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Evaluation of Four Deoxyribonucleic Acid (DNA) Extraction Protocols for DNA Yield and Variation in Restriction Fragment Length Polymorphism (RFLP) Sizes Under Varying Gel Conditions

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ABSTRACT: This study originated from discussions and recommendations of the Technical Working Group on DNA Analysis Methods (TWGDAM). Four bloodstain deoxyribonucleic acid (DNA) extraction protocols and five semen stain DNA extraction protocols were evaluated. Nine laboratories participated in the extraction of DNA from 20 bloodstains and 20 semen stains using each protocol. All blood and semen stains originated from a single donor and were prepared under uniform conditions to permit the direct comparison of DNA yields and restriction fragment lengths. The extracted DNA from approximately 600 bloodstains and 700 semen stains was quantified by yield gel analysis and a slot blot hybridization technique. The extracted DNA was digested and restriction fragment length polymorphism (RFLP) patterns were generated using three single-locus probes. The RFLP sizing data produced from the blood and semen stains were evaluated with respect to (1) DNA extraction method, (2) gel length, (3) agarose type, (4) presence or absence of ethidium bromide in the gel, and (5) fragment sizes obtained from DNA isolated directly from the donor's liquid blood. This study demonstrates conclusively that high-molecular-weight DNA can be isolated using either organic or nonorganic DNA extraction protocols and that the resulting RFLP sizes are highly reproducible regardless of gel length, agarose type, or presence/absence of ethidium bromide.

KEYWORDS: pathology and biology, deoxyribonucleic acid (DNA), restriction fragment length polymorphisms (RFLP), extractions, DNA stain extractions, blood, semen

Currently, restriction fragment length polymorphism (RFLP) analysis is widely accepted by forensic science laboratories for identifying genetic markers in biological materials [*1–6*]. Various biological sources contain the necessary deoxyribonucleic acid (DNA) for RFLP analysis, but in forensic science casework, blood and semen are considerably more prevalent than other sources [*7–9*]. Many studies have shown that blood

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and semen stains provide sufficient high-molecular-weight (HMW) DNA for RFLP analysis, even after being subjected to a wide range of environmental insults [*8–12*]. Several different RFLP procedures have been developed to analyze these stains for DNA banding patterns. All of these protocols use the same basic analytical steps: (1) DNA extraction, (2) endonuclease digestion, (3) electrophoresis, (4) blotting, (5) hybridization, (6) autoradiography, (7) imaging, and (8) interpretation.

The advantages gained by using new RFLP analysis procedures are tempered by the need to use uniform protocols to enhance quality assurance. Quality assurance and legal precedence are of great importance to the forensic science community when developing new protocols such as DNA profiling. Standardized DNA profiling procedures can also benefit the forensic science community in areas such as recordkeeping, proficiency testing, and a national database system [*13*].

The Technical Working Group on DNA Analysis Methods (TWGDAM: a nonregulatory Federal Bureau of Investigation (FBI)-sponsored organization consisting of representatives of crime laboratories at or near implementation of DNA profiling techniques) is striving to bring uniformity to DNA procedures used in crime laboratories in the United States and Canada [*14*]. In this endeavor, the members of TWGDAM have evaluated the various steps of different RFLP procedures for banding pattern reproducibility. In this study, blood and semen stain DNA extraction protocols were identified by TWGDAM for evaluation. The Minnesota Forensic Science Laboratory was designated as project coordinator while six other TWGDAM member laboratories and two private laboratories assisted in various aspects of testing. The procedures evaluated were obtained from the FBI, Lifecodes Corporation, and Cellmark Diagnostics. These procedures follow a similar sequence of steps and techniques; however, there is considerable variation in reagents and sample handling. These protocols were selected because they represent established procedures that are currently used in forensic applications for DNA isolation.

This paper describes each of the extraction methods used for isolating HMW human DNA from dried blood and semen stains and the average yield for each method. The subsequent RFLP analysis using three single-locus probes that recognize highly polymorphic regions of human DNA is compared with RFLP patterns generated from DNA isolated from liquid blood standards. The experiments were designed to evaluate DNA extraction protocols and to assess the effects that different agarose types, gel lengths, and the presence or absence of ethidium bromide have on RFLP patterns. The experiments demonstrate that RFLP patterns from bloodstains, semen stains, and liquid blood can be accurately matched over a wide range of procedural variation.

Materials and Methods

Sample Preparation and Dissemination

One person donated blood and semen for use throughout this study. The DNA from the liquid blood was extracted by three methods and electrophoresed in three lanes on each of two separate gels for each extraction method. The DNA extraction from liquid blood and RFLP analysis were performed at the Minnesota Forensic Science Laboratory. The RFLP patterns generated from the DNA isolated from the liquid blood were compared with RFLP patterns generated from the blood and semen stains. All stains used in this study were prepared at the Minnesota Forensic Science Laboratory. The 25-μL bloodstains and 10-μL semen stains were prepared on clean cotton cloth. All stains were prepared using liquid blood and semen that were mixed constantly during stain preparation to ensure the uniform deposition of material. The stains were dried for 1 h in a fume hood at ambient temperatures and stored for approximately three months at −80°C. Each set of stains was sent by overnight express mail to the laboratories participating in

the DNA extraction phase of the study. The nine laboratories that participated in this study were the following:

