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Deoxyribonucleic Acid (DNA) Analysis by Restriction Fragment Length Polymorphisms of Blood and Other Body Fluid Stains Subjected to Contamination and Environmental Insults

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ABSTRACT: Deoxyribonucleic acid (DNA) restriction fragment length polymorphism (RFLP) profile results were obtained from bloodstains and other body fluid stains subjected to mixture with other body fluids, environmental insults (sunlight and temperature), different substrates (cotton, nylon, blue denim, glass, aluminum, and wood), and contaminants (gasoline, bleach, sodium hydroxide, soil, motor oil, detergent, phosphate salt, glacial acetic acid, and microorganisms). Of the samples that produced profile results, all had profiles that were consistent with those of untreated control samples.

KEYWORDS: forensic science, deoxyribonucleic acid (DNA), blood, body fluids, genetic typing, restriction fragment length polymorphisms

Recombinant deoxyribonucleic acid (DNA) technology has the ability to detect differences between individuals at the level of their DNA. Genetic characterization of evidentiary materials by restriction fragment length polymorphism (RFLP) technology has been implemented in a number of forensic science laboratories around the world and provides those laboratories with a powerful means of characterizing a body fluid, stain, or tissue.

Forensic science evidence can be subjected to a variety of external influences prior to its examination by crime laboratory personnel; therefore, an understanding of the effects

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of environmental insults and of adventitious substances on DNA isolated from blood and other body fluid stains is necessary before this technology can be implemented in casework. The literature contains several examples of this type of research [*1–6*]. Although it can be hypothesized that environmental insults would not produce false positive or false negative results, this study was designed to evaluate the effects of external influences and adventitious substances on the RFLP pattern or profile produced from laboratory-prepared bloodstains and other body fluid stains. These external influences included sunlight, temperature, several substrates, and duration of time. In addition, stains from blood or semen, or both, mixed with adventitious substances were prepared to simulate commonly encountered biological (for example, bacteria, yeast, and other body fluids) and nonbiological (for example, detergents, gasoline, and salts) contaminants found in association with evidentiary items. Finally, blood samples from a variety of nonhuman sources were analyzed to determine the potential of cross-hybridization with an array of single locus probes used for identification.

Materials and Methods

All the samples in this study were analyzed by the RFLP/variable number of tandem repeats (VNTR) method developed at the Federal Bureau of Investigation (FBI) Laboratory [*7,8*]. The single-locus probes (SLPs), size markers, and cell line controls were obtained from a number of commercial sources[3]: D2S44, D16S85, D17S79, molecular-weight size markers, and the K562 cell line were purchased from Lifecodes Corp., Valhalla, New York; D1S7 was purchased from Cellmark Diagnostics, Germantown, Maryland; D4S139 was purchased from Bethesda Research Laboratories, Gaithersburg, Maryland; and D10S28 and D14S13 were purchased from Promega Corp., Madison, Wisconsin. These materials were used in the following studies.

Environmental Studies

Long-Term Exposures to Sunlight

Blood from a known donor was drawn into Vacutainer™ tubes containing no preservatives. Forty stains (50 μL each) were made rapidly on washed cotton cloth and air dried at room temperature for 4 h. The stains were then placed within a greenhouse at ambient outdoor temperatures, beginning in March 1988. Half of the stains were exposed to the diurnal cycle of sunlight and darkness. The other half were maintained at the same temperatures, but exclusively in the dark. Four bloodstains from each set of stains were collected at intervals of 2 weeks for a period of 10 weeks (5 samplings). These stains were stored at −80°C until a time at which all the samples could be analyzed simultaneously. Bloodstains not exposed to these conditions were maintained at −80°C and served as controls for this study. The DNA derived from all the samples was hybridized with an SLP complementary to the genetic locus D2S44.

Short-Term Exposure to Sunlight

Blood from known donors was obtained and made into stains as has just been described. Seventy-two bloodstains were allowed to air dry at room temperature for 2 h and then placed in the same light/dark conditions described above. Samples of three bloodstains from each group were collected every 24 h for a period of 12 days (12 samplings) during July 1988. The stains were stored at −80°C until analyzed. Bloodstains stored at −80°C

[3]The names of commercial manufacturers are provided for identification only, and their inclusion does not imply endorsement by the Federal Bureau of Investigation.

and liquid blood samples served as controls. All the samples were hybridized with SLPs complementary to the genetic loci D2S44, D14S13, D16S85, and D1S80 (provided by R. White, Howard Hughes Medical Institute, Salt Lake City, Utah).

A similar study was performed subsequently in July 1990. During this study, bloodstains exposed to sunlight were maintained in the open. Triplicate samples exposed to the sunlight and triplicate samples maintained at the same temperatures in the dark were collected at 24-h intervals for 10 days. A total of 70 stains, including controls, were analyzed using SLPs complementary to the genetic loci D2S44, D17S79, D1S7, D4S139, and D10S28.

