in a 25 μL final reaction volume containing 1 ng to 2.5 ng of genomic DNA (in a total volume of 10 μL with FAD water completing the volume), 9.5 μL of the AmpFℓSTR PCR Reaction Mix, 5 μL of the AmpFℓSTR *Profiler Plus*™ Primer Set Solution and 0.5 μL of AmpliTaq Gold™ DNA Polymerase (5 U/μL stock). The reaction mixtures were subjected to a hot start at 95°C for 11 min to activate the AmpliTaq Gold™ DNA Polymerase. Amplifications were carried out for 28 cycles using the following parameters: denaturation for 60 s at 94°C, annealing of primers for 90 s at 59°C and extension for 90 s at 72°C. A final extension at 60°C for 45 min, followed by an overnight incubation at room temperature, were also included as these conditions were found necessary to promote the 3′ terminal transferase activity of the AmpliTaq Gold™ DNA Polymerase. All amplifications were conducted in a Perkin Elmer GeneAmp™ PCR System 9600 thermal cycler using thin-walled 0.2 mL MicroAmp™ Reaction Tubes.

The control cell line GM9947A (66; NIST Standard Reference Material #2391 PCR-based DNA Profiling Kit) served as the positive amplification control and FAD water as the negative amplification control.

Analysis of Amplification Products—Analysis of the *Profiler Plus*™ fluorescent amplified products was performed as follows: an aliquot of 2 μL of each PCR reaction was mixed with 0.5 μL of ABI GeneScan™-350 Internal Lane Size Standard (labeled with 6-carboxy-X-rhodamine [ROX, a fluorescent dye from ABI]) and 4 μL of denaturing loading buffer (20 mg/mL blue dextran, 7.3 M urea, 2X TBE, 20 mM EDTA). Following denaturation at 95°C for 2 to 3 min, samples were snap-cooled in ice cold water and 1.6 μL aliquots were loaded on a 4% (19:1) acrylamide:bisacrylamide gel containing 6 M urea (36 cm well-to-read glass plate format), which had been prerun at constant voltage (1000 V) for 30 min and equilibrated to 51°C. Electrophoresis was conducted for 2 h at constant voltage (3000 V) in 1X TBE using an ABI PRISM® 377 DNA Sequencer with the laser set at 40 mW. Allele sizes were determined using the GeneScan® Analysis v.2.1 software and the Local Southern size calling method. Automatic allele designation was achieved using the Genotyper® v.2.1 software (Applied Biosystems Division of Perkin Elmer).

Experimental Designs

Phase 1—A comparative study was undertaken to determine the yield of DNA retrievable from blood drops and bloodprints (prepared with different concentrations of blood) before and after enhancement. One series of blood dilutions from individual B was prepared in FAD water and ranged in concentration from 1:2 to 1:200. Aliquots of 20 μL of these dilutions were then deposited as drops on linoleum and allowed to air dry at room temperature prior to collection and processing for DNA extraction and quantitation. A series of bloody fingerprints was produced on linoleum using different aliquots (5,10,15, and 20 μL) of undiluted blood from individual B, as well as two different blood dilutions (1:10 and 1:50). Fingerprints were left to air dry at room temperature before being processed for DNA extraction and DNA quantitation. Finally, a series of bloody fingerprints was prepared using 20 μL aliquots of diluted blood from individual B (dilutions ranging from 1:2 to 1:200) and was applied to linoleum, allowed to air dry completely at room temperature prior to enhancement with Crowle's Double Stain. Following treatment with Crowle's Double Stain, the bloody fingerprints were swabbed and processed for DNA extraction and quantitation as outlined in previous sections.

Linoleum and Crowle's Double Stain were selected from all possible substrate/chemical combinations because this combination represented one of the most challenging scenarios for recovering DNA from bloody fingerprints. Indeed, a series of destaining steps is required following enhancement with Crowle's Double Stain, Amido Black and Hungarian Red to eliminate the background staining prior to detection of the fingerprints. Blood may be washed off the surface during these steps which would result in a significant reduction in DNA yields. It was anticipated that any other combination of substrate and chemical reagent would provide better, or at least equivalent, DNA yields as those recorded for bloodmarks on linoleum enhanced with Crowle's Double Stain.

Phase 1 was the determinant for providing the range of dilutions to use for Phase 2. Phase 2 was set up with the largest blood dilution suitable for obtaining sufficient DNA for *Profiler Plus*™ analysis. It was deemed important to detect the fingerprints but more crucial to retrieve sufficient DNA for subsequent analysis.

Phase 2—To define the limit of detection of the seven chemicals selected in this study and set the experimental parameters to be used in Phases 3 and 4, two different types of bloodstains were made on various nonporous and porous surfaces (Table 2). One set of stains was composed of 20 μL drops of diluted blood from individual B (1:2 to 1:200, see Table 1) and the second set consisted of bloodprints made with 20 μL aliquots of the same set of blood dilutions. The range of blood dilutions used in this phase was directed by the results obtained in Phase 1. The quantity of DNA retrieved from enhanced bloodprints prepared with 20 μL aliquots of the 1:200 diluted blood was sufficient for one round of *Profiler Plus*™ analysis. Under our experimental conditions, this dilution represented the limit for the evaluation of the sensitivity of the chemical reagents.

Bloodprints were photographed immediately after drying, before and after enhancement, and a semi-qualitative evaluation of the color intensity of the bloodprints was performed. No efforts were made to establish whether visualized fingerprints presented clear details of ridges or other interesting features for identification.

Phase 3—To determine the effects of blood enhancement on subsequent *Profiler Plus*™ typing analysis, bloodprints (10 μL aliquots) from individuals A and B were prepared in duplicate (on five different substrates) using undiluted blood and blood diluted 1:20 (see Table 2). As clothing (85% polyester/15% cotton) did not allow for good chemical penetration during Phase 2, this substrate was not selected in this phase of the study; clothing (65% polyester/35% cotton) was used instead. Likewise, the paper towel (Scott® 2-ply) disintegrated during enhancement with Crowle's Double Stain when tested in Phase 2, so was exchanged for the Xerox-grade paper in this phase of the study. Blue denim and the metal surface were not used in Phase 3. Once dried, the bloodprints on linoleum, glass and painted wood were treated with Amido Black, Crowle's

Double Stain, Hungarian Red, luminol and leucomalachite green. The bloodprints on clothing (65% polyester 35% cotton) were treated with luminol and those applied to laser photocopy paper (Xerox-grade) were treated with DFO and ninhydrin. All bloody fingerprints were either swabbed or cut out of the substrate and DNA was extracted, quantitated, amplified and detected by fluorescence on denaturing polyacrylamide gels as detailed in Materials and Methods. Duplicates were processed as separate samples.

Many controls were included in this phase of the study. Untreated areas from the five surfaces examined (linoleum, glass, wood, clothing and paper) were swabbed and processed for DNA extraction, quantitation and DNA typing analysis. In addition, nonbloody fingerprints from individuals A and B were applied to all five nonporous and porous surfaces tested, swabbed and also processed for DNA extraction and DNA profiling. As well, all blood enhancement reagents were applied to bare surfaces (linoleum, glass, wood, clothing, and paper) and were further processed for DNA extraction and DNA typing.

Phase 4—Bloodprints (10 μL aliquots of undiluted blood) from individuals A and B were prepared in duplicate on the same five surfaces used in Phase 3. Once dried, the bloodprints were either immediately treated with the same seven chemicals used in Phase 3 or were left to air dry in a laminar flow hood or on a bench for another 7 days or 14 days before being chemically enhanced. In order to determine the effect of long-term exposure to enhancing chemicals on subsequent PCR STR analysis, chemically treated fresh and aged bloody fingerprints were stored at room temperature in a laminar flow hood or on a bench for 7, 14 or 54 days before being processed for *Profiler Plus*™ typing analysis. The bloody fingerprints were swabbed and the DNA was extracted, quantitated, amplified and detected by fluorescence as described earlier.

Results and Discussion

Phase 1

As shown in Table 5, the DNA yields obtained from blood drops applied to linoleum varied from 1500 ng (undiluted blood) to 25 ng (1:100 dilution) total DNA. As most DNA typing protocols recommend the use of 1 ng to 2.5 ng of target DNA for routine successful profiling, the quantity retrieved from the blood drops was more than sufficient. DNA yields from fingerprints in blood varied greatly within one series. Fingerprints prepared using 5 μL of undiluted blood provided an abundance of genetic material. The 1:50 dilution yielded sufficient DNA to subject the sample to one or two rounds of typing with the *Profiler Plus*™ STR multiplex system. As the aliquot of blood used to make the bloodprints increased in size (up to 20 μL), the amount of DNA was significantly increased (25 ng with 5 μL aliquots compared to 625 ng with 20 μL aliquots).

A significant drop in DNA yields (2.5 to 4-fold reduction) was noted when the blood was transferred from fingertip to substrate, compared to the situation where the blood was deposited directly on the linoleum as drops. This was anticipated because only a fraction of the blood deposited on the finger will be transferred to the surface. For the same aliquot of undiluted blood (i.e., 20 μL), 1500 ng and 625 ng of DNA were recovered from drops and fingerprints, respectively. The reduction in DNA yield was more pronounced with the 1:50 blood dilution where 40 ng and 10 ng of DNA were recovered from drops and fingerprints, respectively. The chemical enhancement of the bloodmarks using Crowle's Double Stain further reduced the quantity of DNA recovered (compared to untreated prints) by a factor between 2 and 12. The DNA yield obtained for bloodprints made with undiluted blood was 625 ng and 315 ng, before and after enhancement, respectively. Bloodprints prepared with the 1:10 blood dilution yielded 250 ng versus 20 ng of DNA following chemical treatment. A twofold reduction in DNA yield was noted for bloodprints prepared with the higher blood dilution tested (1:50), 10 ng were recovered before enhancement versus 5 ng following treatment. The decrease in DNA yield following enhancement is likely due to some loss of blood cells, hence of genetic material, during the destaining steps carried out in order to reduce the background staining. Despite some loss, the total amount of DNA recovered from the least concentrated bloodprint (1:200) treated with Crowle's Double Stain was approximately 1.5 ng, which is sufficient to generate a complete *Profiler Plus*™ profile. As this staining protocol applied to a nonporous substrate is recognized as one of the most challenging of all seven blood enhancement procedures evaluated, it is anticipated that other methods applied to other types of surfaces with different porosity characteristics would promote better DNA yields. Although not used by the RCMP field identification officers, fixatives such as methanol and sulphosalicylic acid, may also improve the amount of DNA recovered from nonporous surfaces by minimizing the loss of biological material during the destaining steps required in some enhancement protocols (26).

TABLE 5—*DNA yields from blood drops and bloody fingerprints on linoleum before and after enhancement using Crowle's Double Stain.*

	DNA Yield (Total Amount of DNA in ng)					
		Fingerprint in Blood before Enhancement				Fingerprint in Blood after Enhancement
Blood Dilution	Blood Drops 20 μL aliquot	5 μL	10 μL	15 μL	20 μL	20 μL aliquot
Undiluted	1500	25	175	250	625	315
1:2	250	ND	ND	ND	ND	80
1:5	80	ND	ND	ND	ND	40
1:10	50	150	250	125	250	20
1:20	40	ND	ND	ND	ND	10
1:50	40	2.5	2.5	1	10	5
1:100	25	ND	ND	ND	ND	1.5
1:200	Lost	ND	ND	ND	ND	1.5

TABLE 6—*Sensitivity limit of blood enhancement reagents tested on 20 μL blood drops or bloodprints made with undiluted and diluted blood applied on surfaces with different porosity.*