1. (CDOJ) California Department of Justice
2. (CM) Cellmark Diagnostics
3. (VIR) Commonwealth of Virginia Crime Laboratory
4. (FBI) Federal Bureau of Investigation Research and Training Center
5. (LC) Lifecodes Corporation
6. (MD) Metro-Dade Police Crime Laboratory
7. (MN) Minnesota Bureau of Criminal Apprehension Forensic Science Laboratory (sender)
8. (NC) North Carolina State Bureau of Investigation
9. (OR) Orange County Sheriff—Coroner Department

Each of these laboratories was supplied with the following:

1. Specimens consisting of 125 25-μL bloodstains and 125 10-μL semen stains (LC and CM were supplied only enough stains to perform their own DNA extraction procedures).
2. Copies of each of the DNA extraction procedures.
3. A suggestion that laboratories practice each procedure before beginning the actual extraction study.
4. Data sheets to record DNA yield for each sample and the volume used to solubilize the DNA.
5. Instructions for storing all DNA extracts at −20°C and returning all extracted DNA to the Minnesota Forensic Science Laboratory where quantification (yield gel analysis and slot blot hybridization) and RFLP analysis would be carried out.

DNA Isolation from Liquid Blood Standards

Method I—(MMBC) Minneapolis Memorial Blood Center Extraction Procedure [15]—Ethylenediaminetetraacetate (EDTA) tubes containing freshly drawn blood were centrifuged for 20 min at 2000 rpm. The white cell layers were removed and placed in 15-mL conical centrifuge tubes. Red cell lysis buffer [0.144*M* ammonium chloride (NH_4Cl), 0.001*M* sodium bicarbonate ($NaCHO_3$)] was added to a final volume of 9 mL. The samples were incubated at ambient temperatures for 20 min with occasional mixing. The samples were centrifuged for 20 min at 2000 rpm and the supernatants discarded. Three millilitres of nuclei lysis buffer [10m*M* Tris, 400m*M* sodium chloride (NaCl), 2m*M* sodium EDTA (Na_2EDTA) to pH 8.2], 200 μL of 10% sodium dodecyl sulfate (SDS), and 600 μL of proteinase K (2 mg/mL) were added to each tube and incubated overnight at 37°C. Following incubation, each sample received 1 mL of saturated 6*M* NaCl, was shaken for 15 s, and centrifuged at 2500 rpm for 15 min. The supernatants were transferred to new 15-mL conical tubes where the DNA was precipitated with two volumes of room temperature absolute ethanol. The precipitated DNA was spooled from solution, placed in new tubes, and resolubilized in Tris-EDTA (TE) buffer (10m*M* Tris, 0.2m*M* Na_2EDTA, pH 7.5). DNA aliquots were removed for yield gel quantification.

Method II—(FBI) FBI Extraction Procedure [16]—Blood collected in EDTA tubes was frozen at −80°C in 700-μL aliquots. The blood was thawed and 800 μL of 1 × SSC [0.15*M* NaCl, 15m*M* sodium citrate ($Na_3C_6H_5O_7$) pH 7.0] were added. The solution in each tube was mixed, microcentrifuged for 1 min, and the supernatants were removed and discarded. The cellular pellet was washed by adding 1.0 mL of 1 × SSC followed by vortexing and centrifugation for 1 min. The supernatants were removed and discarded. Three hundred seventy-five microlitres of 0.2*M* sodium acetate, 25 μL of 10% SDS, and 5 μL of proteinase K (20 mg/mL) were added to the DNA pellet in each tube. The tubes

were vortexed and incubated at 56°C for 1 h. The DNA was extracted with 120 μL of phenol/chloroform/isoamyl alcohol and precipitated with 1.0 mL of cold absolute ethanol at −20°C for 15 min. The tubes were centrifuged to pellet the DNA. The DNA pellets were washed once with 70% ethanol and dried in a vacuum centrifuge. The pellets were resolubilized in TE buffer and aliquots were removed for yield gel quantification.

Method III—(LCNO) Lifecodes Nonorganic Extraction Procedure [17]—EDTA tubes containing freshly drawn blood were centrifuged for 5 min at 3700 rpm. One millilitre of the white cell layer was removed from each tube, placed into 2-mL vials, and stored at −20°C.[2] The samples were thawed and 1 mL of cold cell lysis buffer [CLB = 0.32*M* sucrose, 10m*M* Tris, pH 7.6, 5m*M* magnesium chloride ($MgCl_2$), 1% Triton X 100] was added. Samples were vortexed for 1 min and centrifuged at 2000 rpm for 5 min. The supernatants were removed and discarded. The CLB procedure was repeated two more times with fresh CLB. Following the CLB wash, 1.0 mL of protein lysis buffer (PLB = 10m*M* Tris, pH 7.4, 10m*M* NaCl, 10m*M* EDTA) was added to each cell pellet. The samples were vortexed to resuspend the pellet, centrifuged at 1500 rpm for 5 min, and the supernatants were removed and discarded. The tubes were placed on ice and 250 μL of master mix [225 μL PLB, 25 μL proteinase K (10 mg/mL)] were added. Samples were transferred to a 65°C heat block for 2 h and removed for brief vortexing every 30 min. Following incubation, the samples were vortexed vigorously for 30 s and microcentrifuged for 2 min. The top 200 μL of sample containing purified DNA were removed and placed into new tubes. Aliquots were removed for yield gel quantification.