This study was performed using actual casework RFLP procedures developed at the FBI Laboratory. The casework RFLP procedures differed from the procedures used in the earlier studies by including the following: (*a*) molecular-weight size markers [0.6 to 23 kilobases (kb)] were used to estimate the DNA fragment size using a computer-assisted semiautomated procedure [*9*]; (*b*) samples were redigested if preliminary tests indicated only partial digestion of the extracted DNA; (*c*) a differential extraction procedure was used in the case of mixed body fluid stains containing semen; (*d*) the quantities of DNA loaded into the analytical agarose gel were controlled (that is, a maximum of 400 ng/lane was used); and (*e*) the K562 cell line control was used in each study.

Time, Temperature, and Substrate

Blood from three individuals was obtained as described above. Four replicate sets of 50-μL bloodstains were prepared on cotton, nylon, denim, glass, wood, and aluminum. Each set of stains was subjected to one of four different temperatures (4, 22, and 37°C and ambient outdoor conditions). Triplicate samples from each individual were harvested at 2 days and at 1, 3, and 5 months from each substrate at each temperature. Therefore, on any given sampling date, 216 bloodstains were taken for analysis, along with control blood samples from the same three individuals. The SLP for D2S44 was used for hybridization with all the samples.

Long-Term Exposure at 37°C

Blood from known donors was aliquoted on washed cotton cloth, air dried, and stored in an incubator at 37°C for 4 years. A portion of the stains was removed for RFLP analysis; this portion was approximately 50 μL of blood from each of the 10 different samples.

Contamination Studies

Nonbiological Contaminants

Experience with forensic science samples indicates that a contaminant will most likely already be on a particular substrate at the time the body fluid is deposited. Blood, obtained as before, from three individuals was prepared as 50-μL stains on cotton cloth which had already been contaminated with 50 μL of one of the contaminants, with the exception of soil. In that case, 50 μL of blood was added to 0.1 g of air-dried soil. The contaminants used in this study were representative of a variety of commonly encountered substances, including unleaded gasoline, motor oil, detergent (10% sodium dodecyl sulfate), chlorine bleach, salt (0.1*M* phosphate buffer), base [1*M* sodium hydroxide (NaOH)], acid (1*M* glacial acetic acid), and soil. This experiment was designed in a manner similar to that described by Budowle and Allen [*10*]. All the stains were allowed to air dry at room temperature for 5 days prior to analysis. Three replicate samples were prepared from

each donor for the 8 contaminants. In addition, uncontaminated bloodstains and each of the contaminants were sampled as controls. The number of bloodstains tested in this study was 104 samples, which included 32 control samples. All the samples were hybridized with an SLP complementary to the genetic locus D2S44.

A similar study was performed subsequently using actual casework RFLP procedures. This later study included a single blood donor, and the stains were prepared as described above. An additional set of samples included blood mixed with soil that had been previously autoclaved in order to destroy soil enzymes. A total of 41 stains, including controls, was analyzed and hybridized with SLPs complementary to the genetic loci D2S44, D17S79, D1S7, D4S139, and D10S28.

Biological Contaminants

Each of four different microorganisms was mixed with 20 samples of blood and 20 samples of semen to determine their effects on RFLP analysis of DNA from these body fluid stains. Two microorganisms used in this study that are found in the vaginal vault are *Staphylococcus epidermidis* and *Candida valida* (yeast).[4] In addition, *Escherichia coli* and *Bacillus subtilis* were used in this study.

The three bacteria were maintained in liquid Luria-Bertani (LB) culture medium [*11*], and the yeast was grown in a liquid Sabouraud medium at the temperatures recommended by the supplier. The approximate cell concentration of each culture was determined by turbidity measurements at 600 nm [*11*]. A sample of each microbe (25 μL) was removed and mixed with blood (50 μL) and semen (10 μL) from known donors. The microbe concentrations ranged from 1.8 million cells/μL to 16 million cells/μL. Five replicate stains were made for each microbe/body fluid mixture on cotton cloth. The stains were air dried and stored at room temperature for 5 days prior to RFLP analysis. Controls consisted of unmixed stains of blood, semen, and the microbes. A total of 56 samples was analyzed at the genetic locus D2S44.

A similar study was performed at a later date, again to simulate actual casework RFLP procedures. This study also utilized *S. epidermidis*, *B. subtilis*, and *E. coli*, but substituted *Candida albicans* for *C. valida*. Blood (50 μL) and semen (10 μL) from a single donor were mixed with estimated concentrations of each microorganism (25 μL), ranging from 0.4 million cells/μL (*B. subtilis*) to 2.5 million cells/μL (*C. albicans*). A total of 56 stains, including blood, semen, and microorganism controls, was analyzed. Each sample was hybridized with SLPs complementary to the genetic loci D2S44, D17S79, D1S7, D4S139, and D10S28.

Mixed Body Fluids

An initial study was performed in which mixed body fluid stains were prepared from semen, blood, urine, saliva, and vaginal secretions previously obtained from separate donors. The stains consisted of 10 μL aliquots from two different body fluids (except vaginal secretions) from separate donors. Ten microlitres of either blood, saliva, urine, or semen from a different donor were added to vaginal swabs. Additional stains were prepared using 1 μL of blood mixed with the other fluids. All the stains were air dried and stored at room temperature for 5 days prior to analysis. Unmixed body fluid stains served as controls. All the samples were hybridized using an SLP complementary to the genetic locus D2S44.