	Blood Drops								Bloodprints							
Surface	Undil.	1:2	1:5	1:10	1:20	1:50	1:100	1:200	Undil.	1:2	1:5	1:10	1:20	1:50	1:100	1:200
							Control in analogous position as Amido Black and other reagent									
Linoleum	4	3	2	2	2	1	1	0	3	2	1	1	1	1	0	0
Clear glass	4	3	3	2	2	1	1	0	2	2	1	1	1	0	0	0
Metal	2	2	1	1	1	0	0	0	2	2	1	1	1	0	0	0
White-painted wood	4	3	3	2	0	0	0	0	3	2	2	2	1	1	1	0
Blue denim	4	3	3	2	1	0	0	0	3	3	2	1	1	0	0	0
85% polyester/ 15% cotton	3	3	3	2	2	1	1	1	2	2	1	1	1	0	0	0
Paper towel (Scott® 2-ply)	3	3	3	2	2	1	1	0	3	3	2	1	1	1	1	1
							Amido Black									
Linoleum	4	3	3	3	3	3	2	2	4	3	2	2	1	1	1	0
Clear glass	4	4	4	4	4	4	4	3	4	4	3	1	2	1	1	1
Metal	4	4	4	4	4	3	3	3	4	4	3	2	1	1	1	0
White-painted wood	4	4	4	4	4	4	3	3	4	3	3	3	2	1	1	1
Blue denim	4	4	4	4	3	3	2	1	4	4	3	3	1	1	1	0
85% polyester/ 15% cotton	4	3	3	3	2	2	1	1	4	3	2	1	1	1	1	1
Paper towel (Scott® 2-ply)	4	4	4	3	3	2	1	1	4	3	3	2	1	1	1	1
							Crowle's Double Stain									
Linoleum	4	4	4	4	3	3	3	2	4	4	3	2	2	1	1	1
Clear glass	4	4	3	3	3	3	3	2	3	3	2	1	1	1	1	1
Metal	4	0	3	0	3	3	3	3	3	3	2	2	1	1	1	1
White-painted wood	4	4	4	3	3	3	3	3	3	3	3	2	2	1	1	1
Blue denim	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0
85% polyester/ 15% cotton	4	4	3	3	3	2	2	1	2	2	1	1	1	0	0	0
Paper towel (Scott® 2-ply)	2	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0
							1,8-diazafluoren-9-one									
Blue denim	0	0	0	0	1	1	0	0	0	0	1	2	2	1	1	1
85% polyester/ 15% cotton	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0
Paper towel (Scott® 2-ply)	0	0	2	2	2	1	0	0	0	1	2	3	3	2	1	1
							Hungarian Red									
Linoleum	2	3	3	4	3	3	2	2	4	4	4	3	2	1	1	1
Clear glass	4	4	4	4	4	3	2	1	4	4	4	2	2	1	1	1
Metal	4	4	3	3	3	2	2	2	4	2	1	1	1	1	0	0
White-painted wood	4	4	3	2	4	3	2	1	4	3	2	2	1	1	1	1
Blue denim	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
85% polyester/ 15% cotton	4	4	3	2	2	2	2	2	3	2	1	1	1	1	0	0
Paper towel (Scott® 2-ply)	3	0	0	0	ND	ND	ND	ND	4	4	2	1	2	1	1	1
							Leucomalachite green									
Linoleum	0	4	4	3	3	3	1	1	3	4	3	2	2	1	1	0
Clear glass	4	4	4	3	2	1	1	1	3	4	3	1	1	1	1	0
Metal	4	4	4	3	3	1	0	0	1	1	1	0	0	0	0	0
White-painted wood	4	4	4	4	3	2	1	1	3	3	3	2	2	1	1	1
Blue denim	4	4	4	4	4	3	2	1	4	4	4	4	3	2	2	1
85% polyester/ 15% cotton	4	3	2	2	2	1	0	0	3	3	3	1	2	1	1	1
Paper towel (Scott® 2-ply)	4	4	3	3	3	2	2	1	4	4	4	4	3	2	2	1

(continued)

TABLE 6—*(continued)*

Surface	Blood Drops								Bloodprints							
	Undil.	1:2	1:5	1:10	1:20	1:50	1:100	1:200	Undil.	1:2	1:5	1:10	1:20	1:50	1:100	1:200
								Luminol								
Linoleum	0	0	0	1	1	2	3	4	4	4	4	3	3	1	2	1
Clear glass	0	0	0	1	2	2	3	4	3	3	4	4	4	4	2	2
Metal	0	0	0	1	1	2	3	2	3	3	4	4	4	3	2	2
White-painted wood	0	0	0	0	0	1	1	1	3	4	4	4	4	3	2	1
Blue denim	1	1	2	4	4	3	3	1	2	3	4	4	3	3	2	1
85% polyester/ 15% cotton	0	1	1	2	1	1	1	1	4	3	4	4	2	1	1	1
Paper towel (Scott® 2-ply)	1	1	2	4	4	3	2	1	2	3	4	4	4	4	2	1
								Ninhydrin								
Blue denim	4	4	3	2	1	0	0	0	4	4	2	1	1	1	0	0
85% polyester/ 15% cotton	4	3	3	2	2	1	0	0	3	2	2	1	2	1	1	1
Paper towel (Scott® 2-ply)	4	4	4	2	1	1	1	0	4	4	4	3	4	2	1	1

Phase 2

Tables 6 and 7 present the results of the sensitivity study performed using the seven chemicals routinely used as indicators of blood in the laboratory or at crime scenes. The designations used in the assessment of the color intensity of the enhanced blood drops and fingerprints in blood ranged from a value of 4 (very intense prints) to a value of 0 (drops or fingerprints that were not visible). Table 6 provides the details of the color spectrum observed for each combination of reagent and surface used. Table 7 reveals the limit of blood detection of the seven chemicals for all of the surfaces tested. As shown, enhancement was successful in the majority of combinations, as the bloodmarks (drops and fingerprints) without chemical treatment were visible only when more concentrated aliquots of blood were applied to the surfaces. Blood drops were more easily detected than bloody fingerprints. This was especially true at the highest blood dilution tested (1:200). This observation was anticipated as the blood in drops is concentrated in a very restricted area; a very different situation prevails for bloody fingerprints. Examples where treatment did not improve detection of the bloodmarks included those combinations involving fingerprints on metal treated with leucomalachite green (see Table 7; 1:20 blood dilution visible before treatment versus 1:5 after treatment), drops or fingerprints on blue denim treated with Crowle's Double Stain (1:20 blood dilution visible before treatment versus 1:2 after treatment), drops on 85% polyester/15% cotton blend fabric treated with leucomalachite green or ninhydrin (1:200 blood dilution visible before treatment versus 1:50 after treatment), fingerprints on paper towel treated with Crowle's Double Stain or Hungarian Red (1:200 blood dilution visible before treatment versus 1:5 and 1:10, respectively, after treatment).

The most sensitive blood enhancement chemical was luminol which revealed highly diluted (1:200) blood drops and bloody fingerprints on all porous and nonporous surfaces selected. The next best reagent was Amido Black. Crowle's Double Stain worked very well on nonporous surfaces revealing highly diluted (1:200) blood drops and bloody fingerprints but performed poorly on the porous substrates selected (particularly in the case of blue denim and paper towel). Hungarian Red gave very good results for nonporous surfaces, with the exception of metal, but performed poorly on two of the three porous surfaces selected (i.e., blue denim and paper towel). These two latter substrates presented a major challenge when tested with enhancement procedures requiring destaining steps, such as Hungarian Red and Crowle's Double Stain. Indeed, the background staining on blue denim was very intense and could not be easily eliminated which prevented the visualization of the bloodmarks. In many instances, the paper towel totally disintegrated in the process which prevented further detection and analysis. Interestingly, enhancement using Amido Black provided much better results with the same surfaces even though destaining steps were also included to visualize the bloodmarks. The reagent DFO, which was only tested on porous surfaces, revealed highly diluted (1:200) blood prints on blue denim and paper towel. However, these surfaces did not permit the visualization of highly diluted blood drops. The limit of detection of blood drops using DFO was a 1:50 blood dilution. The 85% polyester/15% cotton blend fabric, with its shiny characteristics, did not allow good penetration of the chemicals and compromised the blood enhancement process. Overall, combinations involving blue denim, the 85% polyester/ 15% cotton blend fabric or paper towel and DFO did not give the full color spectrum observed using other combinations such as Amido Black and any of the surfaces tested. Leucomalachite green gave very good results for bloodprints on porous surfaces and nonporous substrates, with the exception of metal. Ninhydrin, which was used to enhance bloodmarks on porous surfaces, showed good results only with the 85% polyester/15% cotton blend fabric and paper towel. Bloodmarks on these surfaces were visible using the 1:200 blood dilution, whereas the limit of blood detection on blue denim was 1:20 for drops and 1:50 for fingerprints.

The sensitivity levels established in our study differ somewhat from those reported by Olsen (24) who used clear glass and white

TABLE 7—*Blood dilutions for which bloodmarks were still visible following seven different chemical enhancement procedures.*

	Blood Drops								Bloodprints						
Surface	No Treatment	AB	CDS	DFO	HR	LG	LUM	NIN	No Treatment	AB	CDS	DFO	HR	LG	LUM	NIN
Linoleum	1:100	1:200	1:200	ND	1:200	1:200	1:200	ND	1:50	1:100	1:200	ND	1:200	1:100	1:200	ND
Clear glass	1:100	1:200	1:200	ND	1:200	1:200	1:200	ND	1:20	1:200	1:200	ND	1:200	1:100	1:200	ND
Metal	1:20	1:200	1:200	ND	1:200	1:50	1:200	ND	1:20	1:100	1:200	ND	1:50	1:5	1:200	ND
White painted wood	1:10	1:200	1:200	ND	1:200	1:200	1:200	ND	1:100	1:200	1:200	ND	1:200	1:200	1:200	ND
Blue denim	1:20	1:200	1:2	1:50	0*	1:200	1:200	1:20	1:20	1:100	1:2	1:200	1:50	1:200	1:200	1:50
85% polyester/ 15% cotton	1:200	1:200	1:200	0†	1:200	1:50	1:200	1:50	1:20	1:200	1:20	1:20	1:200	1:200	1:200	1:200
Paper towel (Scott® 2-ply)	1:100	1:200	1:5	1:50	0‡	1:200	1:200	1:100	1:200	1:200	1:5	1:200	1:10	1:200	1:200	1:200

AB = Amido Black, CDS = Crowle's Double Stain, DFC = 1,8-diazafluoren-9-one, HR = Hungarian Red, LG = leucomalachite green, LUM = luminol, NIN = ninhydrin.
*High background staining that could not be removed prevented visualization of blood drops.
†Shiny characteristics of the fabric prevented good penetration of the chemicals.
‡The substrate totally disintegrated during the process.

bond paper, and those presented by Theeuwen et al. (26) who used white opaque glass, white paper and white cotton fabric. These authors found that many of the reagents used in our study were much more sensitive and allowed extremely diluted bloodmarks to be enhanced and visualized (up to 1:16 000 blood dilution). Many reasons may account for this variation: (1) the selection of different porous and nonporous material, (2) enhancement of blood drops versus bloody fingerprints, (3) enhancement of wet bloodmarks versus dried bloodmarks, and (4) different qualification criteria to assess what constitutes a positive result. Furthermore, our sensitivity study was unique in the sense that the upper boundary for the blood dilutions was set at 1:200 so that enough DNA could be recovered to ensure subsequent STR DNA typing analyses. Many of the chemicals included in this study such as Amido Black, Crowle's Double Stain, Hungarian Red, leucomalachite green and luminol would have exhibited higher levels of sensitivity based on the qualification criteria used to assess the color intensity (see Table 6). At 1:200 dilution, the bloody fingerprints or blood drops were still detected in many cases. The actual sensitivity levels for the seven reagents used in our study have been recently reported by Germain and Miller (63).

Table 8 summarizes observations made during this phase of the study and illustrates the most effective enhancement chemical on porous and nonporous substrates following our experimental conditions. This table provides an indication only of the performance of the reagents as other types of porous and nonporous material will have different absorption and diffusion capabilities and show different characteristics for the enhancement chemicals. Our study already indicated that blue denim, the 85% polyester/15% cotton blend fabric and paper towel (Scott® 2-ply) were challenging substrates for many of the enhancement procedures evaluated. Theeuwen et al. (26) recently presented their classification of reagents as best performers for a variety of surfaces. Many of the blood enhancement reagents used in our study were also selected by this group of investigators to reveal bloodmarks on three surfaces showing characteristics that differed from the substrates we selected. These authors used white opaque glass, white paper and white cotton fabric, while clear glass, white paper towel (Scott® 2-ply) and polyester/cotton blend fabric were tested in our study, along with linoleum, metal, white painted wood and blue denim. In addition, luminol was not evaluated in their study, whereas it was shown to be the most sensitive reagent of all seven chemicals surveyed in our evaluation. As a result, Theeuwen's final classification of reagents as best performers for nonporous surfaces was very similar to ours but their list of best performers for porous material differed greatly with the one established in the present study.

