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Validation Studies on the Forensic Analysis of Restriction Fragment Length Polymorphism (RFLP) on LE Agarose Gels Without Ethidium Bromide: Effects of Contaminants, Sunlight, and the Electrophoresis of Varying Quantities of Deoxyribonucleic Acid (DNA)

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ABSTRACT: This study was designed to analyze the effects of sunlight, various contaminants (those found typically in forensic samples) and the electrophoresis of varying quantities of DNA on the restriction fragment length polymorphism (RFLP) patterns produced from DNA isolated from blood and semen stains. The DNA RFLP patterns were obtained following *Hae III* restriction enzyme digestion, low electroendosmotic (LE) agarose gel electrophoresis (in the absence of ethidium bromide), Southern transfer, hybridization with DNA probes detecting highly polymorphic variable number of tandem repeats (VNTRs) and autoradiography. Computer assisted image analyses were used to detect variations in RFLP band sizes in relation to control samples. Comparisons between the samples were made for the presence of high molecular weight DNA, the ability to achieve a complete restriction digestion, and the RFLP fragment sizes obtained. The results demonstrate that high molecular weight DNA can be obtained when blood and semen stains are subjected to environmental and contaminating factors. The RFLP allele sizes were not significantly affected by environmental conditions, contamination factors or by loading varying amounts of DNA. This study serves to further document the reliability and validity of DNA typing for forensic applications.

KEYWORDS: pathology and biology deoxyribonucleic acid (DNA), restriction fragment length polymorphism (RFLP), blood, semen, contaminants, environmental, DNA quantity

As a restriction fragment length polymorphism (RFLP) protocol for DNA analysis is developed, a decision must be made on whether or not to incorporate ethidium bromide into the analytical gel prior to electrophoresis. Numerous studies have been published

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demonstrating that the electrophoresis of varying amounts of DNA, recovered from forensic type samples which had been subjected to contaminants and environmental insults, resulted in no significant band shifts whether or not ethidium bromide was contained in the analytical gels [*1–5*]. However, it has been reported that band shifting may occur if a large excess of DNA is electrophoresed in agarose gels containing ethidium bromide [*6*]. Alternatively, no band shifting was observed when a large excess of DNA was electrophoresed in gels that did not contain ethidium bromide [*6*]. In a previous paper [*7*] we reported that the method of DNA extraction may have an effect on RFLP patterns. In that study, one DNA extraction method yielded RFLP patterns with observable band shifts in non-ethidium bromide gels and not in gels that contained ethidium bromide.

In a National Institute of Standards and Technology (NIST) study involving 17 forensic laboratories [*8*] approximately three quarters of the laboratories used non-ethidium bromide analytical gel electrophoresis for RFLP analysis. In addition, a report released by the National Research Council [*9*] strongly recommends that ethidium bromide not be used in DNA electrophoresis when performing forensic RFLP analysis.

The validation of specific RFLP procedures is required prior to their implementation in the forensic laboratory. This paper documents the effects of contaminants and environmental insults on the measurement of DNA RFLP fragment sizes when using the restriction endonuclease *Hae III* and agarose gel electrophoresis in the absence of ethidium bromide.

In this study, DNA was recovered from blood and semen stains that were subjected to various contaminants and environmental insults. It will be demonstrated that the electrophoresis of *Hae III* restriction digested DNA fragments in agarose gels without ethidium bromide resulted in no significant mobility shifts when compared to blood and semen controls. One contaminant (Derma Soothe lotion) did cause a shift of one allele in the high molecular weight range (above 10kb) for the locus D4S139. As will be demonstrated, this did not present a problem in the interpretation of the results due to the ease of recognition and correction of this situation. This paper serves to further document the previous report of Waye and Fourney [*6*] that the electrophoresis of varying amounts of DNA produced no appreciable band shifts in gels when ethidium bromide was absent. This paper also further validates the reliability of forensic DNA typing.

Materials and Methods

Sample Preparation

One person donated blood and semen for use throughout the contamination and environmental parts of this study. Nine different individuals donated blood for use in the lotion study. The RFLP patterns obtained from the DNA isolated from 50 μL untreated bloodstain controls were compared with the RFLP patterns obtained from treated 50 μL blood and 25 μL semen stains. All stains were prepared using liquid blood and semen that were mixed constantly during stain preparation to ensure the uniform deposition of material.

Extraction from Liquid Blood

Vacutainer (Becton Dickinson) ethylenediaminetetraacetate (EDTA) tubes containing freshly drawn blood were centrifuged for 20 min at 2000 rpm. Approximately 1 mL of the white cell layers were removed and placed in 15 mL conical centrifuge tubes. Red cell lysis buffer [0.144M ammonium chloride (NH_4Cl), 0.001M sodium bicarbonate ($NaHCO_3$)] was added to the white cell layers to make a final volume of 9 mL. The samples were incubated at ambient temperatures for 20 min with occasional mixing,

centrifuged for 20 min at 2000 rpm and the supernatants discarded. To the retained cell pellet, 3 mL of nuclei lysis buffer (10mM tris(hydroxymethyl)-aminomethane (Tris), 400mM sodium chloride (NaCl), 2mM Na_2EDTA to pH 8.2), 200 μL of 10% sodium dodecyl sulfate (SDS), and 600 μL of proteinase K solution [2 mg Pro K/1 mL Pro K buffer (1% SDS, 2mM Na_2EDTA)] were added to each tube and the extracts incubated overnight at 37°C. Following incubation, each sample received 1 mL of saturated NaCl, was shaken for 15 s, and centrifuged at 2500 rpm for 15 min. The supernatants were transferred to new 15-mL conical tubes and the DNA precipitated with 2 volumes of room temperature absolute ethanol. The precipitated DNA was spooled from solution, placed in 1.5 mL tubes, and resolubilized in 400 μL of Tris-EDTA (TE) buffer (10mM Tris, 1mM Na_2EDTA, pH 7.5). Aliquots were removed for yield gel and spectrophotometric quantification. The remaining DNA was stored at 4°C until analyzed.

Extraction from Bloodstains

Each 50 μL bloodstain was cut into small pieces and placed in a 1.5 mL tube with 400 μL of stain extraction buffer (10mM Tris, 10mM EDTA, 0.1M NaCl, 2% SDS) and 10 μL Proteinase K (20 mg/mL). The tubes were centrifuged for 2 s and incubated overnight at 56°C. The samples were then piggyback centrifuged at 3000 rpm for 3 min to remove the liquid from the cloth. The DNA was extracted once with 500 μL of phenol/chloroform/isoamyl alcohol (25:24:1) and precipitated with two volumes of cold absolute ethanol at −20°C for 30 min. The DNA was recovered by centrifugation at 14,000 rpm for 15 min and the pellet washed with 1.0 mL of 70% ethanol and dried in a vacuum centrifuge (1400 rpm for 15 min). Upon observation, if the DNA pellet was discolored or appeared to still contain contaminant, the DNA sample was subjected to a double extraction by resolubilizing the pellet in 500 μL of TE and reextracting the sample with 500 μL of phenol/chloroform/isoamyl alcohol. The DNA was recovered by adding 50 μL of 8.0M lithium chloride (LiCl) and reprecipitating with absolute ethanol as previously described. When the pellets appeared uncontaminated, (either after a single or double extraction), they were resolubilized in 449 μL TE buffer and a 10 μL aliquot was removed for yield gel quantification. The remaining DNA was stored at 4°C until analyzed.

Extraction from Semen Stains

Each 25 μL semen stain was cut into small pieces and placed in a 1.5 mL tube with 400 μL of TNE (10mM Tris, 0.1M NaCl, 1.0mM EDTA), 25 μL 20% Sarkosyl (N-Laurylsarcosine), 75 μL H_2O, and 5 μL Proteinase K (20 mg/mL). The tubes were vortexed and incubated at 37°C overnight. The samples were piggyback centrifuged at 3000 rpm for 3 min to remove the liquid from the cloth. The supernatant (female fraction) was discarded and the sperm pellet and cloth piece combined. One hundred fifty microliters of TNE, 50 μL 20% sarkosyl, 40 μL 0.39M dithiothreitol, 150 μL H_2O, and 10 μL proteinase K were added to the pellet and cloth. The samples were vortexed, incubated at 37°C overnight, and piggyback centrifuged at 3000 rpm to remove liquid from the cloth. The DNA was extracted with 500 μL of phenol/chloroform/isoamyl alcohol and precipitated with 1.0 mL of absolute ethanol at −20°C for 30 min. The DNA was recovered by centrifugation (14,000 rpm for 15 min) and the pellet was washed with 1.0 mL of 70% ethanol and dried in a vacuum centrifuge (1400 rpm for 15 min). The DNA pellets were resolubilized in 459 μL TE buffer and a 10 μL aliquot was removed for yield gel quantification. The remaining DNA was stored at 4°C until analyzed.