A more comprehensive mixed body fluid stain study was performed at a later date using casework RFLP analysis procedures. In this study, different concentrations of blood

[4]Brian Parkin, Metropolitan Police Laboratory, London, England, personal communication, 1988.

and semen were used, mixed with vaginal and oral swabs and each other. Body fluids were obtained from a male and a female donor and mixed in the following ten combinations: (*a*) 50 μL of blood (male) and 25 μL of blood (female), (*b*) 25 μL of blood (male) and 50 μL of blood (female), (*c*) ½ vaginal swab and 80 μL of blood (male), (*d*) ½ vaginal swab and 40 μL of blood (male), (*e*) 1 whole vaginal swab and 15 μL of semen, (*f*) 1 whole vaginal swab and 5 μL of semen, (*g*) 1 whole oral swab (female) and 10 μL of semen, (*h*) 1 whole oral swab (female) and 3 μL of semen, (*i*) 50 μL of blood (female) and 3 μL of semen, and (*j*) 25 μL of blood (female) and 8 μL of semen. Each mixture was represented by three replicate samples and allowed to air dry for 5 days prior to analysis. A differential extraction procedure [*8*] was performed on the triplicate specimens containing semen, and an additional mixed stain was extracted using the standard extraction procedure. A total of 74 stains, including controls, was analyzed in this study and hybridized with SLPs complementary to the genetic loci D2S44, D17S79, D1S7, D4S139, and D10S28.

Cross Reactivity Study—Nonhuman Blood Samples

Dried blood stains from a Celebes ape, Japanese macaque, gorilla, spider monkey, DeBrazza monkey, rabbit, domestic cat, scarlet macaw, dog, ferret, cockatoo, steer, horse, goat, burro, pig, chicken, sheep, and deer were subjected to RFLP analysis. These samples were kindly provided by the National Institutes of Health Animal Center, Poolesville, Maryland. Human DNA samples served as controls. The samples were hybridized with SLPs complementary to the genetic loci D2S44, D14S13, D16S85, and D17S79. A later study also was performed using SLPs complementary to the genetic loci D2S44, D17S79, D1S7, D4S139, and D10S28.

Results and Discussion

Environmental Studies

Bloodstains maintained at ambient outdoor temperatures (within a greenhouse) in light and dark conditions during March through May 1988 were exposed to identical temperatures, which averaged approximately 21°C for the daily high temperature during the 10-week study period. After 8 weeks of exposure to sunlight, the DNA was degraded to such a degree that no RFLP banding patterns could be observed using autoradiography. However, DNA of sufficient molecular weight for analysis was recovered from stains maintained in the dark for the entire 10-week sample period. All the RFLP patterns obtained were consistent with those of the control bloodstains from the same donor.

In the initial short-term sunlight exposure experiment, bloodstains maintained in light and dark conditions during the month of July 1988 were exposed to an average daily high temperature of 41°C and an average daily low temperature of 23°C. The DNA from bloodstains maintained in the sunlight for 12 days was degraded and did not produce RFLP patterns. DNA from samples stored at the same temperature in the dark produced RFLP patterns consistent with those of the control samples. All other stains in this study that produced DNA profile results had results consistent with those for the control stains.

In the subsequent short-term sunlight exposure experiment, bloodstains were exposed to direct sunlight rather than being exposed to sunlight filtered through greenhouse glass. In addition, the average daily high temperature in the second study was approximately 10°C less than that in the previous study. All bloodstains in the second study, regardless of exposure conditions, produced DNA profile results that were consistent with those of the control stains (Fig. 1). A comparison of the estimated fragment sizes between the control stains and the treated stains determined that no treated sample had DNA frag-

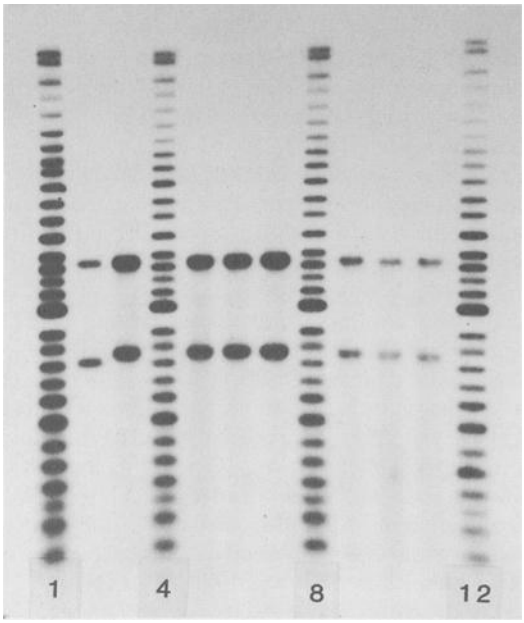

FIG. 1—*DNA profile results for the genetic locus D2S44 for bloodstains maintained in sunlight (Lanes 9, 10, and 11) and in the dark (Lanes 5, 6, and 7) for 9 days. Lanes 1, 4, 8, and 12 contain size markers. Lane 2 is the K562 human cell line control. Lane 3 is the untreated bloodstain control.*

ments that differed from those of the control by more than the 5% matching criterion established by the FBI Laboratory [*12*], and in fact, the maximum observed difference (positive or negative) between the control and treated samples by SLP for any fragment was 1.79% (D2S44), 3.02% (D17S79), 2.67% (D1S7), 4.12% (D4S139), and 1.55% (D10S28). The average difference {±SD} between the control and treated samples by SLP was 0.71% {0.48} (D2S44), 0.94% {0.68} (D17S79), 0.73% {0.58} (D1S7), 1.17% {0.96} (D4S139), and 0.58% {0.37} (D10S28). For all the SLPs used in this study, 80% of all the values for the treated stains were within ±1.5% of the control sample values.