TABLE 8—*Most effective enhancement protocols for nonporous and porous surfaces as established under the present experimental conditions.*

Nonporous Surfaces		Porous Surfaces	
Blood Drops	Bloodprints	Blood Drops	Bloodprints
Amido Black Crowle's Double Stain Luminol Hungarian Red	Crowle's Double Stain Luminol	Amido Black Luminol	Leucomalachite green Luminol

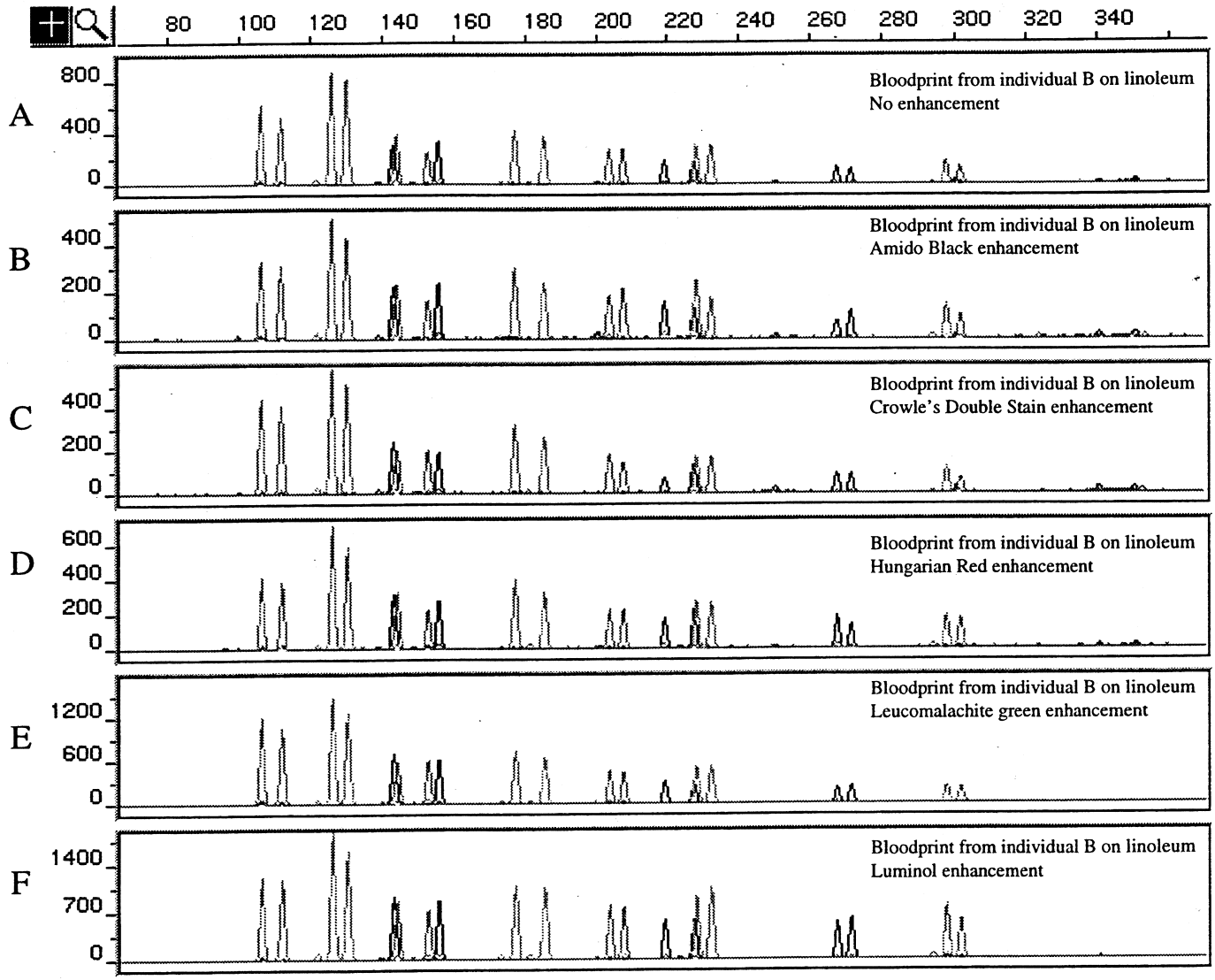

FIG. 1—Profiler Plus™ *profiles from bloodprints of individual B produced on linoleum and enhanced using a variety of chemicals. PCR amplifications were performed using 2.5 ng of template DNA in a 25 μL PCR reaction volume as detailed in Materials and Methods. (A) Control, no enhancement; (B) Amido Black; (C) Crowle's Double Stain; (D) Hungarian Red; (E) Leucomalachite green; (F) Luminol. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

Phase 3

In this series of experiments, fingerprints prepared using the 1:20 blood dilution did not yield sufficient DNA to permit PCR analysis and these samples were not processed any further. Loss of biological material likely took place during the application of the bloodmarks to the various surfaces, during the chemical enhancement procedure and/or during DNA extraction of the blood collected from the surfaces. Therefore, results presented are those of the bloodmarks prepared using undiluted blood.

As noted in Figs. 1 to 4, none of the seven chemical enhancement procedures, tested on a variety of surfaces, had detrimental effects on the PCR amplification of the genetic markers surveyed in the *Profiler Plus*™ multiplex STR system. Regardless of the reagent and the surface examined, the fluorescent signals as well as the allele size measurements remained relatively constant, balanced across the nine STR loci and essentially identical to those of the untreated blood fingerprints (also see Tables 9 and 10). No inhibitory effect on the PCR process or interference with the fluorescence-based detection procedure was observed. No allele dropout or extraneous bands were detected in profiles generated from the DNA of enhanced bloodprints.

Interestingly, although no inhibitory effect on the PCR process was noted, many of the DNA extracts showed a strong coloration following the one-step organic extraction protocol. In most instances, the purification step on the Microcon® -100 exclusion columns was successful in removing the pigments but the time required to process these DNA extracts on the columns was significantly increased compared to the untreated bloodmarks. Indeed, 17 to 32 min were necessary to allow the extracts to pass through

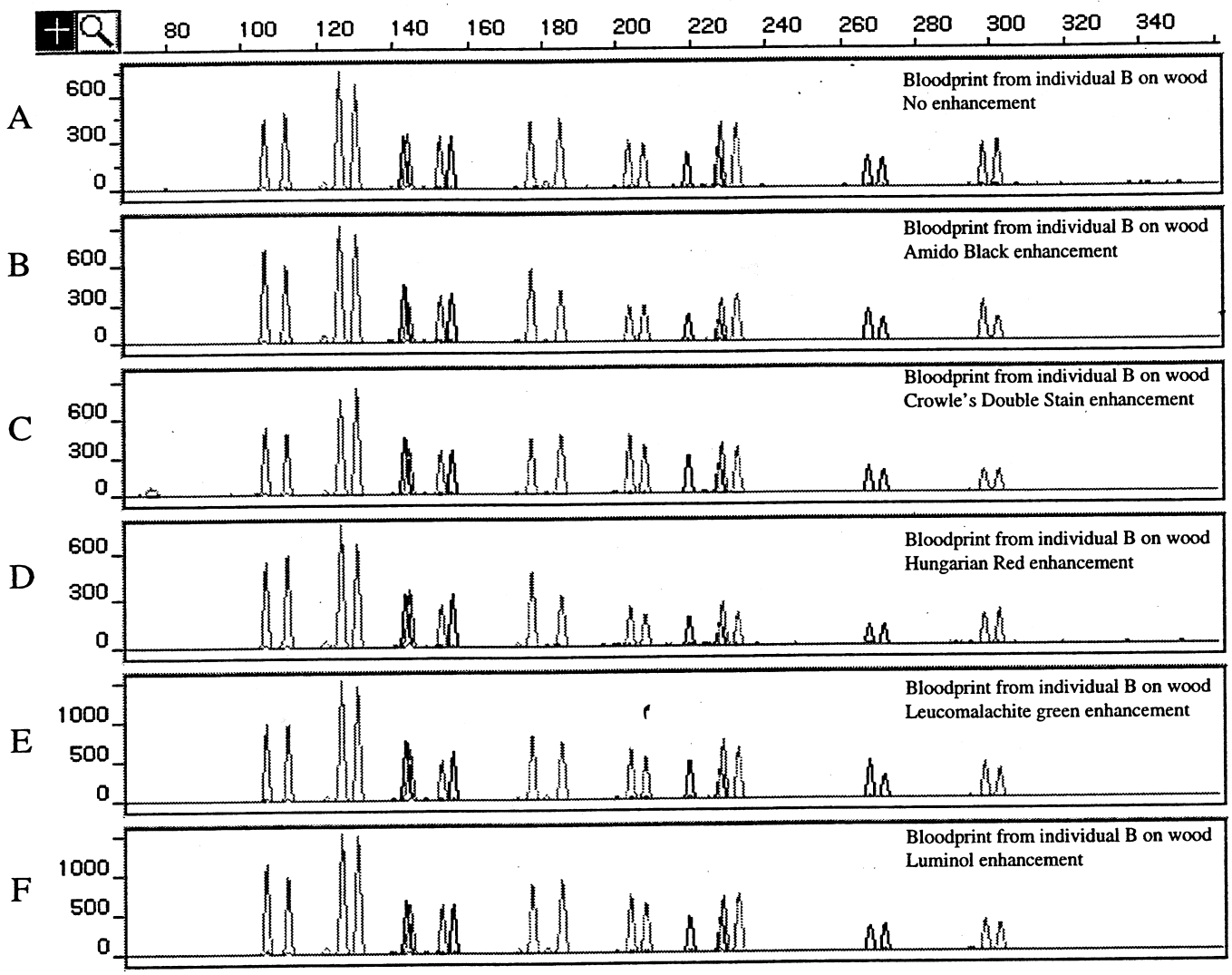

FIG. 2—Profiler Plus™ *profiles from bloodprints of individual B produced on white painted wood and enhanced using five different reagents. PCR amplifications were performed using 2.5 ng of template DNA in a 25 μL PCR reaction volume as detailed in Materials and Methods. (A) Control, no enhancement; (B) Amido Black; (C) Crowle's Double Stain; (D) Hungarian Red; (E) Leucomalachite green; (F) Luminol. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

the membrane for the enhanced bloodmarks compared to 10 min for untreated samples. Routinely, two Microcon® -100 columns were used to obtain the final DNA extracts that would be used for PCR amplification. Samples centrifuged for 10 min had a tendency to saturate the Microcon® -100 membranes with some unidentified agents, preventing the rest of the extracts to pass through them. In these instances, the remaining aqueous solutions were transferred onto new Microcon® -100 columns and spun for another 10 min or longer to isolate the DNA from any potential contaminants. The DNA recovered from both columns for each sample was then pooled. In light of the excellent *Profiler Plus*™ STR typing results that were obtained using the DNAs from the enhanced samples, the difficulties encountered during the extraction process did not impact on their subsequent PCR amplification.

No quantifiable DNA was recovered from any of the control samples included in this phase of the study (see Experimental Design Section; Phase 3 for details) as revealed by the lack of detectable signals on the chemiluminescent slot blot membrane (data not shown). Nevertheless, all controls were subjected to PCR amplification using the *Profiler Plus*™ multiplex to ensure that no fluorescent background signals would be detected. As shown in Fig. 5, seven controls showed minor signals. These included untreated clothing, luminol-treated wood, and untreated nonbloody fingerprints from individuals A and B produced on glass, wood and clothing. In all instances, incomplete *Profiler Plus*™ profiles were observed. The intensity of the signals ranged between 40 relative fluorescent units (RFU) to 150 RFU, i.e., above the threshold limit of detection of alleles set at 40 RFU during our extensive STR validation studies performed at the RCMP forensic laboratory using the *Profiler Plus*™ multiplex STR system. Three samples showed signals for Amelogenin only (panels E, F and G; non-