Yield Gel Analysis of Samples

A 10 μL aliquot of DNA solution from each sample was combined with 2.5 μL of 5X loading buffer (50% glycerol, 30% TE, 20% 0.1M EDTA, 0.1% bromophenol blue) and loaded into wells on a 1% agarose gel containing ethidium bromide (1 μg/mL)[2]. Yield gel quantification standards of 4,6,8,10,15,20,25,30,35, and 50 ng of K562 genomic DNA were loaded in adjacent wells. Electrophoresis was carried out at 5.5 V/cm for 30 min in 1X TAE (0.04M Tris, 0.019M glacial acetic acid, 0.025M EDTA, pH 8.3 at 23°C) buffer. The gels were illuminated with UV light and photographed. The photographs were used to assess the quantity and quality of DNA in the samples.

Restriction Digestion with HAE III

To the remaining 449 μL of each DNA sample, 1 μL of the restriction endonuclease *HAE III* (40 U/μL) and 50 μL of 10X Restriction Buffer [0.5M Tris, 0.1M magnesium chloride ($MgCl_2$), 0.5M NaCl] were added and the samples incubated overnight at 37°C. An additional 1 μL of *HAE III* was then added to each sample and the samples incubated for two hours at 37°C. The DNA was precipitated with 50 μL of 8.0M LiCl and two volumes of −20°C absolute ethanol. The alcohol was removed after centrifugation and the DNA was resolubilized with 16 μL of TE. Two microliters of the samples were electrophoresed as previously described for yield gels.

Photographs were taken and used to assess the completeness of the restriction digestion. If there was evidence of incomplete digestion, the DNA samples were brought back to 449 μL with TE and the restriction digestion repeated.

Resolution of DNA Fragments

Electrophoretic separation of the DNA fragments was accomplished using 12 × 20 cm gels prepared with 1% LE agarose (BRL) and 1X TAE buffer. No ethidium bromide was added. The electrophoresis was carried out in 1X TAE buffer for 16 hours at 26 volts (across gel).

Southern Transfer of DNA

The DNA was transferred from the analytical gel to a nylon membrane (Biodyne B, Pall Biosupport) by first soaking each gel in a solution of 0.5N sodium hydroxide (NaOH) and 0.5M NaCl for 30 min. After denaturing, the DNA was transferred to the membrane support using transfer solution (0.5N NaOH, 0.5M NaCl). The transfer was accomplished by stacking two 3 mm thick sponges, one blot pad (BRL), and one sheet of 3MM paper in a tray containing transfer solution. The gel was inverted and placed on the stack followed by the nylon membrane, one sheet of 3MM paper, and approximately 10 blot pads. After 4 hours of transfer, the membranes were washed in 2X SSPE [0.3M NaCl, 0.02M sodium phosphate (NaH_2PO_4), 2mM EDTA] for 15 min and placed between two sheets of Whatman 3MM paper. The DNA was then fixed to the membrane by baking at 80°C for 30 min.

[2]Ethidium bromide is used in yield and test gels but not in analytical gels where the RFLP fragments are analyzed.

Probes

Six VNTR probes were utilized in this study: YNH24 (Promega; D2S44), MS1 (Cellmark; D1S7), V1 (Lifecodes; D17S79), TBQ7 (Promega; D10S28), CMM101 (Promega; D14S13), and pH-30 (BRL; D4S139). The probe MGB7 (Oncor; D7Z2) which identifies a repeat 2731 bp α satellite DNA fragment [*12*] was also used.

Hybridization

Probes were labeled using the BRL Random Primers DNA Labeling System as recommended by the manufacturer. Up to ten membranes were hybridized in 5 × 30 cm tubes using a Robbins incubator (Model 1040-00-1). Membranes were prehybridized in a solution containing 10% polyethylene glycol, 5x SSPE, 2% SDS, 1 mg/mL heparin, and 250 μg/mL salmon sperm DNA (Lifecodes) at 65°C for 15 min (4 to 5 mL per membrane). This solution was discarded and replaced with 20 mL of a 65°C hybridization solution (same as prehybridization solution) containing denatured (100°C for 10 min) labeled probe [1×10^6 dpm (disintegrations per minute) mL^{-1} of hybridization solution], denatured labeled size ladder probe [(BRL) 5×10^5 dpm mL^{-1}], and denatured salmon sperm DNA (250 μg/mL). Hybridization was carried out at 65°C for 15 to 20 h.

Washing

After removing the hybridization solution from the hybridization tubes, the membranes were briefly rinsed with 150 to 200 mL of wash solution B [2X SSPE, 0.5% SDS]. The liquid was then discarded and 250 mL of wash solution B added to the tubes. The tubes were then incubated with rotation at 65°C for 20 min and the liquid again discarded. The membranes were then removed and individually placed in a wash container with 500–1000 mL of wash solution A [0.1X SSPE, 0.5% SDS] for 20 min at 65°C. This was repeated two more times. The membranes were then blotted and wrapped in Glad cling wrap.

Autoradiography

The membranes were placed between two sheets of Kodak X-OMAT XAR-5 X-ray films and were light-secured inside Kodak X-ray cassettes equipped with dual X-Omatic regular intensifying screens. The films were exposed at −80°C for several hours to days to optimize the sensitivity and resolution of the autoradiograms. The films were developed in a Konica QX-70 automatic film processor.

Image Analysis

Size measurements of the bands were made using a video camera, a uniformly illuminated light box, IBM compatible personal computer and software developed by the FBI [*10*]. The analysis involved automatic lane location, density profile production by lane and band position detection. Standards and size markers run in five of the lanes allowed autoradiogram assessment and determination of fragment length.

Stripping

To allow for multiple hybridizations, probes were stripped from the membranes by incubation for 45–90 min at 65°C with a solution consisting of 55% Formamide, 5%

SDS, and 2X SSPE. The membranes were subsequently rinsed in 200 mL 0.1X SSPE, 0.5% SDS for 1 min at room temperature, blotted to remove excess fluid and either rehybridized immediately or wrapped in Glad cling wrap and stored at 4°C.

Contamination and Environmental Insult Analysis

Preparation and Handling of Samples

One person donated the blood and semen specimens that were used throughout this part of the study. All bloodstains were prepared using freshly drawn blood which contained no preservatives or anticoagulants. All semen stains were prepared from previously pooled ejaculates stored at −20°C, thawed, and used immediately after mixing. All stains were prepared on freshly laundered, rinsed, and dried, 100% cotton cloth.

Contaminants (Dried Stains)

Contaminants were applied so as to saturate individual pieces of clean cloth and then allowed to air dry overnight at room temperature. The contaminants were: soil solution, liquid hand soap, 1:10 dilution of Chlorox liquid bleach, hand lotion, coffee, whole milk, 1M sodium chloride (NaCl) solution, used motor oil, unleaded gasoline, and *Escherichia coli* bacteria (strain HB101, overnight culture, grown in Luria broth). Semen or fresh whole blood were applied to each contaminated cloth in amounts of 25 μL and 50 μL respectively. Ten duplicate blood and semen stains were made on each cloth contaminant. These samples were dried overnight at room temperature and then stored in the dark at room temperature until analyzed. Two bloodstains and two semen stains were cut from each cloth contaminant at intervals of 1, 4, 8, 14, and 28 days. The DNA from these samples was individually extracted, quantified, and subjected to *Hae III* restriction enzyme digestion. One of each of the duplicate blood and semen *Hae III* restriction digested DNA samples was processed through the entire RFLP procedure. The other duplicate DNA samples were stored at −20°C in the event that further testing proved necessary.

Contaminants (Wet)

Ten 50 μL bloodstains and ten 25 μL semen stains were prepared on each of four pieces of clean cloth and dried overnight at room temperature. Each of the four cloth pieces was then saturated with a different contaminant. The contaminants applied were: soil solution, 1:10 dilution of Chlorox liquid bleach, unleaded gasoline, and *E. coli* bacteria (same as above). The cloth samples were dried overnight at room temperature and then stored in the dark at room temperature. Two bloodstains and two semen stains on each cloth contaminant were processed at intervals of 1, 4, 8, 14, and 28 days as previously described for *Contaminants (Dried Stains).*

Environmental

Twenty 50 μL bloodstains and twenty 25 μL semen stains were prepared on clean cotton cloth, dried overnight at room temperature, and placed outdoors where they were exposed to daily temperatures that ranged from 10°C to 35°C. One set of 10 bloodstains and 10 semen stains were subjected to direct sunlight while a second identical set of stains were kept in the dark. Two bloodstains and two semen stains from each set (sunlight and dark) were processed at intervals of 1, 4, 8, 14, and 28 days as previously described for *Contaminants (Dried Stains).*

Standards

Control 50 μL bloodstains and 25 μL semen stains were prepared on clean cotton cloth. The stains were allowed to dry overnight at room temperature, stored in the dark at room temperature, and analyzed at intervals of 1, 4, 8, 14, and 28 days as previously described for the contaminated and environmental insult stains. DNA extracted from fresh liquid blood was *Hae III* restriction digested, stored at −20°C, and analyzed at the same time intervals.