In the time, temperature, and substrate study, the daily high temperature during the 5-month sample period (April–August 1988) for those stains maintained outdoors ranged from 23 to 41°C. All the samples that produced an RFLP profile result were consistent in profile results with the untreated liquid blood samples of the same origin. Stains from all six substrates produced DNA profiles consistent with those of the control samples, even after 5 months. A consistently more intense DNA profile (that is, with more DNA extracted) was obtained from those stains maintained at room temperature in comparison with those maintained at the other temperatures (4°C, 37°C, and ambient outdoor temperature). This was especially true for stains prepared on nonporous substrates. Specimens maintained at 4°C on the nonporous substrates were noticeably moist when collected. The DNA was more difficult to recover from bloodstains produced on cotton and blue denim and maintained at the higher temperatures than from bloodstains on the other substrates. Thus, some of these samples yielded no profiles. All DNA profiles produced from all substrates at all temperatures were consistent with those of the untreated control samples. Only low-molecular-weight DNA could be detected from prepared bloodstains maintained at 37°C for 4 years. No RFLP results were obtained from these samples.

Contamination Studies

In the initial nonbiological contamination study, high-molecular-weight DNA was recovered from all contaminated bloodstained material except for the samples in which blood had been mixed with soil. The DNA in these latter samples could have been degraded by enzymes present in the soil, or the extraction of DNA could have been physically inhibited by soil components, or both factors could have been in operation. No RFLP results were obtained from these soil-contaminated samples. The soil control samples, which contained endogenous DNA, also yielded no RFLP patterns. All other samples produced RFLP results consistent with those of the uncontaminated bloodstains from the same origin.

An analysis of the results from the second nonbiological contamination study showed similar results (Figs. 2 through 4). However, this study clearly demonstrated, since the soil sample was autoclaved first, that the reason RFLP patterns were not obtained was because components of the soil physically inhibited DNA extraction rather than that DNA was degraded by enzymes present in the soil. No RFLP results were obtained from blood mixed with previously autoclaved soil (Fig. 5). A comparison of the estimated fragment sizes of control stains and contaminated stains determined that no contaminated sample had DNA fragments that differed from those of the control by more than the 5% matching criterion. The maximum observed difference (positive or negative) between the control and treated samples by SLP was 1.93% (D2S44), 1.61% (D17S79), 2.30% (D1S7), 1.53% (D4S139), and 1.41% (D10S28). The average difference {±SD} between the control and treated samples by SLP was 0.39% {0.38} (D2S44), 0.49% {0.43} (D17S79), 0.53% {0.40} (D1S7), 0.40% {0.35} (D4S139), and 0.42% {0.37} (D10S28). For all the SLPs used in this study, 97% of all the treated stain values were within ±1.5% of the control sample values.

Blood and semen mixed with either bacteria or yeast produced RFLP profiles consistent with those of uncontaminated body fluid samples. DNA isolated from the purified cultures

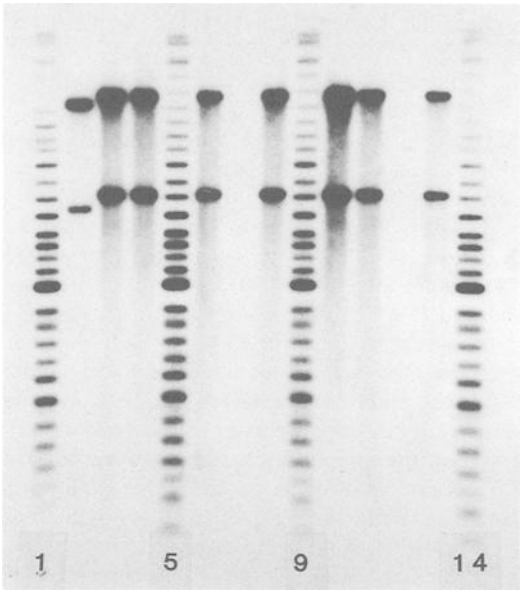

FIG. 2—*DNA profile results for the genetic locus D4S139 for gasoline (Lanes 3, 4, and 6) and motor oil (Lanes 8, 10, and 11) contaminated bloodstains. Lanes 1, 5, 9, and 14 contain size markers. Lane 2 is the K562 human cell line control. Lane 13 is the untreated bloodstain control. Lanes 7 (blank) and 12 (blank) contain control stains of gasoline and motor oil, respectively.*