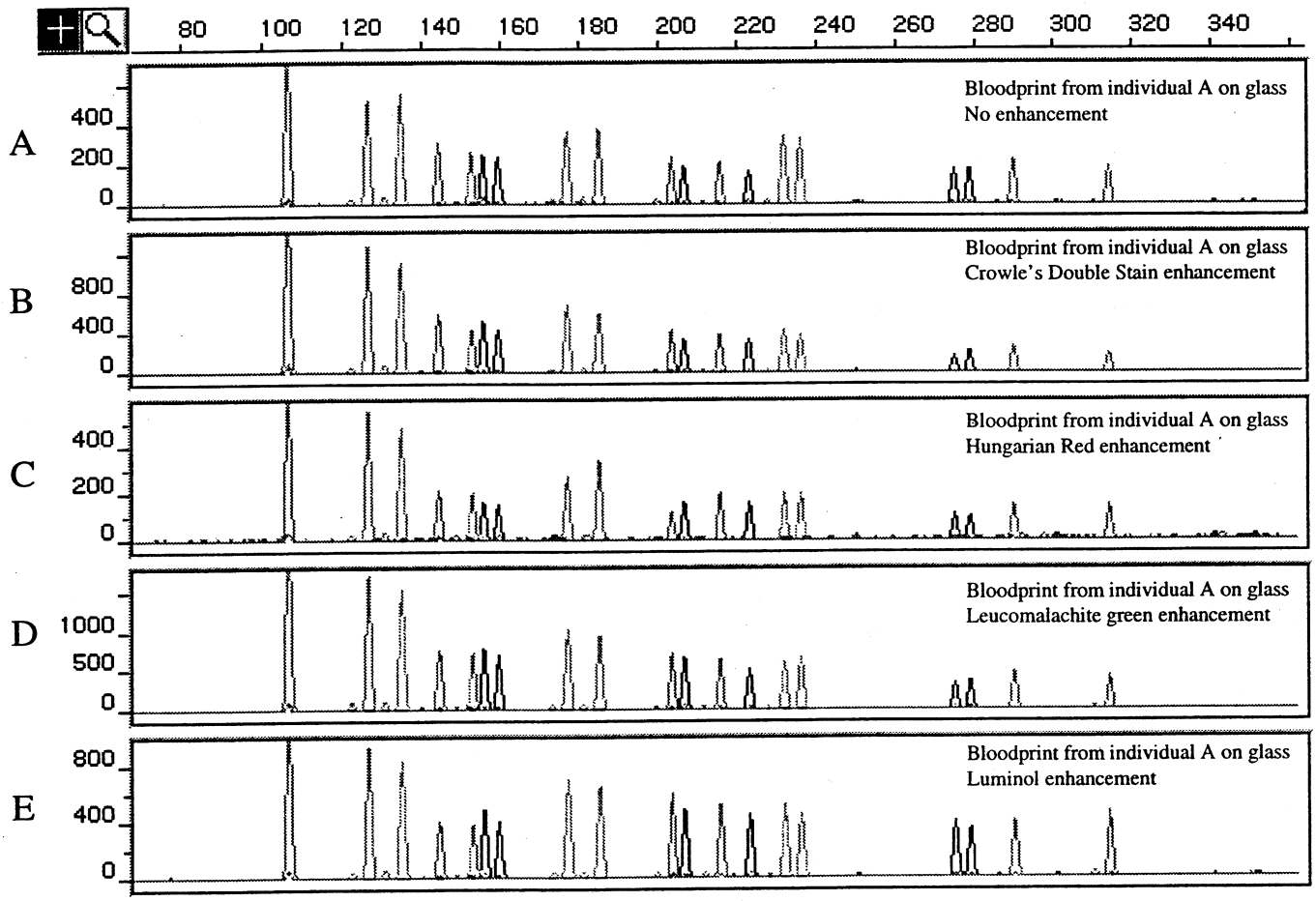

FIG. 3—Profiler Plus™ *profiles from bloodprints of individual A produced on clear glass and enhanced using a variety of chemicals. PCR amplifications were performed using 2.5 ng of template DNA in a 25 μL PCR reaction volume as detailed in Materials and Methods. (A) Control, no enhancement; (B) Crowle's Double Stain; (C) Hungarian Red; (D) Leucomalachite green; (E) Luminol. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

bloody fingerprints from individual A on clothing, nonbloody fingerprints from individual B on wood, and luminol-treated wood). The four remaining samples (panels A to D) showed signals above 40 RFU for the STR systems labeled with FAM (blue dye; D3S1358, HumvWA, HumFGA) or with JOE (green dye; D8S1179, D21S11, D18S51). None of these control samples showed alleles above 40 RFU for the STR systems labeled with NED (yellow dye; D5S818, D13S317, D7S820). Interestingly, in the two situations where excessive pressure was applied by fingers during the preparation of the nonbloody fingerprints (e.g., those applied to glass), the *Profiler Plus*™ profiles observed were almost complete and were consistent with the profiles of the contributor of the fingerprint (panels B and C). These results are in agreement with a recent report by Van Oorschot and Jones (67) which indicated that STR profiles, under some circumstances, could be obtained from epithelial cells left on pens, car keys, telephone receivers, and briefcases. These results further reiterate that caution should be exercised when handling materials or samples that could potentially be submitted to a forensic laboratory for DNA typing analysis. With the judicious use of gloves at crime scenes, evidentiary samples will not show contamination.

Phase 4

Figure 6 presents the *Profiler Plus*™ STR profiles generated using DNA recovered from untreated bloodprints produced on linoleum which was then left at room temperature for 7 days, 14 days and 54 days before DNA extraction and amplification. The four panels show profiles with no allele drop out or additional bands, demonstrating the long-term stability of blood in this particular context. Similar results were obtained using DNA from aged untreated bloodprints produced on wood, clear glass, Xerox-grade paper, and 65% polyester/35% cotton blend fabric (data not shown). Differences noted in the fluorescence intensity originated from differences in the amount of input DNA used for PCR amplification and subtle differences in pipetting during the quantitation step and/or preparation of the DNA samples for PCR amplification and/or preparation of the amplified DNA aliquots for gel electrophoresis and analysis.

Figures 7 and 8 show the *Profiler Plus*™ STR profiles generated using DNA recovered from fresh and aged bloodprints produced on linoleum and subjected to DNA extraction following 7 days, 14 days or 54 days post-enhancement using Amido Black

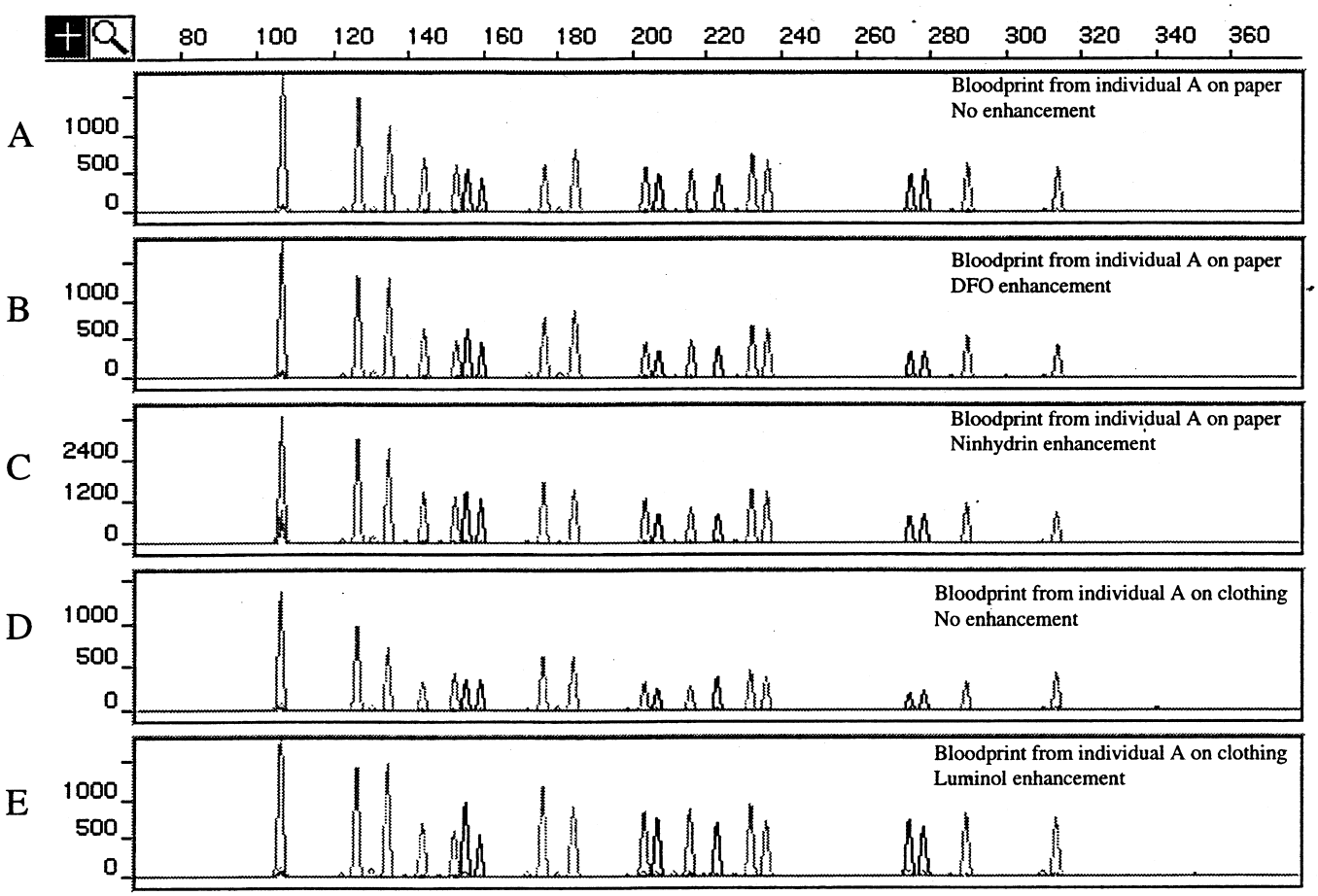

FIG. 4—Profiler Plus™ *profiles from bloodprints of individual A produced on Xerox-grade paper or 65% polyester/35% cotton blend fabric and enhanced using a variety of chemicals. PCR amplifications were performed using 2.5 ng of template DNA in a 25 μL PCR reaction volume as detailed in Materials and Methods. (A) Control sample on paper, no enhancement; (B) Paper, DFO; (C) Paper, ninhydrin; (D) Control sample on clothing, no enhancement; (E) Clothing, luminol. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

TABLE 9—*Allele size measurements for fresh bloodprints from individual A applied to any of five surfaces (before and following enhancement using any of seven chemical reagents).*

	Untreated Bloodprints			Enhanced Bloodprints		
Profiler Plus Loci	Average Size (bases)	Standard Deviation	*n**	Average Size (bases)	Standard Deviation	*n*†
D3S1358	126.47	0.14	19	126.42	0.18	92
	134.66	0.06	19	134.65	0.09	92
HumvWA	176.65	0.18	19	176.75	0.18	92
	184.70	0.14	19	184.78	0.16	92
HumFGA	231.86	0.11	19	231.84	0.14	92
	235.95	0.12	19	235.95	0.13	92
Amelogenin	106.46	0.10	19	106.46	0.12	92
D8S1179	144.11	0.05	19	144.13	0.08	92
	152.59	0.10	19	152.60	0.09	92
D21S11	203.29	0.04	19	203.30	0.06	92
	215.60	0.09	19	215.59	0.10	92
D18S51	289.50	0.12	19	289.54	0.11	92
	313.47	0.07	19	313.51	0.10	92
D5S818	155.44	0.18	19	155.47	0.12	92
	159.32	0.10	19	159.32	0.10	92
D13S317	206.66	0.08	19	206.66	0.11	92
	223.04	0.08	19	223.02	0.12	92
D7S820	274.59	0.11	19	274.67	0.17	92
	278.49	0.13	19	278.52	0.14	92

* *n* represents the number of data points originating from 9 different gels.
† *n* represents the number of data points originating from 11 different gels.

TABLE 10—*Allele size measurements for fresh bloodprints from individual B applied to any of five surfaces (before and following enhancement using any of seven chemical reagents).*

	Untreated Bloodprints			Enhanced Bloodprints		
Prolifer Plus Loci	Average Size (bases)	Standard Deviation	*n**	Average Size (bases)	Standard Deviation	*n*†
D3S1358	126.38	0.16	19	126.37	0.19	102
	130.52	0.12	19	130.51	0.15	102
HumvWA	176.63	0.18	19	176.71	0.17	102
	184.68	0.14	19	184.76	0.15	102
HumFGA	227.81	0.10	19	227.75	0.13	102
	231.90	0.09	19	231.84	0.13	102
Amelogenin	106.46	0.11	19	106.45	0.12	102
	112.19	0.15	19	112.17	0.18	102
D8S1179	144.14	0.10	19	144.14	0.09	102
	152.62	0.08	19	152.61	0.08	102
D21S11	203.30	0.05	19	203.30	0.04	102
	207.41	0.08	19	207.39	0.06	102
D18S51	297.44	0.07	19	297.41	0.12	102
	301.37	0.12	19	301.37	0.09	102
D5S818	143.10	0.09	19	143.11	0.08	102
	155.52	0.12	19	155.50	0.07	102
D13S317	219.07	0.15	19	219.01	0.15	102
	227.16	0.08	19	227.11	0.11	102
D7S820	266.84	0.11	19	266.83	0.14	102
	270.77	0.13	19	270.77	0.16	102

* *n* represents the number of data points originating from 9 different gels.
† *n* represents the number of data points originating from 12 different gels.

and luminol, respectively. Figures 9 and 10 present the electrophoretic tracings obtained using DNA from fresh and aged bloodprints produced on paper and submitted for DNA analysis 7 days, 14 days or 54 days after enhancement using DFO and ninhydrin, respectively. Figures 11 to 14 focus strictly on the long-term exposure to the enhancing chemicals and present the *Profiler Plus*™ profiles generated using DNA from bloodprints produced on linoleum, glass, wood, paper, and clothing, subjected to DNA extraction and amplification 54 days post-enhancement. For all samples examined, the nine STR systems present in the *Profiler Plus*™ multiplex were efficiently amplified, and the fluorescent profiles produced from the chemically treated bloodprints showed no allele drop out or additional bands when compared to profiles obtained from untreated samples. Differences noted in the fluorescence intensity originated from differences in the amount of input DNA used for PCR amplification (1 ng to 2.5 ng) and subtle differences in pipetting during the quantitation step and/or preparation of the DNA samples for PCR amplification and/or preparation of the amplified DNA aliquots for gel electrophoresis and analysis. Clearly, a continuous exposure (up to 54 days) to any of the seven reagents tested did not compromise the STR typing analysis of fresh, 7- and 14-day-old bloodprints. None of the enhancement procedures resulted in alterations in the allele profiles of the two individuals that donated blood for this study. This point is well illustrated in Tables 11 and 12 in which all allele size measurements generated during this phase of the study are compiled.