DNA Quantity

DNA extracted from fresh liquid blood was quantified using a Hewlett-Packard 8451A spectrophotometer and by yield gel assessment of standards and unknowns according to Laber et al. [7]. The quantified DNA was subjected to *Hae III* restriction enzyme digestion and then loaded into wells on 1% LE agarose gels (lacking ethidium bromide) in the following quantities: 20 μg, 10 μg, 5 μg, 1 μg, 500 ng, 250 ng, 100 ng, 50 ng, and 25 ng in 16 μL total sample volume. The gels were processed to obtain RFLP patterns as previously described. The allele sizes obtained from each sample representing a different quantity of DNA were compared to the average allele size obtained from 500 ng DNA control samples.

Results and Discussion

Extraction

The quantities of high molecular weight (HMW) DNA recovered from 50 μL blood and 25 μL semen stains subjected to contamination and environmental insults over a 28 day time interval are shown in Table 1. The quantities of HMW DNA recovered from the untreated blood and semen stain controls stored at room temperature over the same time interval are also shown in Table 1. With some noted exceptions, almost all of the stains subjected to contaminants and environmental factors yielded HMW DNA over the 28 day time interval. However, no measurable HMW DNA was recovered from semen stains that were contaminated with liquid bleach. In addition, the DNA recovered from semen stains exposed to four days of direct sunlight and the DNA recovered from bloodstains exposed to fourteen days of direct sunlight was too degraded to quantify accurately. Also, less HMW DNA was obtained from the semen deposited on cloth contaminated with soil and used motor oil with increasing days of exposure.

In evaluating treated bloodstains for DNA yield over each time interval, approximately 97% of the samples yielded HMW DNA. Their average DNA yield was 372 ng (SD = 106) compared to 424 ng (SD = 49) for the untreated bloodstain controls. In evaluating the treated semen stains for DNA yield over each time interval, approximately 84% of the samples yielded HMW DNA. Their average DNA yield was 375 ng (SD = 84) compared to 598 ng (SD = 126) for the untreated semen stain controls. The data from Table 1 demonstrates that the vast majority of contaminants and environmental insults analyzed in this study had no appreciable effect on DNA yield.

Interestingly, the quantity and quality of DNA extracted from most contaminated stains did not seem to be significantly influenced by the exposure time to the contaminant prior to extraction. In fact, approximately one-third of the contaminated stains yielded greater quantities of DNA after 28 days of exposure than after 1 day of exposure. These data suggest that variation in DNA recovery may be due to normal variation in the method of DNA extraction or yield determination rather than the time a stain was exposed to a

TABLE 1—*Approximate DNA yield (in ng) from contaminated, environmental, and standard (untreated) 50-μL blood and 25-μL semen stains.*

	Time interval in days					
Contaminant-dried	1	4	8	14	28	Mean/SD
Soil + blood	690	690	1150	460	460	690/282
Soil + semen	370	460	180	90	...[b]	275/170
Soap + blood	370	370	180	370	690	396/184
Soap + semen	280	280	460	180	690	378/202
Bleach + blood	370	370	920	690	180	506/295
Bleach + semen	370	370	370	370	180	332/85
Lotion + blood	280	460	690	370	180	396/194
Lotion + semen	690	280	690	280	180	424/246
Coffee + blood	280	370	690	460	280	416/170
Coffee + semen	460	370	370	280	280	352/75
Milk + blood	280	370	690	370	280	398/169
Milk + semen	370	370	460	460	370	406/49
Salt soln. + blood	280	280	370	280	180	278/67
Salt soln. + semen	690	370	460	370	370	452/139
Used motor oil + blood	280	370	460	370	180	332/106
Used motor oil + semen	180	370	460	...[a]	...[b]	336/143
Gasoline + blood	280	280	280	370	180	278/67
Gasoline + semen	690	280	460	370	1150	590/348
E. coli + blood	460	180	460	280	370	350/121
E. coli + semen	460	180	370	460	370	368/114
Mean/SD	406/162	353/109	508/237	362/124	365/254	
Contaminant-wet						
Blood + soil	180	370	370	280	280	296/79
Semen + soil	280	280	180	180	...[b]	230/58
Blood + bleach	180	460	370	370	90	294/153
Semen + bleach	...[b]	...[b]	...[b]	...[b]	...[b]	...[b]
Blood + gasoline	180	180	370	370	180	256/104
Semen + gasoline	180	370	460	460	460	386/122
Blood + E. coli	460	180	370	370	370	350/102
Semen + E. coli	460	180	370	460	460	386/122
Mean/SD	274/132	288/114	355/84	356/99	306/154	
Environmental						
Blood + sunlight	280	280	460	...[a]	...[a]	340/103
Semen + sunlight	690	...[a]	...[a]	...[a]	...[b]	—
Blood + dark	280	280	460	690	180	378/202
Semen + dark	690	180	460	180	180	338/231
Mean/SD	485/236	383/212	460/0	435/361	180/0	
Standards						
Blood stains	460	460	370	460	370	424/49
Semen stains	690	690	460	460	690	598/49
Mean/SD	575/163	575/163	415/64	460/0	530/226	

[a]DNA was too degraded to estimate yield.
[b]DNA was totally degraded or no DNA was recovered.

given contaminant. One scientist performed all the DNA extractions and quantifications thus minimizing variation due to laboratory techniques.

Restriction Endonuclease Digestion

All samples yielding sufficient HMW DNA were digested to completion with *Hae III*. As noted by test gel analysis, all samples indicated complete digestion after a single

TABLE 2—*Average RFLP band sizes (in base pairs) from liquid blood standards (LBS), semen stain standards (SSS), and bloodstain standards (BSS) for the loci shown. Each value was calculated by averaging the base pair sizes obtained from gels run at 1, 4, 8, 14, and 28 day time intervals. Also shown is the greatest positive and negative percent differences from the mean, followed by the time interval in days (in parentheses) when the percent difference was observed.*

	D2S44	D1S7	D17S79	D10S28	D14S13	D4S139	D7Z2
				Band 1			
LBS	1567	5666	1479	3713	3417	10209	2738
% Diff-pos	+0.82(28)	+0.83(28)	+0.88(8)	+0.59(8)	+0.82(28)	+1.50(4)	+0.55(4)
% Diff-neg	−0.42(1)	−0.60(1)	−0.47(1)	−0.67(28)	−0.73(1)	−1.83(14)	−0.58(14)
				Band 2			
LBS	1133	3795	1284	1861	2161	3559	—
% Diff-pos	+0.88(28)	+0.74(28)	+0.78(1)	+0.91(8)	+0.69(28)	+0.56(8)	—
% Diff-neg	−0.71(1)	−0.40(8)	−0.70(1)	−0.64(1)	−0.79(1)	−0.45(8)	—
	D2S44	D1S7	D17S79	D10S28	D14S13	D4S139	D7Z2
				Band 1			
SSS	1576	5690	1489	3714	3418	10382	2738
% Diff-pos	+0.70(14)	+0.60(28)	+0.81(14)	+0.48(28)	+0.50(14)	+1.50(28)	+0.47(28)
% Diff-neg	−0.25(1)	−0.44(4)	−0.81(8)	−0.27(8)	−0.38(4)	−1.20(1)	−0.73(4)
				Band 2			
SSS	1139	3798	1289	1869	2164	3559	—
% Diff-pos	+0.88(14)	+0.47(14)	+0.70(14)	+0.59(14)	+0.55(14)	+0.39(14)	—
% Diff-neg	−0.44(8)	−0.47(8)	−0.39(8)	−0.37(4)	−0.28(28)	−0.56(8)	—
	D2S44	D1S7	D17S79	D10S28	D14S13	D4S139	D7Z2
				Band 1			
BSS	1573	5696	1483	3717	3420	10333	2743
% Diff-pos	+0.51(14)	+0.68(28)	+0.54(14)	+0.78(4)	+0.20(4)	+2.16(28)	+0.36(28)
% Diff-neg	−0.32(1)	−0.53(4)	−0.54(8)	−0.40(8)	−0.38(8)	−1.08(8)	−0.36(4)
				Band 2			
BSS	1131	3802	1287	1865	2168	3571	—
% Diff-pos	+0.97(14)	+0.47(14)	+1.09(14)	+0.48(14)	+0.46(14)	+0.17(28)	—
% Diff-neg	−0.97(8)	−0.34(4)	−1.00(8)	−0.21(8)	−0.23(8)	−0.06(8)	—

addition of enzyme with the exception of DNA recovered from soil contaminated blood and semen stains. Repeated attempts to digest the DNA from these samples failed.