DNA Isolation from Bloodstains

Method I—(CM) Cellmark Diagnostics Extraction Procedure—Each 25-μL bloodstain was cut into small pieces and placed in an Eppendorf tube with 700 μL of Gill buffer (10m*M* Tris, pH 8, 10m*M* EDTA, pH 8, 0.1*M* NaCl, and 2.0% SDS) and 35 μL of proteinase K (20 mg/mL). The samples were incubated overnight at 56°C and piggyback centrifuged to remove the liquid from the cloth. The DNA was extracted once with phenol/chloroform and once with chloroform. One microlitre of glycogen (20 mg/mL) was added to each sample. The DNA was precipitated with two volumes of absolute ethanol overnight at −20°C. The DNA was recovered by centrifugation and the pellet was washed with 700 μL of 80% ethanol and dried in a vacuum centrifuge. The pellets were resolubilized in TE buffer and aliquots were removed for yield gel quantification.

Method II—(FBI REG) FBI Extraction Procedure—Each 25-μL bloodstain was cut into small pieces and placed in a 1.5-mL tube with 400 μL of stain extraction buffer [10m*M* Tris, pH 8, 10m*M* EDTA, 0.1*M* NaCl, 0.039*M* dithiothreitol (DTT),[3] and 2% SDS] and 10 μL of proteinase K (20 mg/mL). The samples were incubated overnight at 56°C and piggyback centrifuged to remove the liquid from the cloth. The DNA was extracted once 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 and the pellet was washed with 1.0 mL of 70% ethanol and dried in a vacuum centrifuge. The pellets were resolubilized in TE buffer and aliquots were removed for yield gel quantification.

Method III—(LCOR) Lifecodes Organic Extraction Procedure—Each 25-μL bloodstain was cut into small pieces and placed into a tube with 935 μL of protein lysis buffer (PLB = 10m*M* Tris, pH 7.4, 10m*M* EDTA, 10m*M* NaCl), 15 μL of proteinase K (10

[2]The Lifecodes procedure was followed with the exception of one modification. In the first step, 1 mL of whole blood was modified to 1 mL of buffy coat.

[3]DTT should be prepared fresh and added to the stain extraction buffer just before use.

mg/mL), and 50 μL of 20% SDS. The samples were placed on a rocking platform in a 37°C incubator for 2 h. Two hundred microlitres of 6*M* sodium perchlorate ($NaClO_4$) were added, and the solution was gently mixed by inverting the tubes several times. The DNA was extracted with equal volumes of phenol, phenol/chloroform/isoamyl alcohol, chloroform/isoamyl alcohol, and dialyzed against three changes of a thousandfold excess of TE buffer (10m*M* Tris, pH 7.4, 1m*M* EDTA). The DNA solution was removed from the dialysis tubes and aliquots were taken for yield gel quantification.

Method IV—(LCNO) Lifecodes Nonorganic Extraction Procedure—Each 25-μL bloodstain was cut into small pieces and placed in a 2-mL microcentrifuge tube with 1.5 mL of cold cell lysis buffer (CLB = 0.32*M* sucrose, 10m*M* Tris, pH 7.6, 5m*M* $MgCl_2$, 1% Triton X 100). The tubes were incubated on ice for 5 to 10 min (with occasional vortexing) and centrifuged for 30 s at 14 000 rpm. The supernatants were removed and discarded. This step was repeated once more with fresh CLB and then once with cold protein lysis buffer (PLB = 10m*M* Tris, pH 7.4, 10m*M* NaCl, 10m*M* EDTA). Following the recovery of the DNA pellet, the tubes were placed on ice and 300 μL of master mix [270 μL PLB, 30 μL proteinase K (10 mg/mL)] were added. The tubes were transferred to a 65°C heat block for 2 h, removed for vortexing every 30 min, and then centrifuged to remove liquid from the sides of the tubes. The supernatants were removed from the fabric, transferred to new 2-mL tubes, and centrifuged to remove particulate matter. The liquid DNA lysates were transferred to new tubes, their volumes were determined, and aliquots were removed for yield gel quantification.