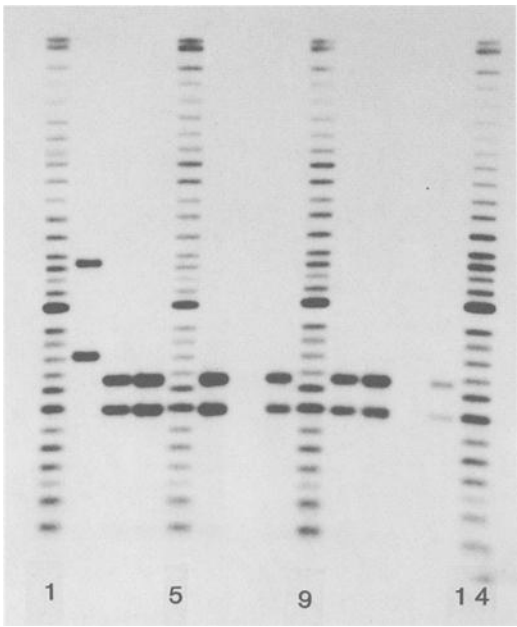

FIG. 3—*DNA profile results for the genetic locus D2S44 for 0.1M sodium phosphate (Lanes 3, 4, and 6) and 1.0M sodium hydroxide (Lanes 8, 10, and 11) contaminated bloodstains. Lanes 1, 5, 9, and 14 contain size markers. Lane 2 is the K562 human cell line control. Lane 13 is the untreated bloodstain control. Lanes 7 (blank) and 12 (blank) contain control stains of 0.1M sodium phosphate and 1.0M sodium hydroxide, respectively.*

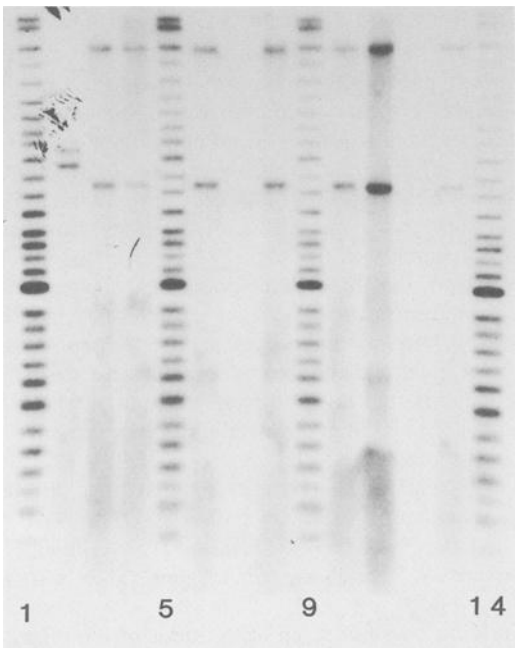

FIG. 4—*DNA profile results for the genetic locus D1S7 for 10% sodium dodecyl sulfate (SDS) (Lanes 3, 4, and 6) and household bleach (Lanes 8, 10, and 11) contaminated bloodstains. Lanes 1, 5, 9, and 14 contain size markers. Lane 2 is the K562 human cell line control. Lane 13 is the untreated bloodstain control. Lanes 7 (blank) and 12 (blank) contain control stains of SDS and bleach, respectively.*

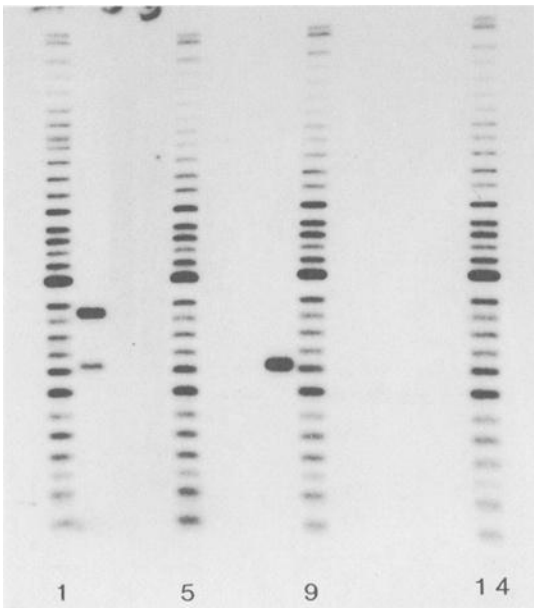

FIG. 5—*DNA profile results for the genetic locus D17S79 for autoclaved soil (Lanes 3, 4, and 6) (blank) contaminated blood samples. Lanes 1, 5, 9, and 14 contain size markers. Lane 2 is the K562 cell line control. Lane 8 is the untreated bloodstain control. Lane 7 is the control sample of autoclaved soil. Lanes 10, 11, 12, and 13 are empty lanes.*

of the microorganism controls did not hybridize with the SLP utilized in either the initial or subsequent analyses (Fig. 6). In the second study, DNA from the *E. coli* samples did hybridize with the size marker probe (a combination of lambda and phi X DNA digests), producing a series of 7 fragments, ranging from approximately 1600 to 600 base pairs in size (Figs. 7 through 9). The *E. coli* probably carried a virus that contains DNA that hybridizes with lambda or phi X DNA or both. This series of bands is a repeatable pattern following *HaE*III digestion and would not be mistaken for a human DNA profile. Also, the pattern shows up repeatedly in the same positions upon subsequent hybridizations with each SLP. DNA from the three other microorganisms used in this study did not hybridize with the size marker probe. As in the earlier study, no SLP used in this study cross-reacted with DNA from any microorganism used. A comparison of the estimated fragment sizes of control stains and contaminated stains determined that no contaminated sample had DNA fragments that differed from those of the control by more than the 5% matching criterion. The maximum observed difference (positive or negative) between the control and treated samples by SLP was 1.77% (D2S44), 2.55% (D17S79), 2.14% (D1S7), 2.53% (D4S139), and 1.80% (D10S28). The average difference {±SD} between the control and treated samples by SLP was 0.58% {0.38} (D2S44), 0.70% {0.53} (D17S79), 0.49% {0.42} (D1S7), 1.01% {0.60} (D4S139), and 0.53% {0.40} (D10S28). For all the SLPs utilized in this study, 92% of all the treated stain values were within ±1.5% of the control sample values.