RFLP Analysis

Standard reference sets of RFLP bands were generated from DNA stored at 4°C which had been extracted from fresh liquid blood and from DNA extracted from control blood and semen stains stored at room temperature. Each sample was analyzed at intervals of 1, 4, 8, 14, and 28 days. The mean RFLP allele size for each set of standard (control) samples and the maximum observed percent difference from the mean was determined and compared at each locus (Table 2). These data show that the allele sizes determined from liquid blood, bloodstains, and semen stains were very reproducible. The maximum observed difference between the mean RFLP band sizes of the standards and any individual band size for all time intervals was less than ±1.1% for alleles smaller than 10kb (D17579, Band 2) and less than ±2.2% for alleles larger than 10kb (D4S139, Band 1). However, when the mean allele size of all standards at each locus were compared at

TABLE 3—*D2S44 band #1: Percent difference comparisons between RFLP band sizes obtained from blood and semen stains subjected to contaminating and environmental factors and the mean RFLP band sizes obtained from the bloodstain standards (1573bp).*

	Time interval in days				
Contaminant-dried	1	4	8	14	28
Soil + blood	—	—	—	—	—
Soil + semen	—	—	—	—	—
Soap + blood	+0.13	−0.32	−0.32	−0.06	+0.13
Soap + semen	−0.06	−0.06	−0.06	+0.06	−0.13
Bleach + blood	0.00	+0.19	−0.19	+0.06	−0.32
Bleach + semen	−0.06	+0.19	+0.45	+0.45	+0.13
Lotion + blood	+0.25	+0.19	+0.51	−0.19	−0.25
Lotion + semen	+0.83	+1.21	−0.06	0.00	+0.76
Coffee + blood	+0.06	−0.32	−0.25	+0.76	+0.32
Coffee + semen	+0.25	−0.32	−0.45	+0.06	−0.06
Milk + blood	+0.38	−0.32	−0.32	+0.38	−0.06
Milk + semen	−0.19	−0.06	−0.06	+0.32	+0.32
Salt soln. + blood	−0.19	−0.45	−0.19	−0.06	−0.06
Salt soln. + semen	−0.06	−0.06	+0.45	+0.19	+0.19
Used motor oil + blood	+0.51	−0.19	+0.06	−0.06	−0.06
Used motor oil + semen	+0.25	−0.19	+0.13	+0.57	—
Gasoline + blood	−0.13	−0.13	0.00	0.00	−0.25
Gasoline + semen	−0.06	+0.45	+0.89	−0.13	+0.06
E. coli + blood	+0.06	−0.19	−0.13	−0.06	−0.06
E. coli + semen	−0.38	−0.32	0.00	+0.64	−0.32
Contaminant-wet					
Blood + soil	—	—	—	—	—
Semen + soil	—	—	—	—	—
Blood + bleach	−1.03	−0.06	—	−0.06	−1.03
Semen + bleach	—	—	—	—	—
Blood + gasoline	+0.13	+0.06	+0.45	+0.06	+0.19
Semen + gasoline	+0.13	+0.51	+0.19	+0.89	+0.19
Blood + E. coli	−0.19	+0.06	−0.51	+0.51	−0.06
Semen + E. coli	−0.06	+0.45	0.00	+0.76	+0.19
Environmental					
Blood + sunlight	+0.13	+0.32	+0.45	+0.32	−0.32
Semen + sunlight	−0.25	+0.19	0.00	+0.19	—
Blood + dark	−0.13	−0.32	+0.25	−0.32	+1.02
Semen + dark	−0.06	+0.25	+0.06	−0.32	+0.76

— no RFLP results were obtained.

days 1 and 28, no significant differences were observed. The mean allele size was 0.27% greater on day 28 than on day 1 for alleles less than 10kb and 0.8% greater on day 28 than on day 1 for alleles over 10kb. The average allele size obtained from DNA derived from the standard bloodstains was used to evaluate the allele sizes obtained from the blood and semen stains that were subjected to the various contaminants and environmental insults. The average allele sizes obtained from the bloodstains were used for comparison because bloodstains are routinely used as controls for case work at the Minnesota Forensic Science Laboratory (MFSL).

Percent difference comparisons[3] were made at six genetic loci between band sizes obtained from blood and semen stains subjected to contaminating and environmental

[3]Treated allele band size minus standard allele band size divided by the standard allele band size multiplied by 100.

TABLE 4—*D2S44 band #2: Percent difference comparisons between RFLP band sizes obtained from blood and semen stains subjected to contaminating and environmental factors and the mean RFLP band sizes obtained from the bloodstain standards (1131bp).*

	Time interval in days				
Contaminant-dried	1	4	8	14	28
Soil + blood	—	—	—	—	—
Soil + semen	—	—	—	—	—
Soap + blood	+0.53	+0.35	+0.18	+0.35	+0.80
Soap + semen	+0.71	+1.06	+0.97	+1.06	+1.06
Bleach + blood	+0.44	+0.97	+0.62	+1.06	+0.80
Bleach + semen	+0.44	+1.06	+0.71	+1.50	+1.33
Lotion + blood	+0.44	+1.15	+1.24	+0.35	+0.80
Lotion + semen	+1.77	+2.92	+0.80	+0.97	+1.95
Coffee + blood	+0.97	+0.80	+0.88	+1.15	+0.97
Coffee + semen	+1.33	+1.33	+0.44	+0.35	+1.06
Milk + blood	+0.88	+0.09	+0.62	+0.97	+0.71
Milk + semen	+0.35	+0.71	+0.35	+1.59	+1.24
Salt soln. + blood	−0.18	+0.27	+0.80	+0.80	+0.97
Salt soln. + semen	+0.35	+1.33	+1.41	+0.97	+1.24
Used motor oil + blood	+0.88	+0.27	+0.53	+0.62	+0.97
Used motor oil + semen	+1.06	+0.71	+1.15	+0.62	—
Gasoline + blood	+1.15	+0.80	+0.97	+1.06	+0.09
Gasoline + semen	+0.80	+1.33	+2.48	+1.50	+0.80
E. coli + blood	+1.33	+0.09	+0.18	−0.88	+0.71
E. coli + semen	+0.09	+1.33	+0.97	+1.24	+1.24
Contaminant-wet					
Blood + soil	—	—	—	—	—
Semen + soil	—	—	—	—	—
Blood + bleach	−0.62	+0.35	—	+1.15	+0.71
Semen + bleach	—	—	—	—	—
Blood + gasoline	+0.44	+0.71	+0.44	+0.35	+0.71
Semen + gasoline	+0.44	+1.59	+1.24	+1.77	+1.77
Blood + E. coli	+0.27	+1.06	+0.53	+1.77	+0.80
Semen + E. coli	+0.27	+1.41	+0.71	+1.86	+1.06
Environmental					
Blood + sunlight	+0.44	+1.24	+1.50	+1.50	+1.77
Semen + sunlight	+0.53	+2.30	+1.68	+1.33	—
Blood + dark	+0.18	+0.62	+1.06	+0.35	+3.27
Semen + dark	+0.27	+1.33	+0.97	+0.97	+1.86

— no RFLP results were obtained.

factors and mean band sizes obtained from the bloodstain standards. An example of this data is shown for the locus D2S44 (Tables 3 and 4). The greatest difference (positive or negative intergel variation) observed between the allele size of the standard and any sample was 3.27% (D2S44) 2.18% (D17S79), 1.89% (D10S28), 3.75% (D1S7) and 1.82% (D14S13). Thus, all differences for the aforementioned loci fell within the MFSL matching criterion of 5% [*11*]. In fact, over 99% of all differences were less than 2%. An example autoradiogram displaying RFLP bands from several insulted samples and appropriate standard samples is shown (Fig. 1).

In contrast, treatment of some of the biological stains with a certain skin care lotion produced shifts in fragment size beyond the MFSL match criteria window. At locus D4S139 (Band 1, 10.3kb) two of the blood stains contaminated with lotion and three of the semen stains contaminated with lotion resulted in differences over 5% (Table 5). However, allele sizes for Band 2 (3.6kb) yielded differences less than 2% (Table 6). The greatest difference observed for any lotion contaminated stain was 10.06%. All other

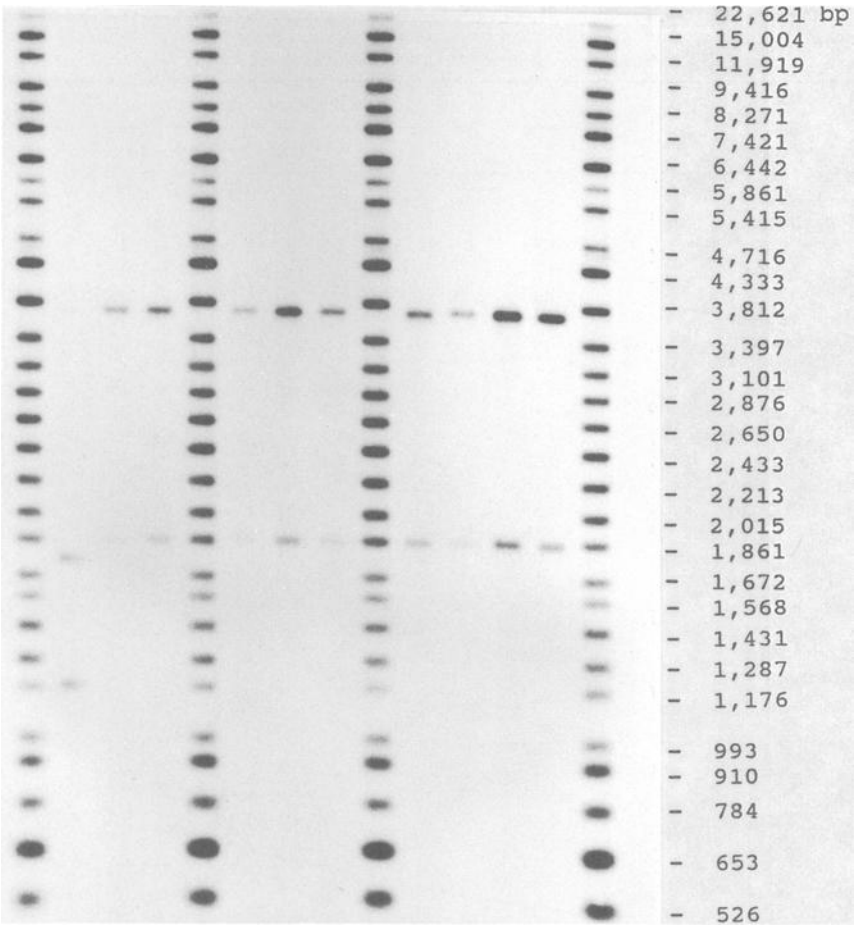

FIG. 1—*Example autoradiograph illustrating the reproducibility of RFLP banding patterns at the D10S28 locus for milk (lanes 3 and 4), salt solution (lanes 6 and 7), used motor oil (lanes 8 and 10), and gasoline (lanes 11 and 12) contaminated bloodstains and semen stains respectively. Lane 13 is the untreated bloodstain control. Lanes 1, 5, 9 and 14 contain BRL 30 band size markers. The fragment sizes of the BRL size marker are indicated on the right. Lane 2 is the K562 human cell line control.*