DNA Isolation from Semen Stains

Method I—(CM) Cellmark Diagnostics Extraction Procedure—Each 10-μL semen stain was cut into small pieces and placed in an Eppendorf tube with 700 μL of Gill buffer (10m*M* Tris, pH 8, 10m*M* EDTA, pH 8, 0.1*M* NaCl, and 2.0% SDS) and 10 μL of proteinase K (20 mg/mL). The samples were incubated overnight at 56°C and piggyback centrifuged to remove the liquid from the cloth. The pellets were washed once with 500 μL of Gill buffer. Five hundred sixty microlitres of TEN buffer (10m*M* Tris, pH 7.6, 1m*M* EDTA, pH 8.0, and 10m*M* NaCl), 70 μL of 10% SDS, 34 μL of 0.8*M* DTT, and 10 μL of proteinase K (20 mg/mL) were added to the cell pellets. The samples were incubated overnight at 56°C. The DNA was extracted once with phenol/chloroform and once with chloroform. Sodium acetate (to a final concentration of 0.2*M*) and 1 μL of glycogen (20 mg/mL) were added to the aqueous phase containing the DNA. The DNA was precipitated with two volumes of absolute ethanol overnight at −20°C. The DNA was recovered by centrifugation and the pellet was washed with 700 μL of 80% ethanol and dried in a vacuum centrifuge. The DNA pellets were resolubilized in TE buffer and aliquots were removed for yield gel quantification.

Method II—(FBI REG) FBI Extraction Procedure—Each 10-μL semen stain was cut into small pieces and placed in a 1.5-mL tube with 400 μL of stain extraction buffer (10m*M* Tris, pH 8, 10m*M* EDTA, 0.1*M* NaCl, 0.039*M* DTT, and 2% SDS) and 10 μL of proteinase K (20 mg/mL). The samples were incubated overnight at 56°C and piggyback centrifuged to remove the 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 and the pellet was washed with 1.0 mL of 70% ethanol and dried in a vacuum centrifuge. The DNA pellets were resolubilized in TE buffer and aliquots were removed for yield gel quantification.

Method III—(FBI DIFF) FBI Differential Extraction Procedure—Each 10-μL semen stain was cut into small pieces and placed in 1.5-mL tube with 450 μL of phosphate buffered saline and 50 μL of 20% sarkosyl. The samples were placed on a rocking platform

at 4°C overnight and then piggyback centrifuged. The supernatants (female fractions) were discarded and the cloth was added back to the pellets. Four hundred microlitres of TNE buffer (10m*M* Tris, 1m*M* EDTA, 0.1*M* NaCl at pH 8.0), 50 μL of 10% SDS, 50 μL of water, and 5 μL of proteinase K (20 mg/mL) were added to the cloth and DNA pellets. The tubes were incubated at 37°C for 2 h, piggyback centrifuged, and the pellets saved. The cloth and supernatants (female fraction) were discarded. One hundred fifty microlitres of TNE, 100 μL of 10% sarkosyl, 40 μL of 0.39*M* DTT, 100 μL of water, and 10 μL of proteinase K (20 mg/mL) were added to the DNA pellets. The solutions were mixed and incubated at 37°C for 2 h. The DNA was extracted with an equal volume 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 and the pellet was washed with 1.0 mL of 70% ethanol and dried in a vacuum centrifuge. The DNA pellets were resolubilized in TE buffer and aliquots were removed for yield gel quantification.

Method IV—(LCOR) Lifecodes Organic Extraction Procedure—Each 10-μL semen stain was cut into small pieces and placed in a tube with 935 μL of phosphate buffered saline, 15 μL of proteinase K (10 mg/mL) and 50 μL of 20% SDS. The samples were placed on a rocking platform and incubated at 37°C for 2 h. The DNA was recovered by centrifugation and each DNA pellet was resuspended in 800 μL of phosphate buffered saline and 175 μL of a mixture consisting of 100 μL sarkosyl, 10 μL 1*M* DTT, 15 μL proteinase K (10 mg/mL), and 50 μL 0.5*M* EDTA. The tubes were placed on a rocking platform in a 65°C incubator for 2 h. Two hundred microlitres of 6*M* sodium perchlorate were added and the solutions were gently mixed by inverting the tubes several times. The DNA was extracted once with phenol, once with phenol/chloroform/isoamyl alcohol, and once with chloroform/isoamyl alcohol, each in equal volumes to the DNA solution. Following extraction, the DNA was dialyzed against three changes of a thousandfold excess of TE buffer (10m*M* Tris, pH 7.4, 1m*M* EDTA). The DNA solution was removed from the dialysis tubes and aliquots were taken for yield gel quantification.

Method V—(LCNO) Lifecodes Nonorganic Extraction Procedure—Each 10-μL semen stain was cut into small pieces and placed in a 2-mL microcentrifuge tube with 1.5 mL of cold cell lysis buffer (CLB = 0.32*M* sucrose, 10m*M* Tris, pH 7.6, 5m*M* $MgCl_2$, and 1% Triton X 100). The tubes were incubated on ice for 5 to 10 min (with occasional vortexing) and centrifuged for 30 s at 13 000 × *g*. The supernatants were removed and discarded. This was repeated once more with CLB and then once with cold protein lysis buffer (PLB = 10m*M* Tris, pH 7.4, 10m*M* NaCl, 10m*M* EDTA). Following the recovery of the DNA pellet, the tubes were placed on ice and 300 μL of master mix [270 μL PLB, 30 μL proteinase K (10 mg/mL)] were added. The tubes were transferred to a 65°C heat block for 2 h with brief vortexing every 30 min and then centrifuged to remove liquid from the sides of the tubes. The supernatants were removed from the fabrics and discarded. Three hundred microlitres of master mix [267μL PLB, 3 μL 0.5*M* DTT, 30 μL proteinase K (10 mg/mL)] were added to each tube. The samples were incubated for 2 h in a 65°C heat block, removed for vortexing every 30 min, and then centrifuged to remove liquid from the sides of the tubes. The supernatants were removed from the fabric, transferred to new 2-mL tubes, and centrifuged to remove the particulate matter. The liquid DNA lysates were transferred to new tubes, their volumes were determined, and aliquots were removed for yield gel quantification.