RFLP patterns could be obtained from each donor of the mixed body fluid samples, which contained vaginal secretions, saliva, blood, and semen. No profiles were observed from urine or small quantities (1 μL) of blood during the initial study. In the more comprehensive study, the mixing of blood and other body fluids did not alter the RFLP band positions, in comparison with those of control samples from the same donors, in

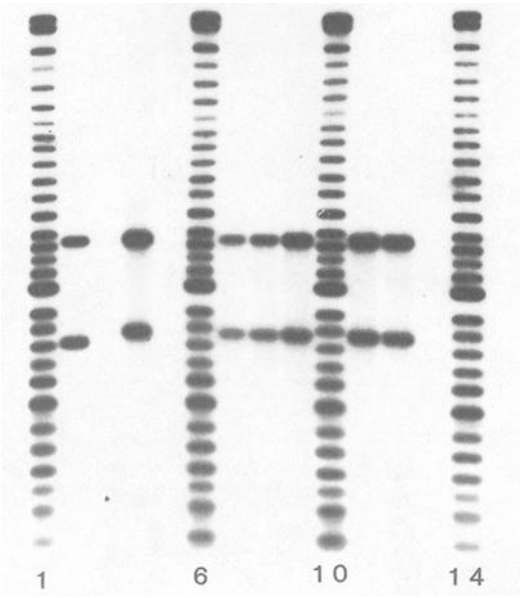

FIG. 6—*DNA profile results for the genetic locus D2S44 for yeast (Lanes 7, 8, 9, 11, and 12) contaminated semen stains. Lanes 1, 6, 10, and 14 contain size markers. Lane 2 is the K562 cell line control. Lane 4 is the uncontaminated semen stain control. Lane 3 (blank) is the yeast control. Lanes 5 and 13 are empty lanes.*

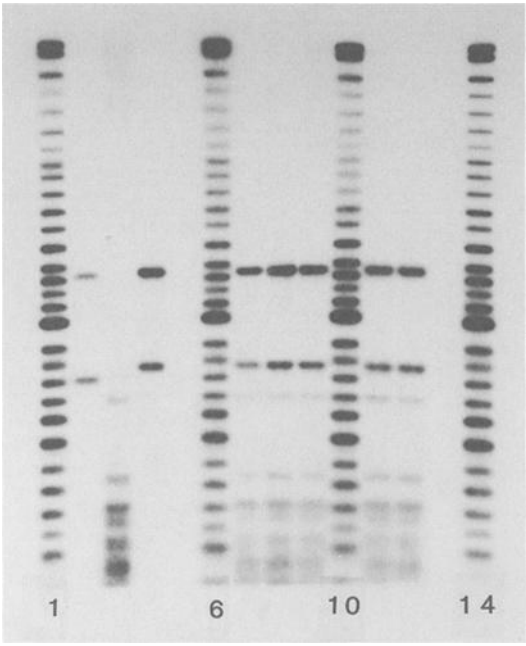

FIG. 7—*DNA profile results for the genetic locus D2S44 for bacterium (*E. coli*) (Lanes 7, 8, 9, 11, and 12) contaminated bloodstains. Lanes 1, 6, 10, and 14 contain size markers. Lane 2 is the K562 cell line control. Lane 3 is the bacterium control. Lanes 5 and 13 are empty lanes.*

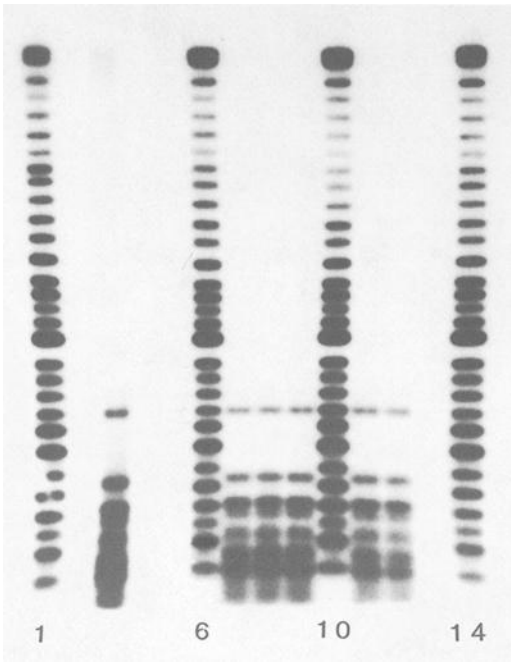

FIG. 8—*DNA profile results for a membrane identical to that in Fig. 7. However, this membrane was only hybridized with the size marker probe. No hybridization occurred with human VNTR markers.*