samples analyzed in this study at the D4S139 locus gave results similar to the previously mentioned probes, thus other contaminants had no significant effect on fragment sizes even in the HMW range (over 10kb). That is, >99% of the comparisons between treated samples and the bloodstain standards were less than 2%.

Lotions Study

Initially, the only brand of lotion used as a contaminant was Derma Soothe (Davron, Minnetonka, MN). To date, Derma Soothe is supplied by catalog order, thereby limiting its general use in the average domestic setting and the potential for this contaminant appearing in forensic casework. However, since some of the Derma Soothe contaminated stains resulted in RFLP fragments that showed differences over the 5% match criterion,

TABLE 5—*D4S139 band #1: Percent difference comparisons between RFLP band sizes obtained from blood and semen stains subjected to contaminating and environmental factors and the mean RFLP band sizes obtained from the bloodstain standards (10343bp).*

	Time Interval in Days				
Contaminant-Dried	1	4	8	14	28
Soil + blood	—	—	—	—	—
Soil + semen	—	—	—	—	—
Soap + blood	−0.67	−0.68	−0.89	+0.37	+0.56
Soap + semen	−0.66	+0.25	+0.11	−0.55	+0.32
Bleach + blood	−0.73	+0.07	−0.44	+0.12	−0.47
Bleach + semen	+0.50	+0.41	+0.65	+0.44	+0.18
Lotion + blood	−8.51	−4.67	−7.96	−4.56	−4.01
Lotion + semen	−10.06	−7.23	−4.20	−3.16	−8.49
Coffee + blood	+0.10	−0.53	−0.17	+0.63	+0.53
Coffee + semen	+1.04	+0.65	+0.85	+0.41	+0.53
Milk + blood	−0.74	−0.49	−1.44	−0.21	+0.25
Milk + semen	−1.32	+0.05	−0.85	+0.44	−0.17
Salt soln. + blood	−0.42	−1.97	−0.92	−1.14	−0.08
Salt soln. + semen	−1.31	−0.72	+1.09	−0.83	+1.78
Used motor oil + blood	−1.03	−0.75	−0.84	−0.59	−0.11
Used motor oil + semen	+0.28	+0.20	+0.13	—	—
Gasoline + blood	−0.03	−0.08	+0.25	−0.05	−0.75
Gasoline + semen	−0.15	+0.96	+1.88	−0.49	−0.60
E. coli + blood	−0.91	−1.53	−1.36	−0.74	−0.32
E. coli + semen	−0.24	−0.24	−0.69	−2.03	−0.42
Contaminant-wet					
Blood + soil	—	—	—	—	—
Semen + soil	—	—	—	—	—
Blood + bleach	—	−0.87	—	−1.63	+0.55
Semen + bleach	—	—	—	—	—
Blood + gasoline	—	−0.57	−0.08	−0.53	+0.50
Semen + gasoline	—	+0.44	+0.56	+0.44	+0.52
Blood + E. coli	−0.89	−0.65	−1.36	−0.40	+0.61
Semen + E. coli	−0.23	+0.18	−0.73	+0.76	+0.89
Environmental					
Blood + sunlight	—	−1.18	−0.09	+0.09	—
Semen + sunlight	—	−0.55	+0.75	—	—
Blood + dark	—	−0.90	+0.95	−0.19	+2.10
Semen + dark	—	+0.03	+0.05	+0.46	+3.15

—no RFLP results were obtained.

a study involving several different brands of lotion was conducted to determine their possible effect on RFLP fragment sizes. In addition, Derma Soothe lotion was subjected to further testing with blood from several individuals.

Derma Soothe Lotion

Blood from nine individuals was used to prepare bloodstains on cloth saturated with Derma Soothe lotion as previously described. The individuals selected exhibited allele sizes ranging from 2kb to 18kb for the loci selected. The contaminated stains were processed as previously described with two exceptions; 1) Only the probes MS1 (D1S7), pH-30 (D4S139) and the monomorphic probe (D7Z2) were used and 2) The DNA was analyzed for band size variation following a single organic DNA extraction and a double organic DNA extraction (see methods).

The greatest difference between the band sizes obtained from the standards and lotion

TABLE 6—*D4S139 band #2: Percent difference comparisons between RFLP band sizes obtained from blood and semen stains subjected to contaminating and environmental factors and the mean RFLP band sizes obtained from the bloodstain standards (3574bp).*

	Time Interval in Days				
Contaminant-Dried	1	4	8	14	28
Soil + blood	—	—	—	—	—
Soil + semen	—	—	—	—	—
Soap + blood	+0.17	−0.68	−0.37	−0.11	−0.34
Soap + semen	−0.59	−0.96	−0.87	−0.51	−0.96
Bleach + blood	−0.42	−0.45	−0.48	−0.14	−0.39
Bleach + semen	−0.48	−0.76	−0.11	−0.45	−0.28
Lotion + blood	−0.51	−0.06	−0.76	−0.28	−0.56
Lotion + semen	−0.76	−1.62	−0.37	−0.65	−0.51
Coffee + blood	+0.06	−0.25	−0.37	+0.36	−0.14
Coffee + semen	+0.34	−0.45	−0.37	−0.20	−0.28
Milk + blood	−0.56	−0.22	−0.42	+0.03	−0.31
Milk + semen	−0.56	−0.90	−0.53	−0.17	−0.45
Salt soln. + blood	−0.45	−0.53	0.00	+0.28	−0.25
Salt soln. + semen	−0.82	−0.99	−0.42	−0.14	−0.48
Used motor oil + blood	−0.20	−0.39	−0.25	0.00	−0.17
Used motor oil + semen	−0.73	−0.59	−0.03	—	—
Gasoline + blood	+0.11	−0.25	−0.03	+0.14	−0.59
Gasoline + semen	−0.53	−0.99	+0.06	−0.11	−0.65
E. coli + blood	+0.03	−0.08	−0.70	−0.45	−0.08
E. coli + semen	−0.51	−1.05	−1.13	−0.51	−0.37
Contaminant-wet					
Blood + soil	—	—	—	—	—
Semen + soil	—	—	—	—	—
Blood + bleach	—	−0.28	—	−0.20	−0.37
Semen + bleach	—	—	—	—	—
Blood + gasoline	—	+0.08	+0.25	−0.34	−0.22
Semen + gasoline	—	−0.53	+0.03	−0.51	−0.65
Blood + E. coli	−0.22	−0.42	−0.62	+0.45	−0.11
Semen + E. coli	−0.11	−0.53	−0.68	−0.28	+0.17
Environmental					
Blood + sunlight	—	−0.59	−0.68	−0.70	—
Semen + sunlight	—	−0.90	−1.02	—	—
Blood + dark	—	−0.03	−0.06	−0.25	+0.03
Semen + dark	—	−0.25	−0.65	−0.48	+0.28

— no RFLP results were obtained.

contaminated bloodstains when subjected to a single organic extraction was 8.73% (D1S7), 13.24% (D4S139) and 0.84% (D7Z2). When subjected to a double organic extraction, the greatest difference was 1.55% (D1S7), 2.84% (D4S139) and 0.92% (D7Z2) (Tables 7 and 8). These data indicate that a second organic extraction can be effective in reducing or eliminating possible migrational differences caused by some contaminants. It is also evident that the monomorphic probe, with a constant value of 2731bp, was not effective in detecting a band shift in a significantly different region of the gel.