Yield Gel Analysis of Samples

The nine laboratories participating in the TWGDAM extraction study submitted the DNA extracts they obtained from each of the 20 blood and 20 semen stains by the various extraction methods to the Minnesota Forensic Science Laboratory. All samples were run

on yield gels under identical conditions at the Minnesota laboratory. Two percent of the total DNA from each extract was loaded onto a 0.8% agarose gel containing ethidium bromide. Electrophoresis was carried out at 150 V for 45 min in electrophoresis buffer containing ethidium bromide. Yield gel quantification standards of 2, 4, 6, 8, 10, 15, 20, and 25 ng of K562 human DNA were loaded on each gel and used to estimate the quantity of HMW DNA in each test sample. High-resolution photographs [20 by 25 cm (8 by 10 in.)] were taken of each yield gel and visual quantification estimates were made from the photographs.

Slot Blot Hybridization

Slot blot hybridization was carried out as described by Waye et al. [*18*] using a BIO-RAD slot blot apparatus. One percent and two percent aliquots of the total DNA from each extract were loaded into adjacent lanes. Slot blot quantification standards were prepared from K562 human DNA and run on each blot at concentrations of 0.3, 0.6, 1.2, 2.5, 5, 10, 20, and 40 ng per lane. All membranes were hybridized to the primate-specific probe p17H8 (locus D17Z1). Quantification was performed by visually matching the intensity of the test sample bands to the intensity of the standard bands. An example slot blot autoradiograph is shown in Fig. 1.

Restriction Endonuclease Digestion

DNA extracts isolated by the same procedure and laboratory were combined if necessary, so that each digestion was performed on approximately 750 ng of DNA. When combined samples resulted in volumes in excess of 449 μL, the volumes were reduced by using Centricon microconcentration columns (Amicon). Prior to restriction, a yield gel was run on all combined samples to verify the quantity of HMW DNA present. The samples [449 μL of DNA extract, 50 μL of 10 × restriction buffer, and 1 μL of Hae III

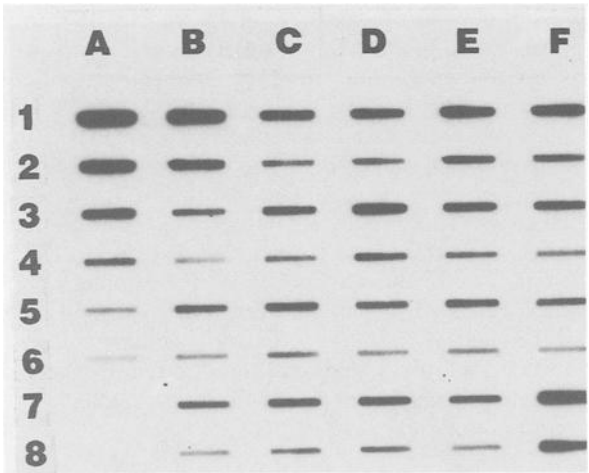

FIG. 1—*An example slot blot autoradiograph derived from one laboratory's DNA extracts of 20 bloodstains. The DNA was extracted using the FBI semen stain extraction procedure and hybridized with the primate-specific probe p17H8 (locus D17Z1). Lane A contains serial dilutions of K562 quantification standards. Lanes B through F were generated using 2% (odd numbered rows) and 1% (even numbered rows) of the total DNA recovered from each stain.*

restriction endonuclease (40 U/μL, American Allied Biochemical)] were incubated for 2 h at 37°C. The DNA was precipitated with 21 μL of 6*M* sodium perchlorate and 1 mL of cold absolute ethanol. Test gels were run to verify complete digestion. Samples that did not completely restrict were subjected to a second, and if necessary, a third restriction digestion.

Electrophoresis and Transfer

Electrophoretic separation of the DNA fragments involved three different analytical gel conditions:

(1) A 1% 12 by 20-cm low electroendosmosis (LE) agarose gel (ultraPURE, Bethesda Research Laboratories).
(2) A 1% 12 by 20-cm medium electroendosmosis (ME) agarose gel (Seakem, FMC) supplemented with 0.5 μg/mL ethidium bromide.
(3) A 1% 12 by 16-cm ME agarose gel (Seakem, FMC) supplemented with 0.5 μg/mL ethidium bromide.

Electrophoresis was performed in 1 × TAE (0.04*M* Tris, 0.019*M* glacial acetic acid, 0.025*M* EDTA) buffer. Gels supplemented with ethidium bromide were electrophoresed in buffer containing 0.5 μg/mL ethidium bromide. Electrophoresis was carried out at 27 V for 16 h or until the loading buffer indicator dye approached 2 cm from the gel end.