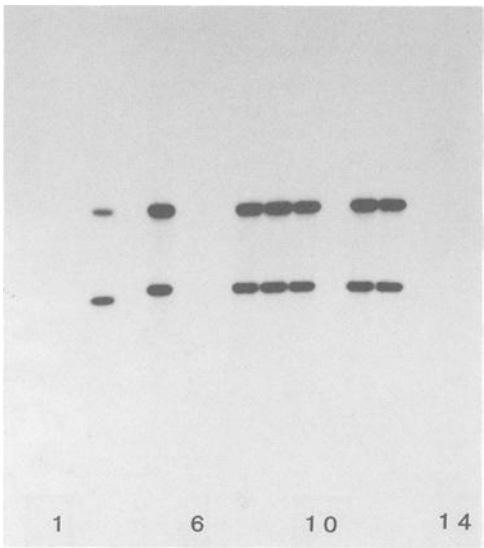

FIG. 9—*DNA profile results for a membrane identical to that in Fig. 7. However, this membrane was only hybridized with the probe complementary to the D2S44 locus. No size marker probe was used in the production of this autoradiograph.*

DNA samples extracted together (Fig. 10) or differentially (Fig. 11). A comparison of the estimated fragment sizes of control stains and mixed stains determined that no mixed sample had DNA fragments that differed from those of controls by more than the 5% matching criterion. The maximum observed difference (positive or negative) between the control and mixed samples for both donors by SLP was 2.13% (D2S44), 2.01% (D17S79), 3.88% (D1S7), 3.63% (D4S139), and 2.86% (D10S28). The average difference {±SD} between the control and mixed samples for both donors by SLP was 0.65% {0.50} (D2S44), 0.64% {0.47} (D17S79), 1.21% {0.86} (D1S7), 1.20% {0.90} (D4S139), and 0.93% {0.62} (D10S28). For all SLPs used in this study, 81% of all mixed stain values were within ±1.5% of the control sample value.

When blood samples from nonhuman sources were analyzed, it was determined that certain primates (Celebes ape, Japanese macaque, gorilla, and DeBrazza monkey) produced RFLP patterns when hybridized with the SLPs used here. DNA isolated from domestic and wild game animal bloods did not produce DNA profiles at these loci, with one exception. Profile results for the genetic locus D17S79 for the different bird species and domestic cat would exhibit multiple DNA fragments after long autoradiography exposures.

Measurement Variation Comparisons in All Studies

With more than 1600 measurements of treated DNA samples, an analysis was performed to determine the extent of measurement variation of treated samples from control samples. A total of 86% of all within-gel measurements were within ±1.5% of the control sample values (Fig. 12). No variation exceeded 4.5%, and 32 treatment measurements did not vary at all from those of the control samples.

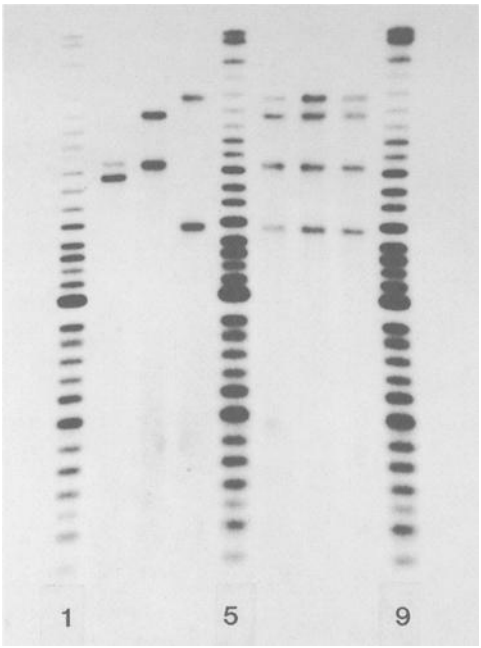

FIG. 10—*DNA profile results for the genetic locus D1S7 for stains containing blood from two sources (Lanes 6, 7, and 8). The mixed stains contained 25 μL of blood from the female donor and 50 μL of blood from the male donor. Lanes 1, 5, and 9 contain size markers. Lane 2 is the K562 cell line control. Lane 3 and 4 are the female and male donor controls, respectively.*

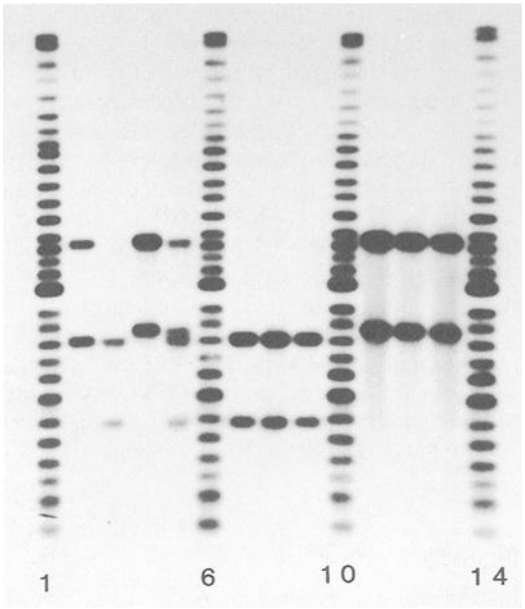

FIG. 11—*DNA profile results for the genetic locus D2S44 for stains containing a mixture of semen and vaginal fluid. Three mixed stains were differentially extracted and are represented by Lanes 7, 8, and 9 (female fraction) and Lanes 11, 12, and 13 (male fraction). Lanes 1, 6, 10, and 14 contain size markers. Lane 2 is the K562 cell line control. Lanes 3 and 4 are the female and male donor controls, respectively. Lane 5 contains a mixed stain using the nondifferential extraction procedure.*