Other Lotions

Eighteen different lotions that were in common household use by laboratory personnel were tested for their potential effect on forensic DNA typing. Cloth pieces were saturated with mineral oil, glycerol, Derma Soothe and each of the eighteen other skin care products listed in Table 9. Blood was applied to cloth pieces as previously described (the

TABLE 7—*RFLP band sizes obtained from single and double organic DNA extractions from bloodstains contaminated with Derma Soothe lotion compared to the mean RFLP band sizes obtained from the untreated bloodstain standard. The data was generated from nine individuals for the locus D1S7. Comparisons were also made between the monomorphic RFLP bands (locus D7Z2) obtained from the lotion stains and the published monomorphic probe value of 2731bp.*

	Single organic extraction		D1S7	D7Z2	Double organic extraction		D1S7	D7Z2
Donor #	Control stain	Lotion stain	Percent difference	Percent difference	Control stain	Lotion stain	Percent difference	Percent difference
1	10265	9527	−7.75	+0.11	10287	10191	−0.94	−0.15
	3593	3573	−0.56		3581	3585	+0.11	
2	4598	4501	−2.16	+0.66	4617	4629	+0.26	+0.59
	3669	3634	−0.96		3680	3668	−0.33	
3	5440	5383	−1.06	+0.26	5424	5401	−0.43	+0.11
	3218	3205	−0.41		3211	3212	+0.03	
4	3872	3831	−1.07	+0.22	3866	3861	−0.13	+0.59
	3298	3300	+0.06		3296	3308	+0.36	
5	7223	7025	−2.82	+0.48	7206	7156	−0.70	+0.33
	4197	4155	−1.01		4174	4174	0.00	
6	7683	7420	−3.54	+0.37	7641	7594	−0.62	−0.22
	2161	2156	−0.23		2147	2149	+0.09	
7	9053	8521	−6.24	+0.84	9054	8916	−1.55	+0.15
	5726	5581	−2.60		5729	5703	−0.46	
8	10131	9318	−8.73	+0.84	10164	10105	−0.58	−0.04
	3607	3571	−1.01		3598	3589	−0.25	
9	12029	11215	−7.26	+0.59	11848	11762	−0.73	+0.92
	3931	3871	−1.55		3901	3910	+0.23	

TABLE 8—*RFLP band sizes obtained from single and double organic DNA extractions from bloodstains contaminated with Derma Soothe lotion compared to the mean RFLP band sizes obtained from the untreated bloodstain standard. The data was generated from nine individuals for the locus D4S139. Comparisons were also made between the monomorphic RFLP bands (locus D7Z2) obtained from the lotion stains and the published monomorphic probe value of 2731bp.*

	Single organic extraction		D4S139	D7Z2	Double organic extraction		D4S139	D7Z2
Donor #	Control stain	Lotion stain	Percent difference	Percent difference	Control stain	Lotion stain	Percent difference	Percent difference
1	11687	10885	−7.37	+0.11	11771	11772	+0.01	−0.15
	5288	5181	−1.68		5289	5257	−0.61	
2	8128	7604	−6.89	+0.66	8134	8000	−1.68	+0.59
	3752	3703	−1.32		3746	3750	+0.11	
3	13314	12200	−9.13	+0.26	13275	12909	−2.84	+0.11
	4867	4833	−0.70		4879	4837	−0.87	
4	7671	7338	−4.54	+0.22	7638	7607	−0.41	+0.59
	6256	6069	−3.08		6247	6227	−0.34	
5	4071	4035	−0.89	+0.48	4074	4074	0.00	+0.33
	3622	3609	−0.36		3625	3625	0.00	
6	7235	7035	−2.84	+0.37	7214	7163	−0.71	−0.22
	5335	5251	−1.60		5321	5291	−0.57	
7	18077	15964	−13.24	+0.84	17809	17805	−0.02	+0.15
	4901	4800	−2.10		4881	4897	+0.33	
8	11624	10786	−7.77	+0.84	11768	11630	−1.19	−0.04
	5268	5120	−2.89		5263	5272	+0.17	
9	2927	2910	−0.58	+0.59	2921	2924	+0.10	+0.92
	2575	2560	−0.59		2566	2568	+0.08	

TABLE 9—*Percent difference comparisons between RFLP band sizes obtained from single donor bloodstains contaminated with nineteen types of lotion, mineral oil, and glycerol and the mean RFLP band sizes obtained from the bloodstain standards. Single and double DNA extractions were performed and band sizes generated for the loci D1S7 (5696bp, 3802bp) and D4S139 (10343bp, 3574bp) and D7Z2 (2731bp).*

Lotion Type	Sgl. D1S7	Sgl. D4S139	Sgl. D7Z2	Dbl. D1S7	Dbl. D4S139	Dbl. D7Z2
1	−0.53	−0.33	+0.51	−0.90	−2.29	+0.51
	−0.37	−0.08		−0.16	−0.51	
2	−0.55	−0.37	+0.44	−0.65	−2.95	+0.22
	+0.13	−0.08		−1.01	−0.82	
3	−0.55	−0.38	+0.40	−1.03	−2.19	0.00
	−0.37	−0.08		−0.85	−1.07	
4	+0.09	+0.87	+0.84	−0.46	−0.48	+0.59
	−0.24	+0.06		−0.03	−0.06	
5	+0.04	+1.01	+0.84	−0.16	+0.52	+0.92
	+0.29	+0.17		+0.18	−0.25	
6	−0.57	+2.29	+0.84	+0.16	+0.27	+0.70
	+0.42	−0.14		−0.13	+0.03	
7	+0.07	+3.03	+0.66	+0.53	+2.63	+1.57
	+0.26	+0.06		+0.60	+0.87	
8	+0.23	+1.09	+0.11	−0.65	−0.25	+0.29
	−0.08	−0.31		−0.05	−0.22	
9	+0.39	+1.36	−0.04	−0.26	−0.95	+0.29
	+0.53	+0.17		−0.16	−0.42	
10	−0.21	+0.01	+0.33	−0.78	−0.91	+0.70
	+0.03	+0.06		−0.16	−0.42	
11	−0.21	−0.19	+0.18	−1.12	−1.59	+1.06
	0.00	−0.06		−0.53	−0.76	
12	−0.16	−0.22	+0.95	−0.76	−0.89	+0.48
	+0.50	+0.37		−0.37	−0.53	
13	−0.11	−0.17	+0.37	−0.14	−0.98	+0.73
	+0.50	−0.08		−0.42	−0.59	
14	+0.62	+1.16	+0.77	+0.18	+0.80	+0.62
	+1.06	+0.45		+0.37	+0.14	
15	+1.12	+0.87	+0.81	−0.16	+0.05	+0.51
	+0.37	+0.25		+0.16	−0.08	
16	+0.25	−0.69	+0.48	+0.48	−0.75	+0.44
	−0.34	+0.11		−0.05	−0.34	
17	+0.81	−0.29	+0.55	−0.21	−0.50	+0.59
	+0.18	+0.39		0.00	−0.08	
18	+0.37	+0.31	+1.21	−0.19	−0.50	+0.70
	+0.42	+0.76		+0.13	−0.53	
19	+0.51	−0.27	+0.88	−0.94	−2.22	+0.37
	+0.18	+0.62		+0.13	−0.20	
20	+0.05	−0.57	+0.88	−0.48	−1.41	+0.37
	+0.29	+0.51		0.00	−0.31	
21	−3.08	−12.27	+1.21	−1.23	−3.03	+0.37
	−0.85	0.00		−0.13	−0.45	

NOTE: Key for Lotion Types:
1. St. Ives—Collagen Elastin
2. Neutrogena—Emulsion
3. Nivea
4. Eucerin
5. Nivea—Ultra Moisture Creme
6. Vaseline—Intensive Care
7. Lady Stetson
8. Clinique—Dramatically Different Moisturizing Lotion
9. Lander—Honey and Almond Lotion
10. Hook's—Cocoa Butter Lotion
11. Vaseline—Intensive Care—Hand and Nail Formula
12. Lubriderm
13. Nivea—After Sun
14. Jergens—Aloe and Lanolin
15. Coppertone—Tan Care
16. Vidal Sasson—Hand and Body
17. Finesse—Moisture Body Lotion
18. Hotel I.C.M.
19. Mineral Oil
20. Glycerol
21. Derma Soothe