The DNA was transferred to nylon membrane (Biodyne B, Pall BioSupport) using a 0.4*M* NaOH transfer solution. After 6 h of transfer, membranes were washed in a 2 × SSC, 0.2*M* Tris solution (pH 7.5) for 15 min, blotted between Whatman 1MM papers, and baked in a vacuum oven between two sheets of Whatman 3MM paper for 30 min at 80°C.

Probes

Three DNA probes which recognize single-locus variable number tandem repeats (VNTRs) were utilized in this study. Probes specific to DNA loci D2S44 (YNH24, Promega Corp.), D10S28 (TBQ7, Promega Corp.), and D17S79 (V1, Lifecodes Corp.) were labeled according to the Promega Prime-a-Gene Labeling System.

Hybridization

Up to six membranes were hybridized per 5- by 30-cm bottle using a Robbins incubator (Model 1040-00-1). Membranes were prehybridized at 65°C in a solution containing 10% PEG, 0.15 × SSPE, and 7% SDS for 15 min. This solution was discarded and replaced with 20 mL of a 65°C hybridization solution consisting of labeled probe [1 × 10^6 dpm (disintegration per minute) mL^{-1} of hybridization solution], labeled ladder λ (2 × 10^5 dpm mL^{-1}) and ΦX174 (2 × 10^4 dpm mL^{-1}) DNA, herring sperm DNA (250 μg/mL), and 0.2 *M* NaOH (100 μL). Hybridization was carried out at 65°C for 15 to 20 h. Following incubation, the membranes were washed three times to a final stringency of 0.1 × SSC + 0.1% SDS and wrapped, while moist, in Glad cling wrap.

Autoradiography

The membranes were placed between two Kodak X-OMAT XAR-5 X-ray films and were light-secured inside Kodak X-ray cassettes with X-Omatic regular intensifying screens. The cassettes were stored at −80°C. The film was developed on a Konica QX-70 film processor.

Stripping

To perform subsequent hybridizations with different probes, the membranes were placed in stripping solution (55% Formamide, 2 × SSPE, 1% SDS) for 45 to 90 min at 65°C. The membranes were rinsed (0.1 × SSC + 0.1% SDS) and rehybridized with the next probe.

Results and Discussion

Extraction

The efficient recovery of HMW genomic DNA is essential to successful DNA typing in forensic science specimens. The quantity of HMW DNA recovered by four bloodstain and five semen stain DNA extraction procedures is given in Table 1. The laboratories that did not perform that extraction method (LC and CM performed their own extraction methods) are indicated. The prior experience of each laboratory with each extraction procedure varied from none to routine use. Most of the laboratories had not used the Cellmark extraction procedure or the Lifecodes nonorganic extraction procedure before this study. The data in Table 1 show that all of the extraction methods are capable of yielding sufficient HMW DNA for RFLP testing. The Lifecodes nonorganic extraction method had the advantage of not using toxic chemicals and producing high and consistent DNA yields; however, the DNA extracts produced by four of the eight laboratories performing this procedure were very discolored and gave rise to DNA band shifts in the RFLP patterns. This will be addressed later.

At the time of this study, the FBI bloodstain DNA extraction procedure did not specify that the DTT must be prepared fresh and added to the stain extraction buffer just before each extraction. The DTT was added to the buffer at the time the buffer was prepared and used over a period of time. As a result, many of the bloodstain DNA extracts received from the participating laboratories showed considerable DNA degradation (Fig. 2B). This is reflected as lower DNA yields when the extracts were quantified by yield gel analysis and much higher DNA yields when analyzed by slot blot hybridization. Additional samples were analyzed by the Minnesota Forensic Science Laboratory to assess the use of DTT in the bloodstain buffer. The Minnesota laboratory determined that bloodstain extraction buffer containing freshly prepared DTT performed comparably to bloodstain extraction buffer without DTT. In addition, the Minnesota laboratory (extracting DNA from duplicate samples) obtained 78% higher DNA yields without DTT than with DTT that was not freshly prepared.

In addition to the yield gel quantification, the extracted DNA from the blood and semen stains were subjected to slot blot hybridization with the primate-specific probe P17H8. The quantification results of the slot blot hybridization are given in Table 2. In comparing Table 1 and Table 2, it can be observed that DNA from several of the samples was detected by the slot blot procedure where no DNA was visible on a yield gel. In general, the slot blot method indicated yields three to five times higher than the DNA yields determined by the yield gels. The difference between the two methods is most likely due to the greater sensitivity exhibited by slot blot hybridization in detecting partially degraded human DNA, which in low concentrations may not be visible on a yield gel. In a few samples, the slot blot results indicated less DNA than did the yield gels. This may have been due to bacterial DNA contamination, although this seems unlikely since all stains were prepared from fresh blood on clean cloth and dried immediately. All slot blot and yield gel standards were cross-checked with each other and with commercial standards to verify that all standards used were functioning properly. In the analysis of the yield gel and slot blot data, it is evident that the two methods allow

TABLE 1—*Average DNA yield (in nanograms) determined by ethidium bromide gel electrophoresis for sets of twenty 25-μL blood and twenty 10-μL semen stains; one set was tested by each laboratory for each extraction method.*