Although complete *Profiler Plus*™ profiles were generated from blood exposed to chemicals for 54 days, a slight descending gradient in fluorescent signal (i.e., a decrease in intensity from left to right in the electrophoretic tracings) was observed when bloodprints produced on linoleum or glass were enhanced using Crowle's Double Stain and Hungarian Red (Figs. 11 and 12, panels C and D). A similar trend was noted for both individuals involved in the study. Untreated bloodprints also showed a slight imbalance of signal from the smallest to largest STR loci but it was not as pronounced as that noted for the enhanced bloodprints. This pattern implies DNA degradation which results in more efficient amplification of the smaller STR loci than the larger STR loci. These results suggest that a longer exposure (i.e., over 54 days) to the chemicals may eventually lead to STR locus drop out which, in turn, would result in the generation of partial profiles or no profiles depending on the surface type and actual exposure time period. This phenomenon appears to be surface- and chemical-dependent as the same reagents on a different surface did not show the same trend (compare Figs. 11 and 12, panels C and D with Fig. 13, panels C and D).

Our results complement those of Hochmeister et al. (60) whose study employed a totally different set of enhancement reagents (cyanoacrylate, Rhodamine 6G and Ardrox™) and surfaces (razor blade and plastic foil), and showed no adverse effects on the PCR amplification of the D1S80 marker system. Our long-term exposure data also complement results reported by Stein et al. (40) indicating that successful STR analysis could be performed on ninhydrin-treated white paper stored at room temperature for a post-enhancement period of 56 days. These authors also tested long-term exposure to other agents such as cyanoacrylate (on razor blades and plastic foils) and gentian violet (on the sticky surfaces of adhesive tapes) and found no deleterious effects on the subsequent analysis of STRs.

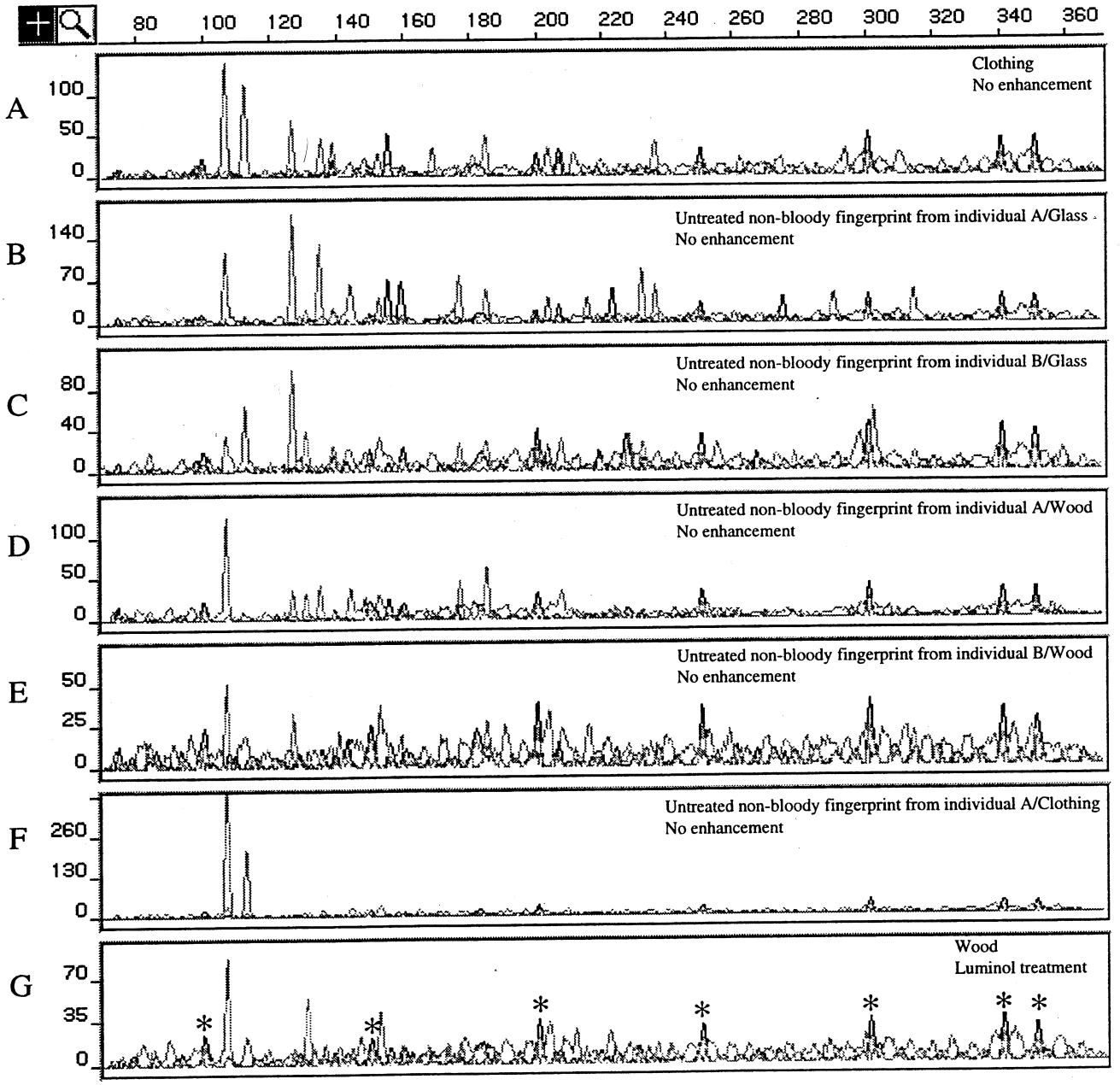

FIG. 5—*Fluorescent signals observed using the* Profiler Plus™ *STR amplification system on control samples. PCR amplifications were carried out despite the lack of human DNA signals on the slot blot membrane during DNA quantitation. Ten μL aliquots of DNA extracts were used in a 25 μL PCR reaction volume as detailed in Materials and Methods. (A) Clothing, no enhancement; (B) Nonbloody fingerprint from individual A on clear glass, no enhancement; (C) Nonbloody fingerprint from individual B on clear glass, no enhancement; (D) Nonbloody fingerprint from individual A on white painted wood, no enhancement; (E) Nonbloody fingerprint from individual B on white painted wood, no enhancement; (F) Nonbloody fingerprint from individual A on 65% polyester/35% cotton blend fabric, no enhancement; (G) White painted wood, luminol treatment. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. Peaks labeled with an asterisk (see panel G) represent the internal lane size standard GeneScan-350 [ROX] which show up in the NED (yellow) spectrum because of the incapability of the matrix to correct for the overlap at this low fluorescence intensity. These peaks can be observed in the same positions in all panels.*

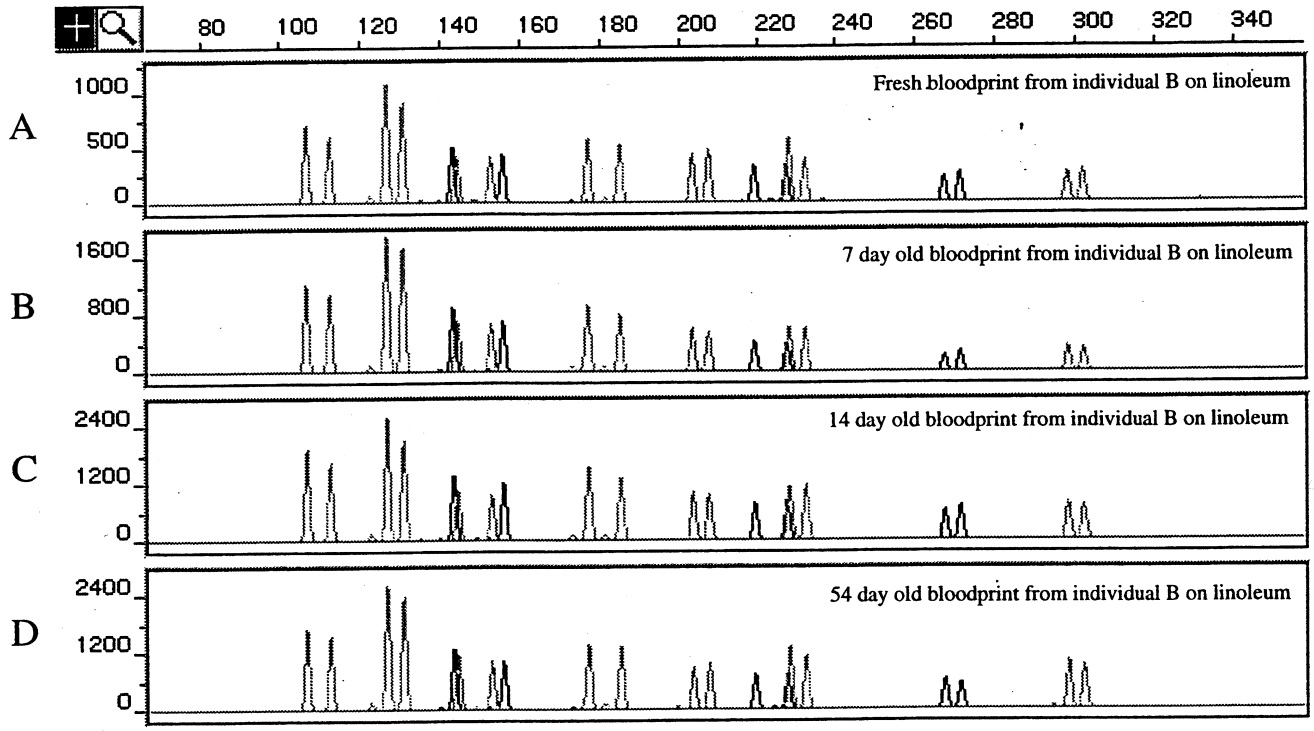

FIG. 6—Profiler Plus™ *profiles from untreated fresh and aged bloodprints of individual B produced on linoleum. PCR amplifications were performed using 1 ng to 2.5 ng of template DNA in a 25 μL PCR reaction volume as detailed in Materials and Methods. (A) Fresh bloodprint; (B) 7-day-old bloodprint; (C) 14-day-old bloodprint; (D) 54-day-old bloodprint. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

Conclusion

Crime scene investigators now have access to a wide variety of chemicals for the enhancement of latent fingerprints in blood. As DNA typing technologies became more sophisticated and more sensitive, much smaller bloodmarks have been submitted for genetic analysis. As casework exhibits are often shared between forensic identification specialists and forensic DNA specialists, we sought to determine the effects of seven enhancement chemicals on the subsequent STR DNA typing of bloody fingerprints prepared on five different surfaces. The results presented in this report indicate that none of the chemicals examined had a deleterious effect, on a short-term basis, on the PCR amplification of nine STR systems plus the gender determination marker, Amelogenin. In all instances, the fluorescent signals were similar and the size measurements of all alleles remained constant and identical to those of the untreated blood. No allele dropout or extraneous bands were detected in profiles generated from the DNA of enhanced bloodprints. Aged bloodprints (7-days-old and 14-days-old) enhanced and exposed to the blood reagents for up to 54 days yielded accurate and reliable results using the *Profiler Plus*™ multiplex system. Only two enhancement chemicals (Crowle's Double Stain and Hungarian Red) indicated a possible negative effect on DNA typing analysis when in contact with samples for 54 days. Forensic identification specialists can therefore utilize enhancement in order to reveal latent fingerprints without fear of compromising subsequent DNA typing results. However, the decision as to which enhancement method is selected in any particular case may impact on the possibility of future submission for DNA analysis. Our study revealed that some loss of biological material will take place with enhancement, specifically with procedures that require destaining steps, such as Crowle's Double Stain, Hungarian Red and Amido Black. In situations where the bloodprints are very small, the loss of blood cells during enhancement may result in insufficient amounts of DNA which, in turn, would jeopardize the DNA analysis. Although enhancement does not preclude the obtention of excellent STR results, it may, when employed on limited samples, have dire consequences and compromise crucial and limited evidentiary samples. Caution is therefore recommended when using an enhancement technique on bloodprints to ensure that sufficient biological material is retained by the substrate for possible future DNA submissions.