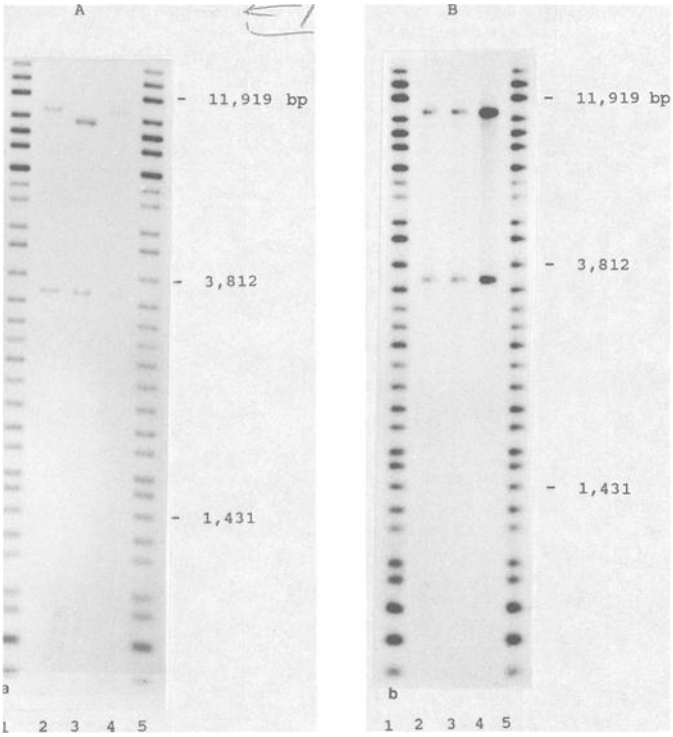

FIG. 2—*Two autoradiographs illustrating the difference between the RFLP banding patterns at the D4S139 locus for Derma Soothe lotion contaminated bloodstains that were analyzed after a single organic extraction, lane 3A, and after a double organic extraction, lane 4B. Lanes 1 and 5 contain BRL 30 band size markers (see figure 1 for complete sizing). Lanes 2A and 4A are glycerol contaminated bloodstains and the untreated bloodstain control respectively. Lanes 2B and 3B are mineral oil and glycerol contaminated bloodstains respectively. Fragment sizes of selected BRL size marker bands are indicated on the right.*

same blood donor was used as in the previously described studies reported in Tables 1 to 6). After exposure to the various skin care products, bloodstains were subjected to a single and double organic extraction and the resulting fragment sizes compared to the mean fragment sizes of the bloodstain standards (Table 9). A Derma Soothe lotion sample was also reanalyzed (sample 21, Table 9). The greatest difference in band size from the bloodstain standard for any of the twenty lotion contaminated bloodstains using a single organic extraction (Derma Soothe excluded) was 1.12% (D1S7) (Coppertone-Tan Care) and 3.03% (D4S139) (Lady Stetson). With a double organic extraction greatest differences were 1.12% (D1S7) (Vaseline Intensive Care) and 2.95% (D4S139) (Neutrogena-Emulsion). Derma Soothe contaminated samples again showed a substantial migrational differences with a single organic extraction 3.08% (D1S7) and 12.27% (D4S139). After a double organic extraction, the band sizes obtained showed no significant variation from those obtained from the bloodstain standards 1.23% (D1S7) and 3.03% (D4S139). All band size differences obtained from the monomorphic probe (D7Z2) were less than 2% from the published value (2731). As previously discussed, the monomorphic probe failed to detect the potential band shift in the 10kb region of the gel that occurred with Derma Soothe lotion. On the other hand, the fragment sizes obtained from all other skin care products analyzed in this study were within established match criteria (MFSL) when

TABLE 10—*D7Z2: Percent difference comparisons between RFLP band sizes obtained from blood and semen stains subjected to contaminating and environmental factors and RFLP band sizes obtained from the blood and semen standards with the published RFLP band size of the monomorphic probe (2731bp).*

	Time Interval in Days				
Contaminant-Dried	1	4	8	14	28
Soil + blood	—	—	—	—	—
Soil + semen	—	—	—	—	—
Soap + blood	+0.37	+0.29	−0.18	+0.55	+0.33
Soap + semen	+0.37	+0.15	+0.18	+0.33	−0.59
Bleach + blood	+0.66	+0.62	+0.18	+0.70	—
Bleach + semen	+0.48	+0.70	+0.29	+0.33	—
Lotion + blood	+0.81	+0.73	+0.44	+0.44	—
Lotion + semen	+1.46	−0.55	+0.07	+0.44	—
Coffee + blood	+0.84	+0.77	+0.66	+1.39	+0.51
Coffee + semen	+0.77	+0.51	+0.70	+0.29	+0.37
Milk + blood	+0.29	−0.11	+0.33	+0.48	+0.33
Milk + semen	−0.07	−0.18	+0.22	+0.51	+0.18
Salt soln. + blood	+0.29	−0.11	+0.22	+0.62	+0.33
Salt soln. + semen	+0.07	−0.48	+0.33	+0.70	+0.07
Used motor oil + blood	+0.33	+0.22	+0.48	+0.77	—
Used motor oil + semen	0.00	+0.18	+0.48	—	—
Gasoline + blood	+0.48	+0.55	+0.88	+0.62	−0.18
Gasoline + semen	+0.44	−0.62	+1.28	+0.26	−0.26
E. coli + blood	+0.44	+0.33	+0.22	+0.73	—
E. coli + semen	+0.48	+0.73	−0.15	+1.46	+0.15
Contaminant-west					
Blood + soil	—	—	—	—	—
Semen + soil	—	—	—	—	—
Blood + bleach	−0.11	+0.73	−0.48	+0.29	—
Semen + bleach	—	—	—	—	—
Blood + gasoline	+0.55	+0.26	+0.44	+0.70	+0.70
Semen + gasoline	+0.26	−0.29	+0.84	+0.81	+0.29
Blood + E. coli	+0.70	+0.51	+0.22	+1.10	+0.33
Semen + E. coli	+0.33	+0.44	+0.22	+0.18	+0.59
Environmental					
Blood + sunlight	+0.44	+0.26	+0.18	−0.07	—
Semen + sunlight	+0.18	+0.59	+0.04	—	—
Blood + dark	+0.18	+0.37	+1.06	+0.37	—
Semen + dark	+0.15	+0.15	+0.51	+0.26	+1.03
Standards					
Liquid blood standard	+0.18	+0.81	+0.29	−0.33	+0.26
	−0.07	+0.26	+0.73	+0.40	+0.15
	+0.15	−0.04	+0.04	+0.15	+0.66
	+0.40	+0.66	+0.22	+0.18	+0.26
Semen stain standard	+0.62	−0.48	−0.18	+0.66	+0.73
Blood stain standard	+0.70	+0.07	+0.11	+0.55	+0.81

— no RFLP results were obtained.

analyzed after a single extraction. An autoradiogram illustrating the effect of the second extraction on RFLP fragment size for the Derma Soothe lotion contaminated bloodstain is shown (Fig. 2).

Monomorphic Probe

All samples were analyzed with the monomorphic probe MGB7, specific to the D7Z2 locus. Some of the D7Z2 data has already been discussed (Lotion Studies Tables 7, 8

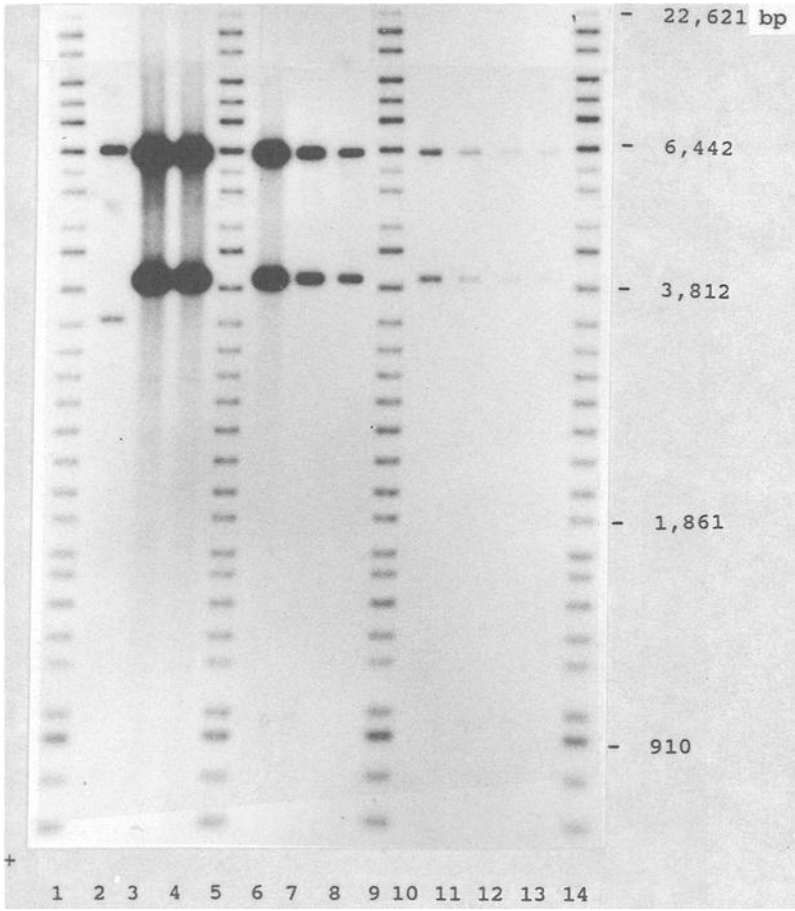

FIG. 3—*Example autoradiograph for the locus D4S139 illustrating the effect of loading different amounts of DNA in a lane. Lanes 3, 4, 6, 7, 8, 10, 11, 12 and 13 contain 20μg, 10μg, 5μg, 1μg, 500ng, 250ng, 100ng, 50ng and 25ng of DNA respectively. Lane 2 is the K562 human cell line control. Lanes 1, 5, 9 and 14 contain BRL 30 band size markers (see figure 1 for complete sizing). Fragment sizes of selected BRL size marker bands are indicated on the right.*

and 9). Comparisons were also made between the monomorphic fragment size obtained from each blood and semen standard (untreated) and each blood and semen stain subjected to contamination and environmental influences and the published fragment size (2731 bp) [*12*] of the monomorphic probe (Table 10). The greatest difference observed between the published monomorphic probe value and any sample in this study was 2.34%.