Extraction Method			Participating Laboratory									Average[a]	Range
			A	B	C	D	E	F	G	H	I		
CM		blood	250	193	50	242	165	[b]	267	262	183	201	50–267
CM		semen	103	0	25	53	0	[b]	117	112	106	86	0–117
FBI	DIFF	semen	76	0	[b]	127	[b]	[b]	127	72	[b]	101	0–127
FBI	REG	blood	103	0	147	190	250	[b]	195	235	[b]	186	0–250
FBI	REG	semen	100	0	180	242	320	[b]	50	810	[b]	283	0–810
LC	OR	blood	105	298	40	232	[b]	100	82	290	[b]	163	40–298
LC	OR	semen	245	226	131	202	[b]	257	180	0	[b]	210	0–257
LC	NO	blood	300	300	300	400	222	235	137	172	[b]	258	137–400
LC	NO	semen	173	190	162	260	232	65	260	318	[b]	208	65–318

[a]Laboratories that obtained zero DNA yield for a procedure were not included in the average yield for that method.
[b]Test was not performed by that laboratory.

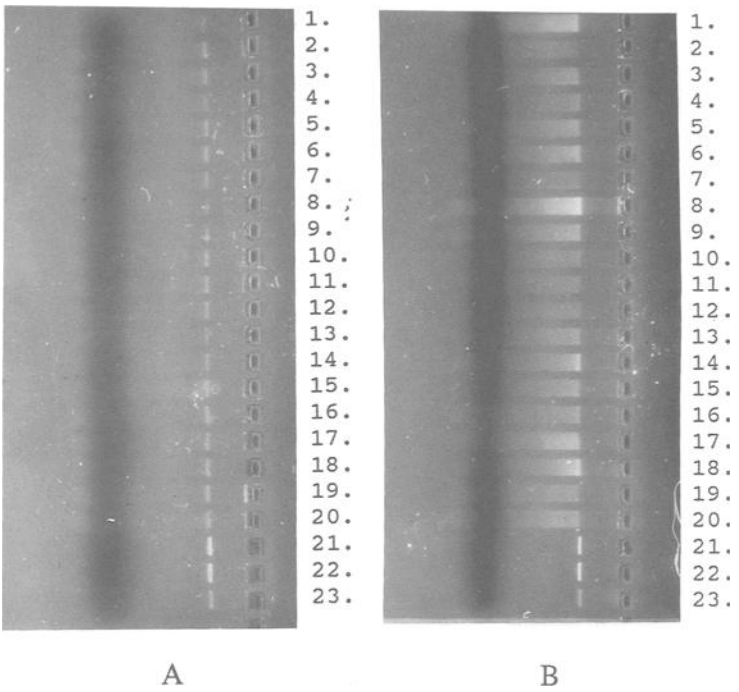

FIG. 2—*Effect of stain extraction buffer with freshly prepared DTT added just before extraction* (A) *and stain extraction buffer previously containing DTT* (B) *on high-molecular-weight DNA yield-by-yield gel electrophoresis. Each lane (1–20) in each figure represents 2% of the total DNA extracted from a 25-μL bloodstain. Lanes 21 and 22 contain 12 and 14 ng of λ DNA quantification standard and Lane 23 is a 10-ng K562 quantification standard. Additional quantification standards were used in other lanes (not shown).*

for a better estimation of the amount of usable HMW human DNA that is contained in a sample.

Restriction Endonuclease Digestion

All of the DNA extraction procedures tested yielded HMW DNA that could be completely restricted with the enzyme Hae III. Approximately 90% of the DNA extracts yielded a complete restriction with the first restriction digestion. The remainder of the DNA extracts restricted completely during the second digestion with the exception of some of the extracts generated by the Cellmark procedure. These samples required a third restriction digest. No explanation was found for the difficulty in the restriction of these extracts.

RFLP Analysis

Locus D2S44—A standard reference set of RFLP bands was generated from DNA extracted from fresh liquid blood. All RFLP patterns derived from the various DNA stain extraction procedures were compared with this reference set. The average allele sizes obtained from DNA derived from the liquid blood were used to evaluate the allele sizes obtained from the blood and semen stains. The RFLP sizing data for the two alleles of each sample are given in Tables 3 and 4 for the probe YNH24 (locus D2S44). The

TABLE 2—*Average DNA yield (in nanograms) determined by slot blot hybridization for sets of twenty 25-μL blood or twenty 10-μL semen stains; one set was tested by each laboratory for each extraction method.*

Extraction Method			Participating Laboratory									Average	Range
			A	B	C	D	E	F	G	H	I		
CM		blood	1125	525	243	822	168	[a]	875	1350	117	653	117–1125
CM		semen	406	275	235	195	48	[a]	262	446	487	294	48–487
FBI	DIFF	semen	256	145	[a]	439	[a]	[a]	550	203	[a]	318	145–550
FBI	REG	blood	550	175	486	525	500	[a]	875	525	[a]	519	175–875
FBI	REG	semen	875	181	925	1700	2000	[a]	362	1450	[a]	1070	181–2000
LC	OR	blood	231	1125	97	468	[a]	275	182	95	[a]	353	95–1125
LC	OR	semen	181	387	1962	500	[a]	1850	500	112	[a]	784	112–1962
LC	NO	blood	950	1537	1175	1375	425	662	512	625	[a]	907	425–1537
LC	NO	semen	932	1315	1450	1250	1000	1000	825	1631	[a]	1175	825–1631

[a]Test was not performed by that laboratory.