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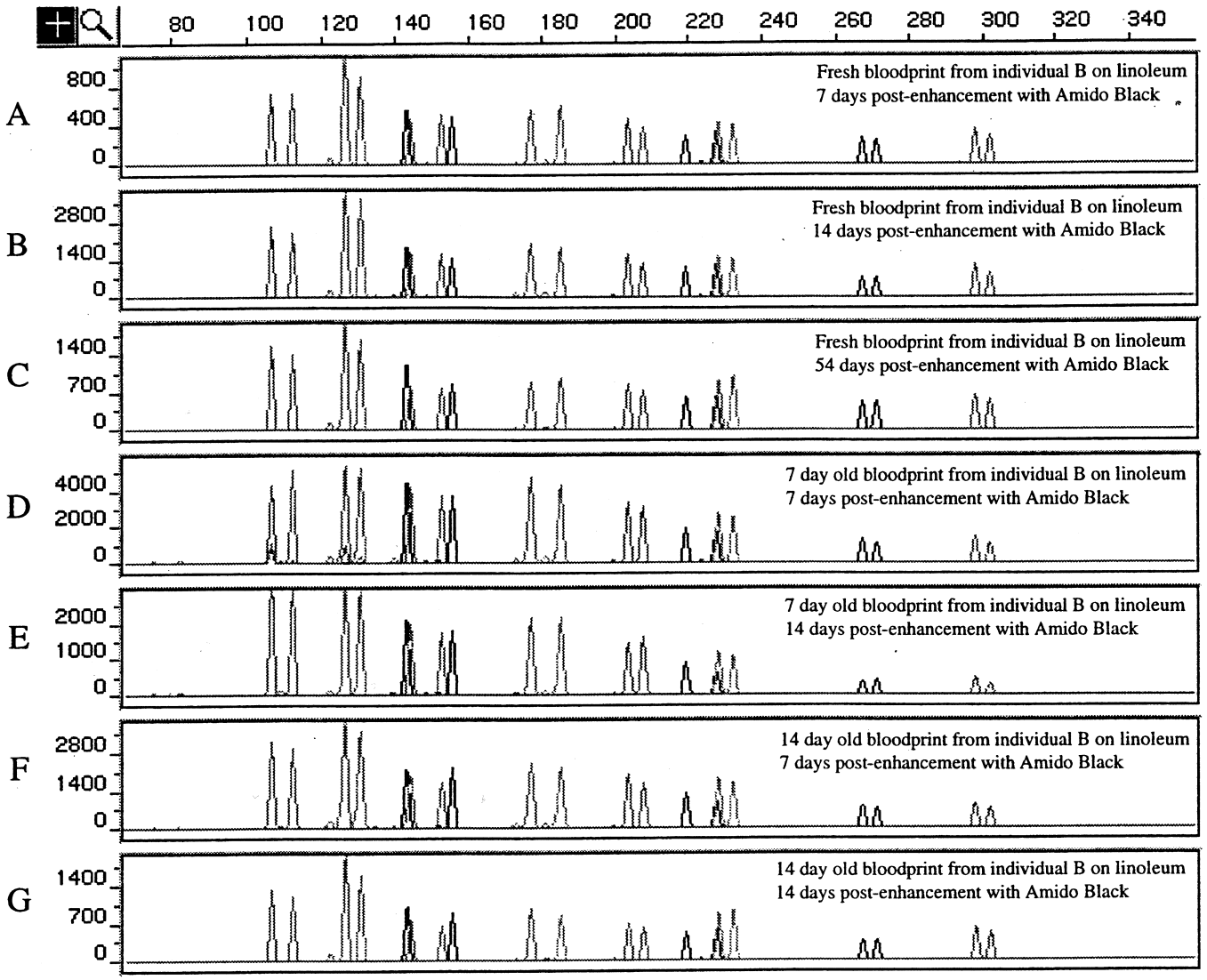

FIG. 7—Profiler Plus™ *profiles from fresh and aged bloodprints of individual B produced on linoleum and submitted for DNA analysis 7 days, 14 days or 54 days post-enhancement with Amido Black. PCR amplifications were performed using 1 ng to 2.5 ng of template DNA in a 25 μL PCR reaction volume as detailed in Materials and Methods. Panels A to G, as noted in the figure captions. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

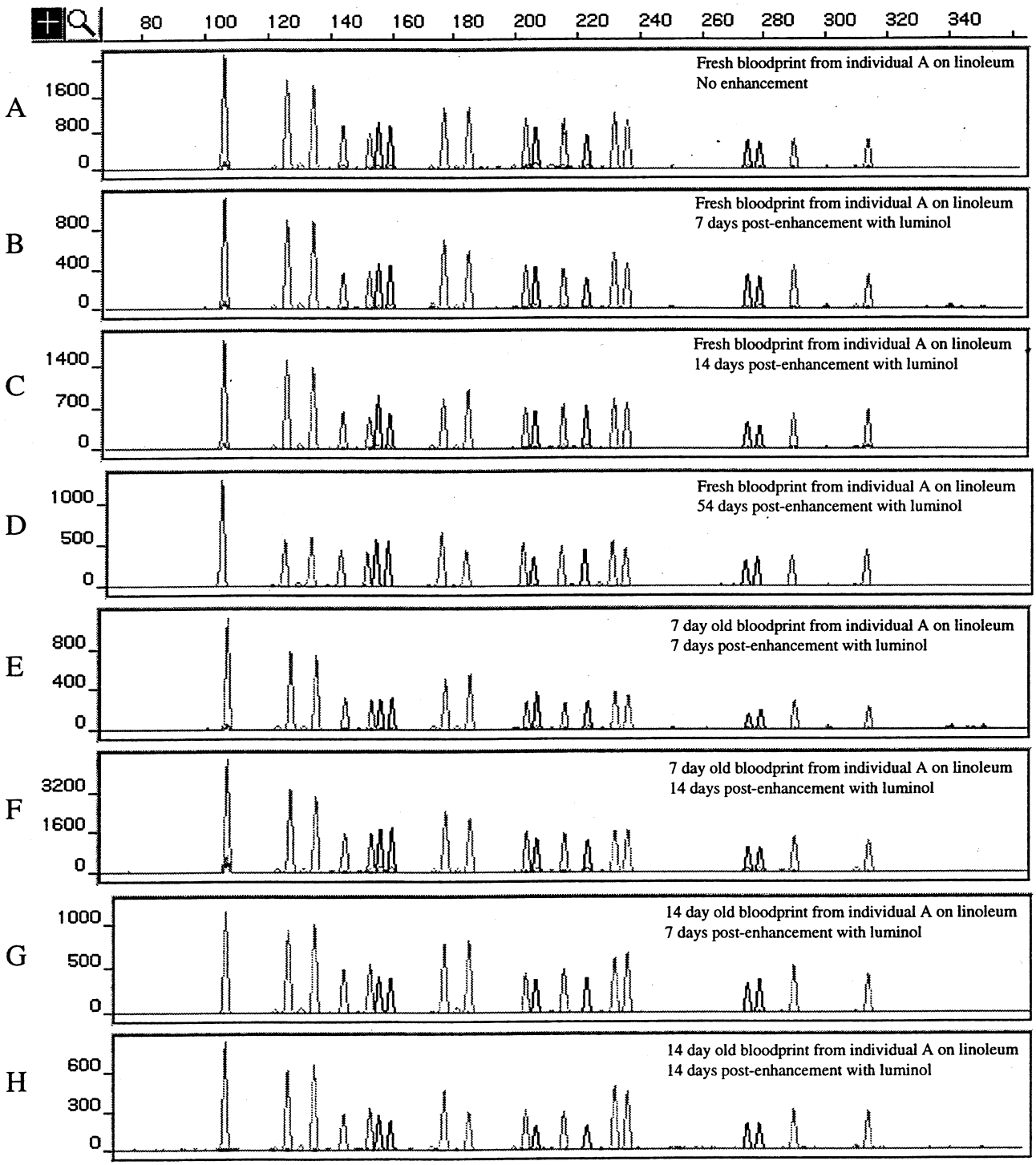

FIG. 8—Profiler Plus™ *profiles from fresh and aged bloodprints of individual A produced on linoleum and submitted for DNA analysis 7 days, 14 days or 54 days post-enhancement with luminol. PCR amplifications were performed using 1 ng to 2.5 ng of template DNA in a 25 μL PCR reaction volume as detailed in Materials and Methods. Panels A to H, as noted in the figure captions. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

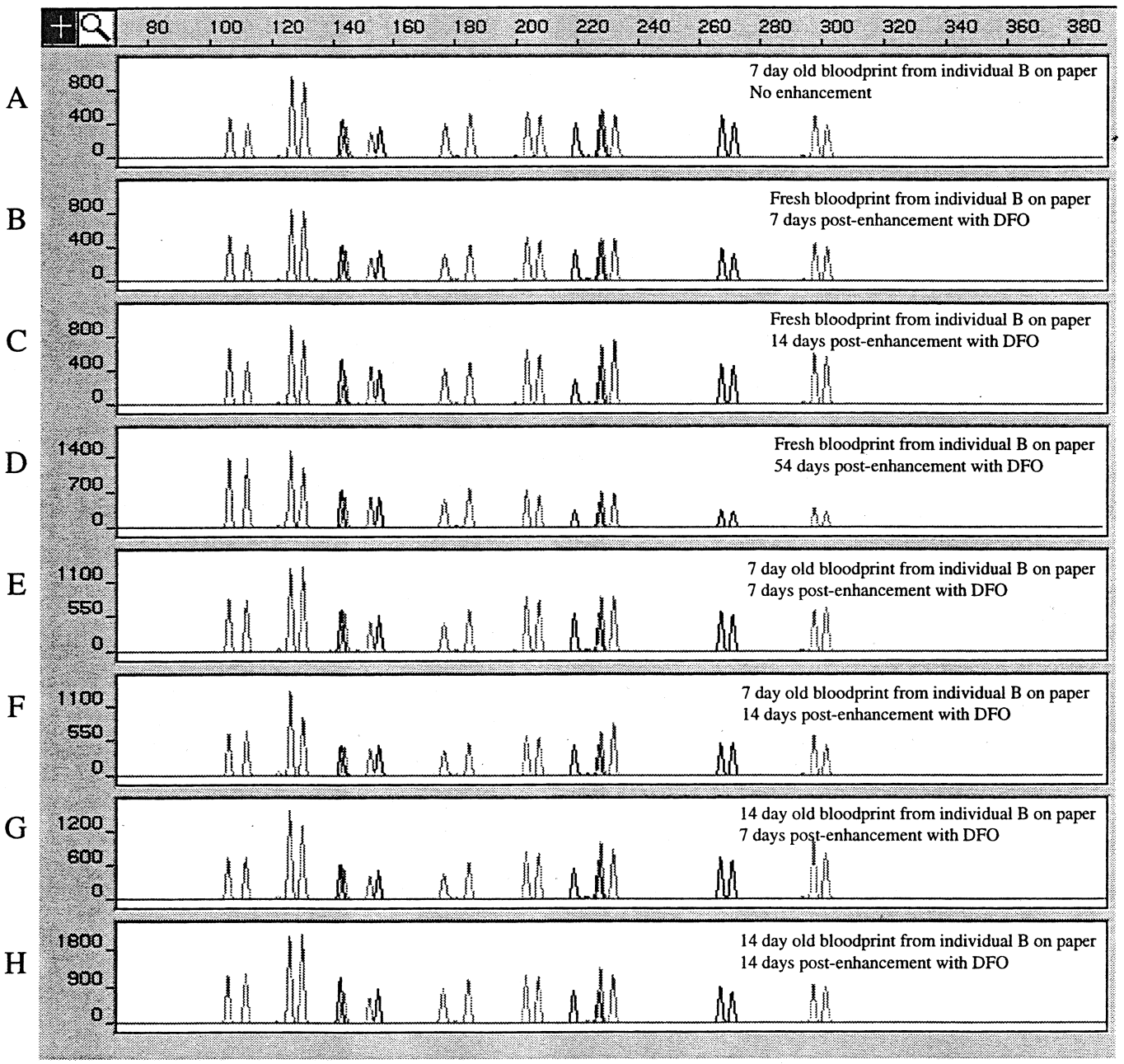

FIG. 9—Profiler Plus™ *profiles from fresh and aged bloodprints of individual B produced on paper and submitted for DNA analysis 7 days, 14 days or 54 days post-enhancement with DFO. PCR amplifications were performed using 1 ng to 2.5 ng of template DNA in a 25 μL PCR reaction volume as detailed in Materials and Methods. Panels A to H, as noted in the figure captions. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