Cell Line Control

A K562 human cell line control DNA sample was analyzed on each gel and compared to the average MFSL established cell line value. All comparisons varied by less than ±1.5%.

TABLE 11—*Percent difference comparisons between the RFLP band sizes obtained from loading various quantities of DNA per lane and the average RFLP band sizes from the bloodstain standards run with 500 ng of DNA per lane. Each DNA quantity was analyzed on two membranes.*

	Membrane #1								
	D2S44		D1S7		D14S13		D4S139		D7Z2
Quantity of DNA (ng) loaded	3082*	1580	9954	7254	2862	1714	6381	3940	2731
20,000	0.00	+0.57	+2.04	+0.67	+0.32	+0.35	+0.31	−0.05	+0.40
10,000	0.00	+0.38	−0.59	+1.01	+0.25	+0.76	+0.25	+0.48	+0.29
5,000	+0.23	+0.06	−0.01	+0.57	+0.39	+0.23	−0.24	+0.13	+0.22
1,000	0.00	−0.13	−0.19	+0.55	+0.07	0.00	−0.09	+0.23	+0.26
500	−0.20	+0.13	−0.36	−0.08	+0.18	+0.06	+0.09	−0.13	+0.29
250	0.00	+0.38	−0.32	+0.22	+0.11	+0.35	−0.16	−0.23	+0.51
100	0.00	+0.32	−0.09	+0.33	+0.32	+0.23	−0.53	−0.46	+0.18
50	0.00	+0.06	+0.15	+0.45	+0.17	+0.06	−0.28	−0.18	−0.11
25	0.00	0.00	+0.42	+0.58	+0.42	−0.06	−0.64	+0.13	+0.04
					Membrane #2				
20,000	0.00	+0.82	+2.04	+1.04	+0.14	+1.11	0.00	−0.13	+0.51
10,000	+0.20	+0.57	+0.14	+0.54	+0.21	+0.41	+0.16	+0.13	+0.66
5,000	0.00	+0.25	+0.65	+0.40	−0.18	0.00	−0.78	−0.10	+0.22
1,000	0.00	−0.32	−0.17	−0.24	−0.28	−0.23	0.00	−0.03	+0.11
500	0.00	0.00	+0.04	−0.07	0.00	0.00	+0.13	−0.02	+0.48
250	+0.20	+0.06	+0.98	+0.51	0.00	−0.18	+0.02	+0.20	+0.40
100	+1.24	+0.25	+2.60	−1.52	+1.19	−0.53	+1.10	+1.48	+1.43
50	+0.52	+0.44	+2.23	+0.61	+0.14	−0.53	+0.13	+0.13	+0.51
25	−0.16	0.00	+0.79	+0.95	+0.25	−0.53	+0.48	+0.30	+0.11

*average RFLP band size (bp).

DNA Quantity

The differences between fragment sizes obtained from the electrophoresis of different quantities of DNA and the mean fragment sizes obtained from the electrophoresis of 500 ng of DNA are shown in Table 11. The greatest differences (positive or negative) observed between the 500 ng standard and any samples were 1.24% (D2S44), 2.60% (D1S7), 1.19% (D14S13), 1.48% (D4S139) and 1.43% (D7Z2). Thus no significant differences in band sizes were observed in the electrophoresis and analysis of different quantities of DNA ranging from 20 μg to 25 ng in agarose gels without ethidium bromide. This demonstrated that when 20 to 40 times the targeted quantity of DNA was loaded in a lane, no significant effect on band position resulted. This data is in agreement with the previously reported results of Waye and Fourney [6].

An analysis of the total number of allele measurements that were larger and smaller than the mean standard allele values was conducted for the contaminated and environmentally insulted samples, the Derma Soothe lotion samples, and the other lotion samples (Table 12). For all sample types, the number of allele measurements larger or smaller than the mean varied at different loci. No pattern of variation was identified by probe or allele size. For the contaminated and environmentally insulted samples, 729 of the allele measurements were larger and 752 were smaller than the standard values. For the Derma Soothe lotion samples that were subjected to a single organic extraction, 35 of 36 of the measured alleles were smaller than the standard values. When the Derma Soothe lotion samples were subjected to a double organic extraction, 21 of the 36 allele measurements were smaller than the standard values. For the various other lotion samples, there were 67 allele measurements larger and 97 allele measurements smaller than the standard values. In total, there were 809 allele measurements larger and 905 allele

TABLE 12—*The number of allele measurements obtained from blood and semen stains subjected to contaminating and environmental factors that were larger (higher base pair values) and smaller (lower base pair values) than the mean RFLP band sizes obtained from the bloodstain standards.*

Probe	Standard allele (BP)	Allele measurements larger than standard	Allele measurements smaller than standard	No difference	Comments
		Treated samples			
D2S44	1573	63	63	7	Single extraction
	1131	130	3	0	
D4S139	10333	49	73	0	Single extraction
	3571	17	103	2	
D1S7	5696	30	95	2	Single extraction
	3802	44	77	5	
D17S79	1483	93	22	15	Single extraction
	1287	100	21	9	
D10S28	3717	52	81	0	Single extraction
	1865	61	54	18	
D14S13	3420	64	64	4	Single extraction
	2168	26	96	10	
TOTAL		729	752	72	
		Derma soothe lotion samples			
D1S7	Varied 9 individuals	1	17	0	Single extraction
D1S7	Varied 9 individuals	6	11	1	Double extraction
D4S139	Varied 9 individuals	0	18	0	Single extraction
D4S139	Varied 9 individuals	6	10	2	Double extraction
TOTAL		13	56	3	
		Various lotion samples			
D1S7	3802	12	9	0	Single extraction
	1483	14	6	1	
D1S7	3802	4	17	0	Double extraction
	1483	6	13	2	
D4S139	10333	10	11	0	Single extraction
	3571	13	7	1	
D4S139	10333	5	16	0	Double extraction
	3571	3	18	0	
TOTAL		67	97	4	

measurements smaller than the standard allele values. This suggests that the overall effect of the treated samples on allele distribution above and below the standard values was insignificant. In addition, the magnitude of the deviation from the standard values was very small, with greater than 99% of the deviations being less than ±2%.

Conclusions

The results presented in this paper, where non-ethidium bromide analytical gels were employed, are in substantial agreement with the findings of Adams et al. [*1*] when electrophoresis was carried out in analytical gels containing ethidium bromide. Adams et al. [*1*] reported that with more than 1600 measurements of treated DNA samples, 95% of all measurements (intragel) were within ±2% of the control sample value and no

TABLE 13—*The number of allele measurements obtained from blood and semen stains subjected to contaminating and environmental factors and the number of allele measurements obtained from the bloodstain standards that were larger (higher base pair values) and smaller (lower base pair values) than the published RFLP band size of the monomorphic probe. Column 4 shows the observed mean monomorphic probe RFLP band size and percent difference between the mean monomorphic probe RFLP band sizes of the samples tested and the published RFLP band size of the monomorphic probe (2731bp).*

	Monomorphic probe (locus D7Z2)		
Sample	Allele measurements larger than monomorph	Allele measurements smaller than monomorph	Observed mean D7Z2 value in BP
Treated	112	13	2741 (+0.38%)
Derma soothe single extraction	9	0	2744 (+0.48%)
Derma soothe double extraction	6	3	2738 (+0.25%)
Other lotions single extraction	20	1	2748 (+0.63%)
Other lotions double extraction	21	0	2747 (+0.60%)
Standards	25	5	2739 (+0.30%)

samples exceeded the 5% match criterion. In this study, with more than 2200 measurements of treated DNA samples, 99% of all measurements (intergel and intragel) were within ±2% of the control sample value and no sample (with one exception as previously discussed) exceeded the MFSL 5% match criterion. These studies show that the electrophoresis of DNA in either ethidium bromide containing gels or in non-ethidium bromide gels will produce very reproducible results over a wide range of contaminated and insulted samples.

In this study, a single band shift did occur for one allele, exposed to a specific contaminant. The other alleles for this sample yielded results well within the 5% match criterion. Forensic scientists schooled in the procedures of DNA typing regard band shifting to be of minimal consequence when declaring DNA fragment matches. Band shifts occur infrequently, however, if a band shift does occur, it can be easily detected by the use of several VNTR probes spanning wide molecular weight ranges. Well-defined DNA controls with expected fragment sizes act as benchmarks for calibration of the entire DNA typing process from extraction to electrophoretic separation and measurement of the final allelic values. If a band shift does occur, the subsequent treatment of the DNA by a second extraction can be effective in eliminating the shift.

As noted by the Office of Technology Assessment report [*13*] and substantiated by the authors of this paper, scientists schooled in the procedures of DNA typing, following a program with rigid quality assurance guidelines [*14*], will produce valid and reliable DNA typing results for forensic casework. This has been further substantiated from the comparisons of allele sizes obtained from victim's control bloodstains and vaginal swab samples taken after sexual assaults in over 100 forensic cases analyzed at the MFSL where no comparisons exceeded the 5% match window (unpublished data). The 2200 independent measurements carried out in this study clearly demonstrate the effectiveness of gaining important forensic evidence from otherwise potentially difficult biological samples.

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