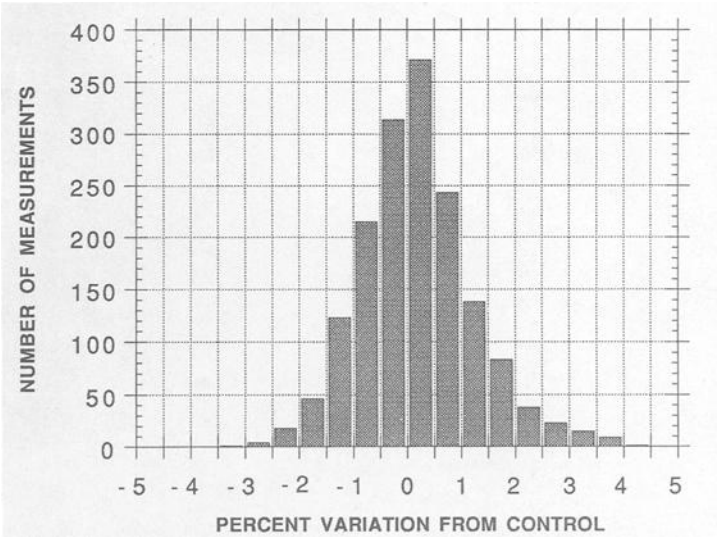

FIG. 12—*Percentage of the measurement variation of treated samples from control values in the mixed body fluid study, biological contaminant study, chemical contaminant study, and sunlight study.*

TABLE 1—*Comparison of the three body fluid donors by probe to determine maximum measured difference, in base pairs (bp), between DNA fragments (treated or controls) in all studies.*

Source	Upper Band, bp	Difference, %	Lower Band, bp	Difference, %
Source A				
D2S44	2993		1906	
	2908	2.8	1856	2.6
D17S79	1328		825	
	1286	3.2	794	3.8
D1S7	7235		3296	
	6963	3.8	3209	2.6
D4S139	7746		6544	
	7386	4.6	6259	4.4
D10S28	4791		. . .	
	4597	4.0	. . .	
Source B				
D2S44	1827		1191	
	1783	2.4	1171	1.7
D17S79	1665		1327	
	1635	1.8	1294	2.5
D1S7	6390		4582	
	6112	4.4	4436	3.2
D4S139	8805		3412	
	8412	4.5	3327	2.5
D10S28	4691		2963	
	4523	3.6	2869	3.2
Source C				
D2S44	1604		1353	
	1573	1.9	1329	1.8
D17S79	1560		. . .	
	1529	2.0	. . .	
D1S7	10061		3874	
	9793	2.7	3799	1.9
D4S139	7102		3780	
	6968	1.9	3713	1.8
D10S28	2271		. . .	
	2236	1.5	. . .	

The variation in measurements is expected to be greater in between-gel comparisons than in within-gel comparisons. Therefore, taking into account each of the three DNA donors for all studies (that is, between-gel studies), a comparison was made between the extreme values of any sample measurement (treated or control) for each locus. All comparisons of these extreme values fell within the 5% matching criterion established by the FBI Laboratory (Table 1).

Conclusions

The validation studies described here demonstrate that evidentiary samples exposed to a wide variety of external influences can yield DNA that gives a valid and reliable RFLP pattern. Furthermore, if the DNA has been too adversely affected by these influences, no RFLP pattern is obtained. Interpretable RFLP profiles did not change when body fluid stains were exposed to the environmental conditions or contaminants described here. The DNA profiles produced from the test samples were consistent with those of the control samples or no profiles were produced.

This study was not designed to consider all possible contaminants or environmental insults, but a number of extreme conditions were tested to evaluate the robustness of

the RFLP typing system. At no time were false positive or false negative results produced. Further support for the reliability of the typing system has been repeatedly demonstrated by RFLP analysis of actual casework samples in the DNA Analysis Unit of the FBI Laboratory. Because all possible conditions cannot be experimentally tested, a comparison of vaginal fluid DNA (predominantly epithelial cells) from sexual assault evidence with the DNA of known blood samples taken from the victims of these crimes was done to demonstrate the reliability of the RFLP technique in forensic science examinations. In an examination of more than 100 cases involving sexual assault evidence, no DNA profiles produced from the non-sperm portion of the DNA extract taken from the evidence differed beyond the established matching criterion from that of the known blood sample of the victim [*12*].

When performed properly under similar conditions and using the necessary quality controls throughout the process, DNA analysis of forensic science evidence by the RFLP procedure can provide the forensic examiner with a reliable method for characterization of body fluid stains.

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