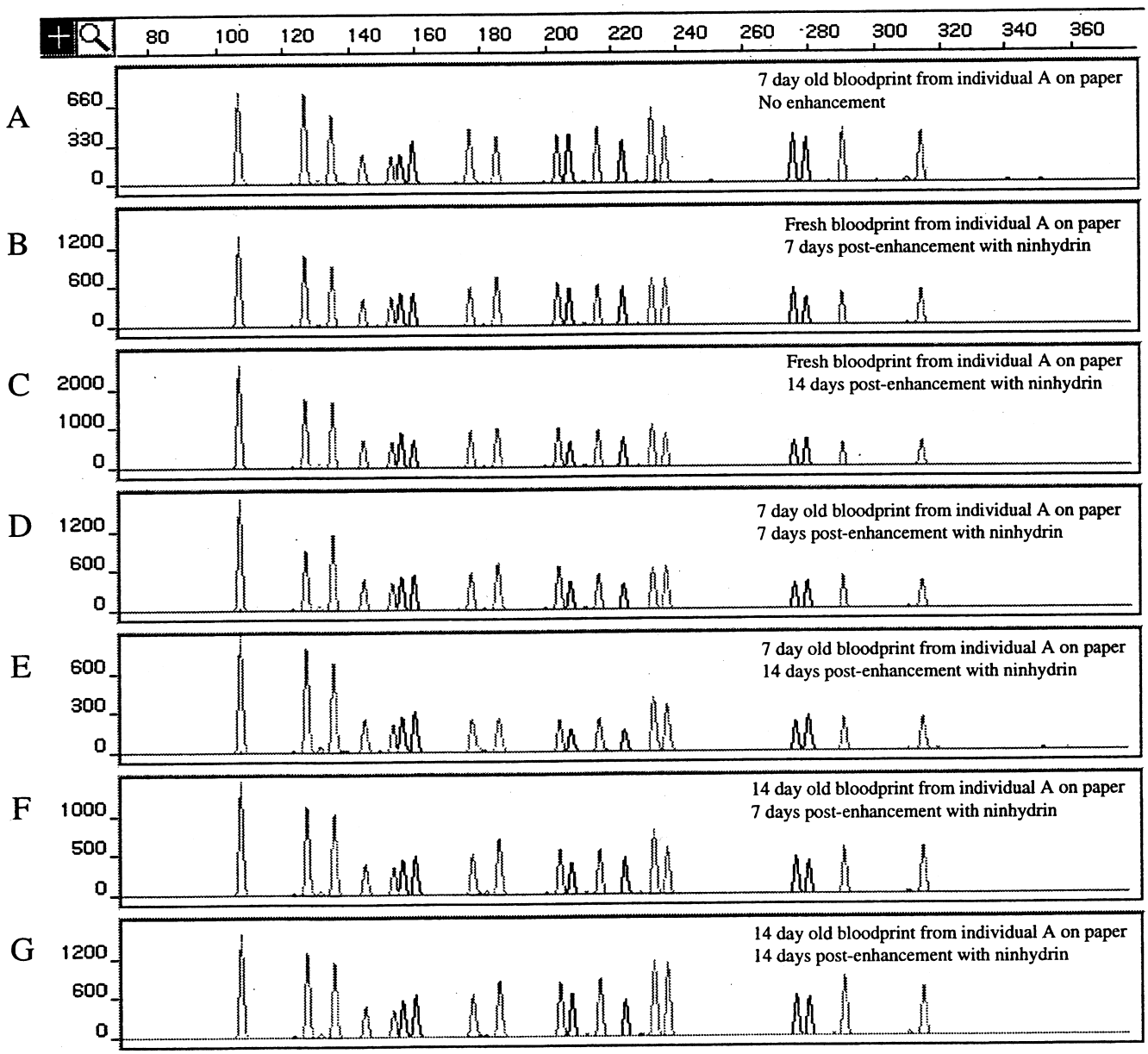

FIG. 10—Profiler Plus™ *profiles from fresh and aged bloodprints of individual A produced on paper and submitted for DNA analysis 7 days or 14 days post-enhancement with ninhydrin. PCR amplifications were performed using 1 ng to 2.5 ng of template DNA in a 25 μL PCR reaction volume as detailed in Materials and Methods. Panels A to G, as noted in the figure captions. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

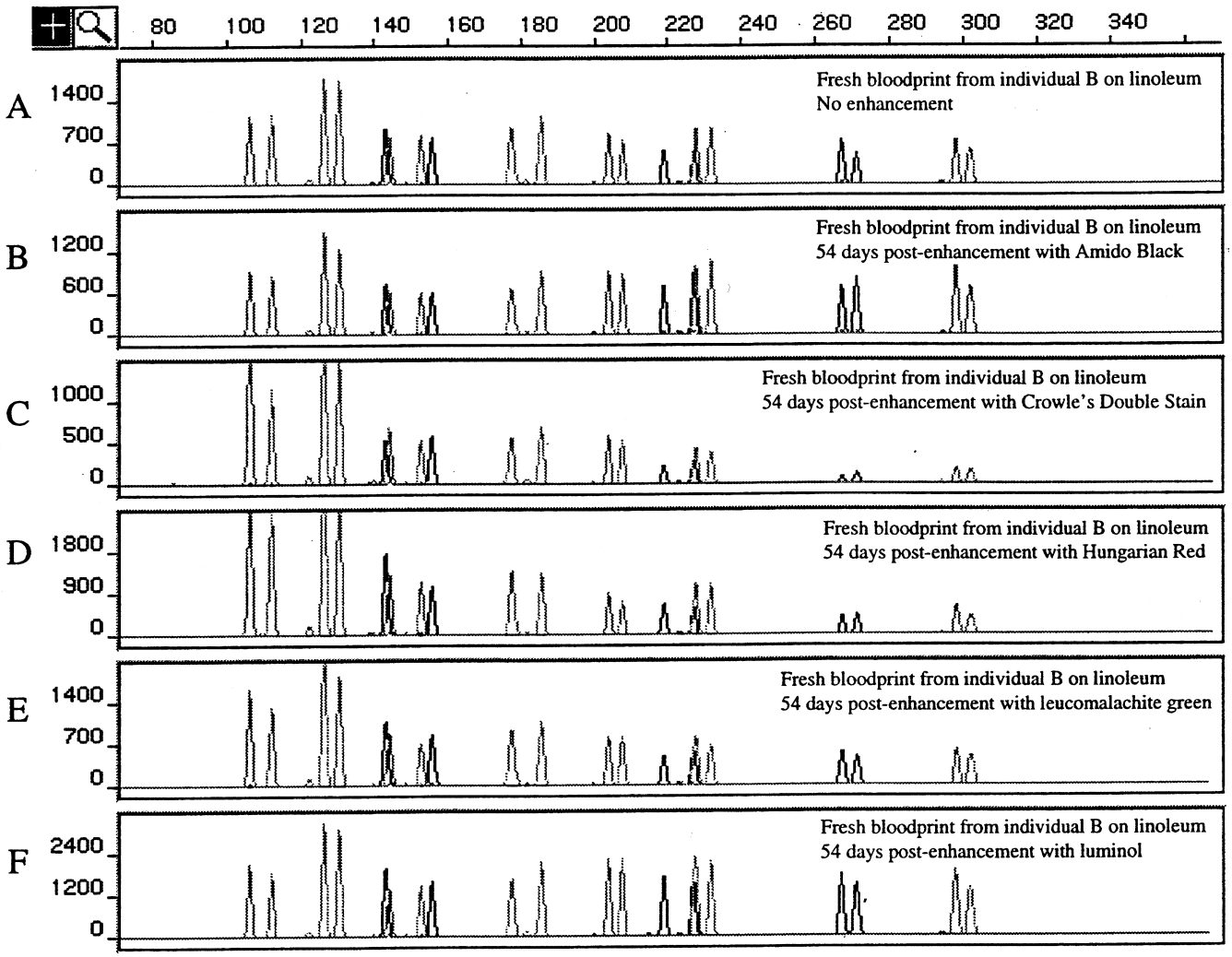

FIG. 11—Profiler Plus™ *profiles from fresh bloodprints of individual B produced on linoleum and subjected to continuous exposure (54 days) to five different enhancing chemicals. PCR amplifications were performed using 1 ng to 2.5 ng of template DNA, with the exception of bloodprints enhanced with Crowle's Double Stain where 0.3 ng of DNA was used. All PCR reactions were done in 25 μL volume as detailed in Materials and Methods. (A) Fresh bloodprint, no enhancement; (B) Amido Black; (C) Crowle's Double Stain; (D) Hungarian Red; (E) Leucomalachite green; (F) Luminol. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

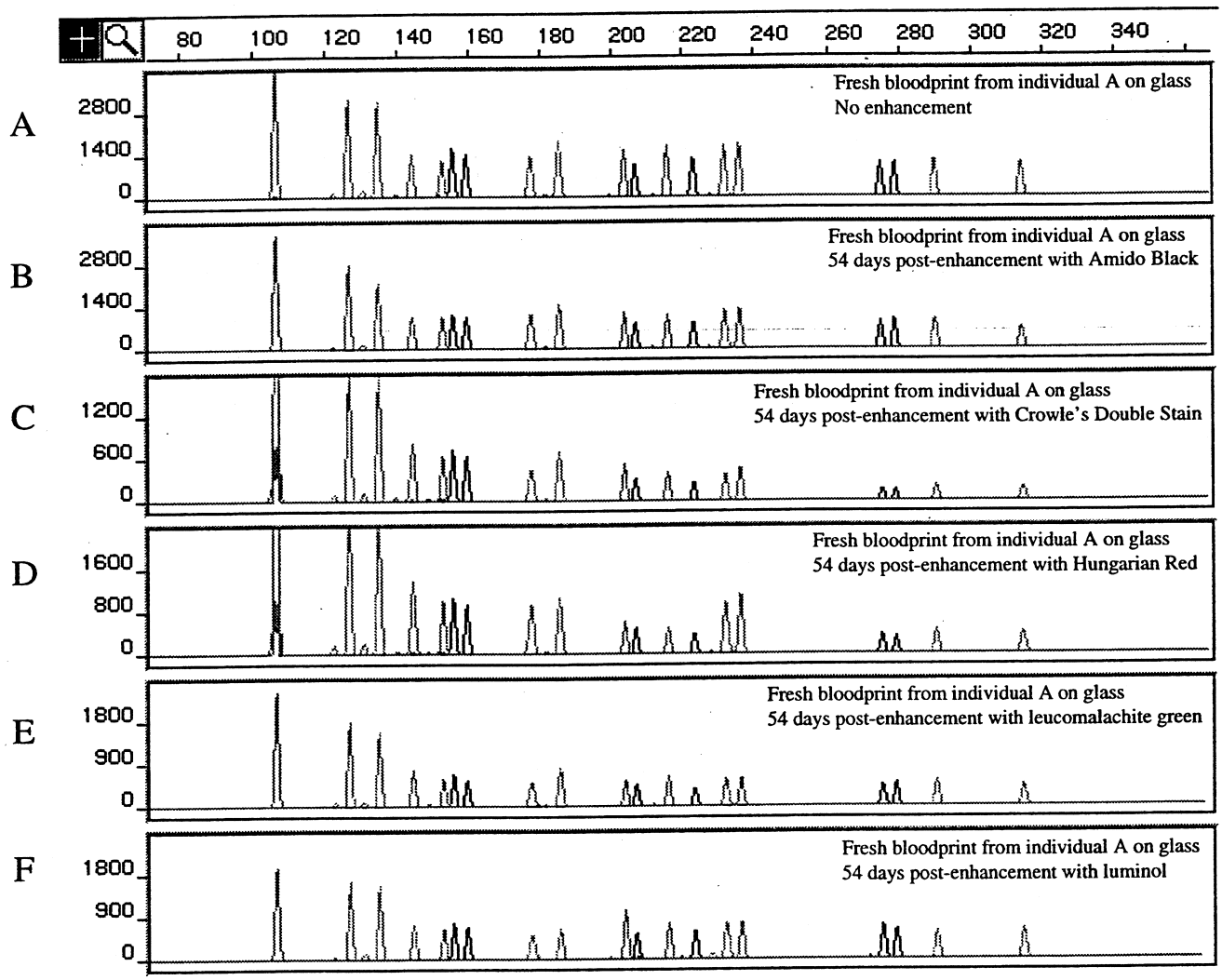

FIG. 12—Profiler Plus™ *profiles from fresh bloodprints of individual A produced on clear glass and subjected to continuous exposure (54 days) to five different enhancing chemicals. PCR amplifications were performed using 2 ng to 2.5 ng of template DNA, with the exception of bloodprints enhanced with Crowle's Double Stain where 0.6 ng of DNA was used. All PCR reactions were done in 25 μL volume as detailed in Materials and Methods. (A) Fresh bloodprint, no enhancement; (B) Amido Black; (C) Crowle's Double Stain; (D) Hungarian Red; (E) Leucomalachite green; (F) Luminol. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

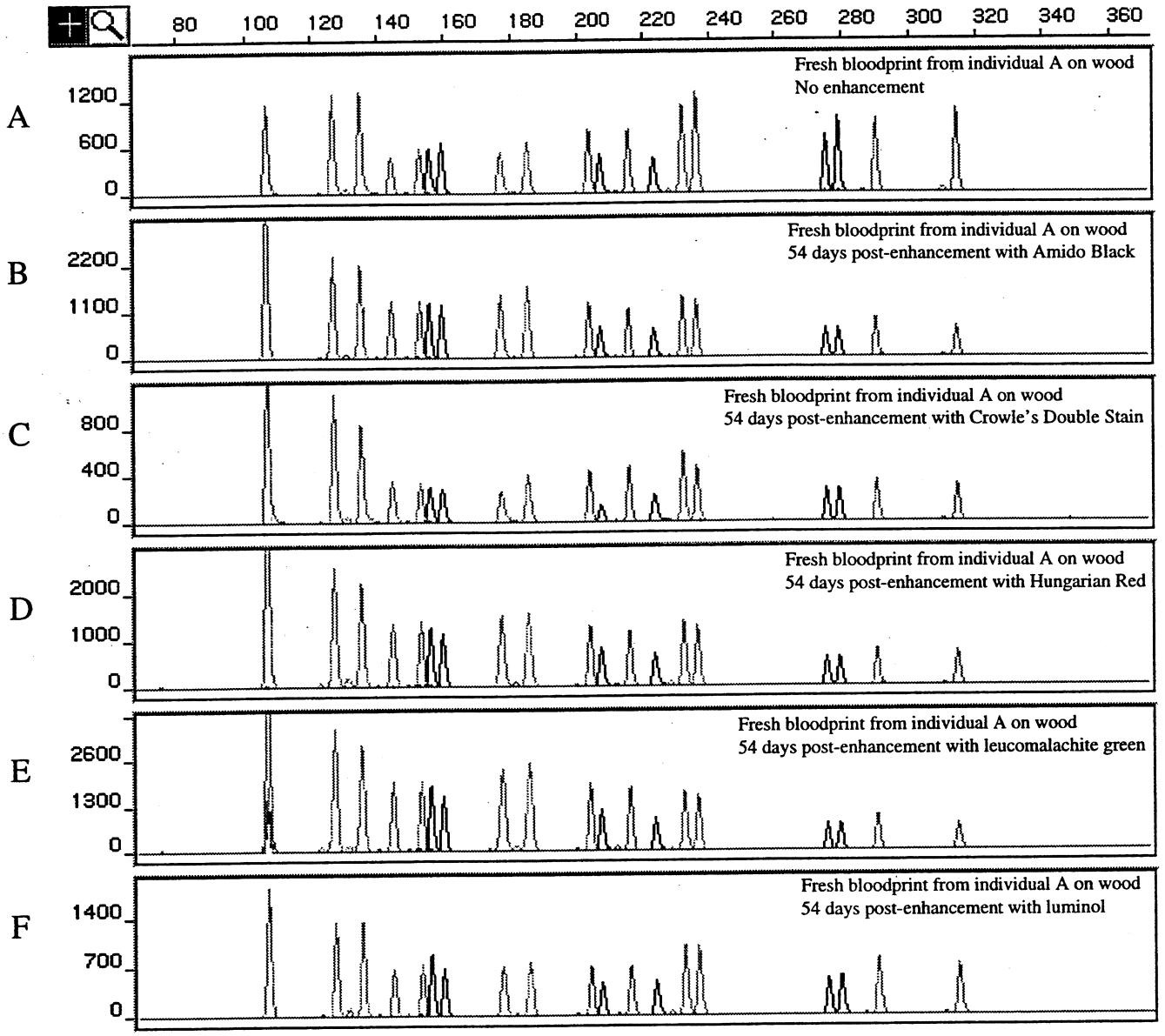

FIG. 13—Profiler Plus™ *profiles from fresh bloodprints of individual A produced on white painted wood and subjected to continuous exposure (54 days) to five different enhancing chemicals. PCR amplifications were performed using 1 ng to 2.5 ng of template DNA in 25 μL PCR reaction volume as detailed in Materials and Methods. (A) Fresh bloodprint, no enhancement; (B) Amido Black; (C) Crowle's Double Stain; (D) Hungarian Red; (E) Leucomalachite green; (F) Luminol. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

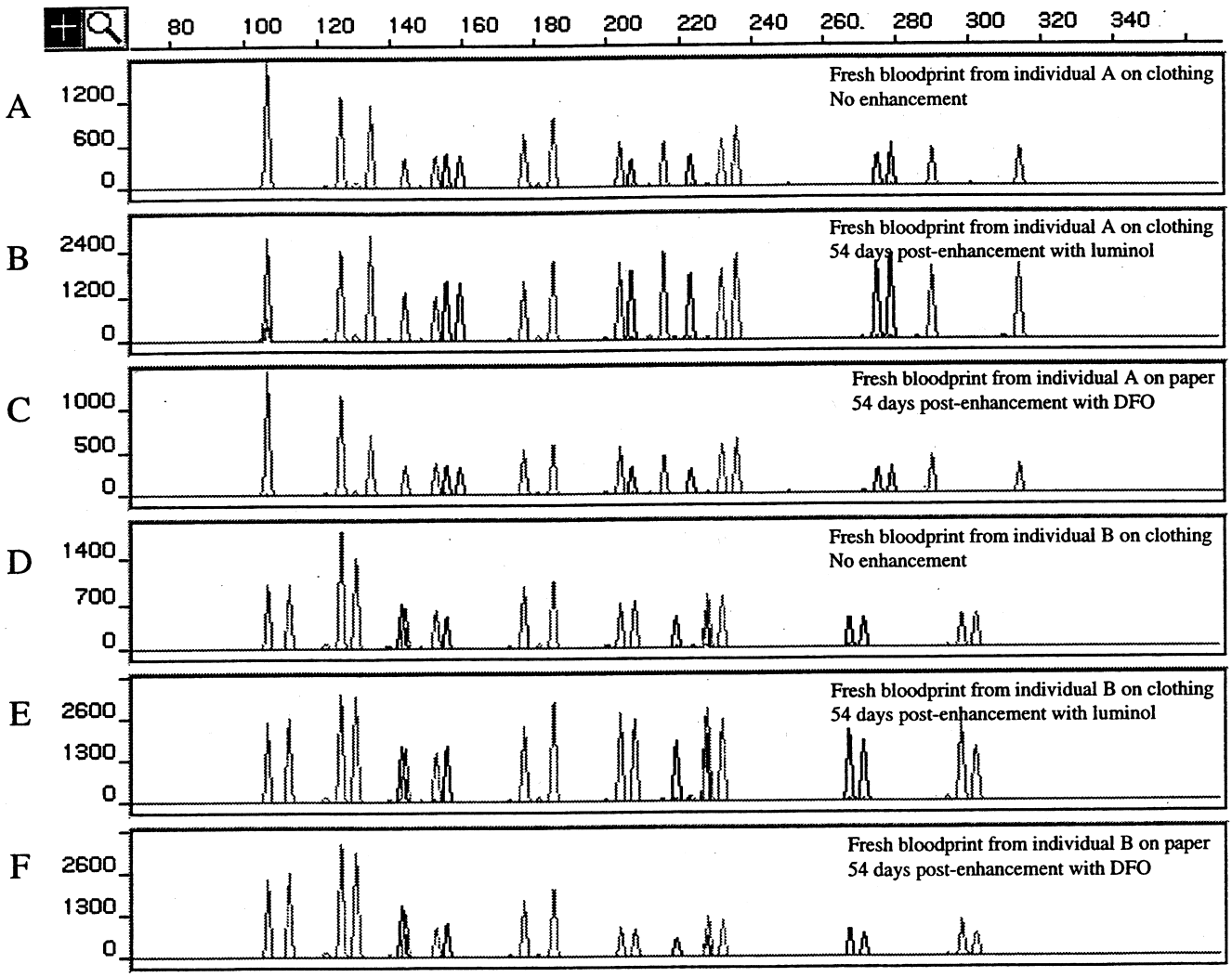

FIG. 14—Profiler Plus™ *profiles from fresh bloodprints of individuals A and B produced on 65% polyester/35% cotton blend fabric or Xerox-grade paper and subjected to continuous exposure (54 days) to two different enhancing reagents. PCR amplifications were performed using 2 ng to 2.5 ng of template DNA in 25 μL PCR reaction volume as detailed in Materials and Methods. Panels A to F, as noted in the figure captions. Each panel depicts the relative fluorescence intensity (RFU, left margin) and the size estimate in bases (top margin) derived from the internal lane size standard GeneScan-350 [ROX] using the ABI GeneScan® Analysis version 2.1 software. The genetic markers observed from left to right, in order of size, are: Amelogenin, D3S1358, D8S1179, D5S818, HumvWA, D21S11, D13S317, HumFGA, D7S820, and D18S51.*

TABLE 11—*Allele size measurements for aged (7- and 14-day-old) bloodprints from individual A applied to any of five surfaces following long-term exposure (7, 14 or 54 days) to any of seven blood enhancement chemicals.*

	Untreated Bloodprints			Enhanced Bloodprints		
Profile Plus Loci	Average Size (bases)	Standard Deviation	*n**	Average Size (bases)	Standard Deviation	*n*†
D3S1358	126.54	0.09	28	126.51	0.17	130
	134.67	0.07	28	134.69	0.10	130
HumvWA	176.82	0.08	28	176.82	0.11	130
	184.84	0.06	28	184.86	0.09	130
HumFGA	231.77	0.21	28	231.80	0.17	130
	235.87	0.19	28	235.86	0.23	130
Amelogenin	106.57	0.10	28	106.56	0.16	130
D8S1179	144.13	0.04	28	144.15	0.07	130
	152.63	0.05	28	152.64	0.08	130
D21S11	203.32	0.05	28	203.32	0.05	130
	215.59	0.05	28	215.58	0.09	130
D18S51	289.52	0.11	28	289.53	0.13	130
	313.43	0.19	28	313.48	0.17	130
D5S818	155.52	0.04	28	155.53	0.06	130
	159.34	0.06	28	159.34	0.07	130
D13S317	206.65	0.07	28	206.66	0.08	130
	222.96	0.16	28	223.00	0.14	130
D7S820	274.60	0.10	28	274.64	0.15	130
	278.46	0.10	28	278.49	0.13	130

* *n* represents the number of data points originating from 7 different gels.
† *n* represents the number of data points originating from 10 different gels.

TABLE 12—*Allele size measurements for aged (7- and 14-day-old) bloodprints from individual B applied to any of five surfaces following long-term exposure (7, 14 or 54 days) to any of seven blood enhancement chemicals.*

	Untreated Bloodprints			Enhanced Bloodprints		
Profile Plus Loci	Average Size (bases)	Standard Deviation	*n**	Average Size (bases)	Standard Deviation	*n*†
D3S1358	126.51	0.11	31	126.46	0.17	151
	130.66	0.12	31	130.59	0.14	151
HumvWA	176.83	0.06	31	176.83	0.10	151
	184.85	0.06	31	184.87	0.08	151
HumFGA	227.64	0.21	31	227.70	0.18	151
	231.76	0.20	31	231.80	0.18	151
Amelogenin	106.58	0.10	31	106.55	0.15	151
	112.29	0.05	31	112.29	0.19	151
D8S1179	144.14	0.06	31	144.16	0.07	151
	152.63	0.04	31	152.63	0.07	151
D21S11	203.32	0.05	31	203.32	0.06	151
	207.41	0.06	31	207.41	0.08	151
D18S51	297.35	0.08	31	297.38	0.11	151
	301.31	0.08	31	301.37	0.12	151
D5S818	143.13	0.06	31	143.15	0.06	151
	155.52	0.04	31	155.53	0.06	151
D13S317	218.92	0.14	31	218.96	0.14	151
	227.06	0.15	31	227.10	0.16	151
D7S820	266.76	0.09	31	266.79	0.16	151
	270.70	0.09	31	270.72	0.17	151

* *n* represents the number of data points originating from 6 different gels.
† *n* represents the number of data points originating from 10 different gels.

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