TABLE 3—*D2S44 band No. 1 sizing data (in base pairs) of the liquid blood standard for three gel conditions and three extraction methods; three extractions were performed by each method and autoradiographs generated from duplicate membranes (A and B).*[a]

Extraction Method	16-cm ME Agarose + ETBR A	16-cm ME Agarose + ETBR B	20-cm ME Agarose + ETBR A	20-cm ME Agarose + ETBR B	20-cm LE Agarose + NO ETBR A	20-cm LE Agarose + NO ETBR B	Average	% Difference Between High and Low
MMBC[b]	4023	4027	4067	4019	4078	4049	4044	1.5
MMBC	4038	4019	4058	4027	4076	4076	4049	1.4
MMBC	4037	4025	4047	4014	4069	4083	4046	1.7
FBI	4034	4066	4065	4045	4057	4090	4060	1.4
FBI	4025	4050	4037	4026	4074	4099	4052	1.8
FBI	4043	–	4060	4054	4057	4068	4056	0.6
LCNO[c]	4040	4057	4071	4046	4080	4099	4066	1.5
LCNO	4059	4043	4083	4044	4080	4083	4065	1.0
LCNO	4023	4051	4045	4062	4054	4092	4055	1.7
Average	4036	4042	4059	4037	4069	4082	. . .	. . .
% Difference between high and low	0.9	1.2	1.1	1.2	0.6	1.2	. . .	. . .

[a]Average allele size of the liquid standard = 4055.
[b]Minneapolis Memorial Blood Center.
[c]Lifecodes Nonorganic.

TABLE 4—*D2S44 band No. 2 sizing data (in base pairs) of the liquid blood standard for three gel conditions and three extraction methods; three extractions were performed by each method and autoradiographs generated from duplicate membranes (A and B).*[a]

Extraction Method	16-cm ME Agarose + ETBR A	16-cm ME Agarose + ETBR B	20-cm ME Agarose + ETBR A	20-cm ME Agarose + ETBR B	20-cm LE Agarose + NO ETBR A	20-cm LE Agarose + NO ETBR B	Average	% Difference Between High and Low
MMBC	1834	1823	1819	1819	1820	1826	1824	0.8
MMBC	1826	1829	1821	1822	1819	1828	1824	0.5
MMBC	1814	1829	1828	1826	1820	1821	1823	0.8
FBI	1823	1822	1818	1820	1820	1821	1821	0.3
FBI	1809	1825	1824	1822	1820	1832	1822	1.3
FBI	1827	–	1825	1829	1826	1823	1826	0.3
LCNO	1834	1824	1820	1826	1832	1836	1829	0.9
LCNO	1828	1819	1823	1822	1829	1827	1825	0.5
LCNO	1823	1832	1818	1819	1826	1839	1826	1.2
Average	1824	1825	1822	1823	1824	1828	. . .	. . .
% Difference between high and low	1.4	0.7	0.5	0.5	0.7	1.0	. . .	. . .

[a]Average allele size of the liquid standard = 1824.

data show that the allele sizes determined for the liquid blood standard were reproducible within 2.5% regardless of the extraction method, gel length, or the presence or absence of ethidium bromide. Fifty-four values were obtained and averaged to develop the liquid blood standard value. These data show that RFLP patterns obtained from the various liquid blood DNA extraction methods and gel conditions are comparable for such purposes as case work, population data basing, and profiling

The RFLP sizing data for the DNA extracted by the nine laboratories using four bloodstain DNA extraction protocols, five semen stain DNA extraction protocols, and three gel conditions are given in Tables 5, 6, 7, and 8 for the probe YNH24. The results presented in this paragraph represent all data obtained with the exception of data obtained through the Lifecodes nonorganic bloodstain DNA extraction procedure. These results were inconsistent with the other data and will be addressed separately later in the paper. The data show that the allele sizes obtained from the DNA extracted by each laboratory were reproducible within 2.5% across all parameters tested. Each allele size in Table 5, 6, 7, and 8 for the probe YNH24 was compared with the appropriate average allele size of the liquid standard given in Tables 3 and 4. An example of this type of comparison is given in Table 9. The data indicate less than a 2% variation from the standard value. This same trend holds for all the data obtained. These data demonstrate that liquid blood, bloodstains, and semen stains give reproducible allele sizing data. In addition, within the limits of the parameters tested, the method of extraction, the gel length, or the presence or absence of ethidium bromide in the gel have negligible effects on the allele sizing data.

A K562 human cell line control was run on each gel. The cell line sizing data were compared with the Minnesota Forensic Science Laboratory's cell line average value obtained from population studies. All comparisons varied by less than 2.5%.

To decrease the effects of measurement error, each autoradiograph was read by two different individuals. Computer analysis was used to compare sizing data between the two readers. Note that sizing variation was less than 2% between readers and the majority (90%) of the assessments were within 1% of each other. An example of this data comparison is given in Table 10 and attests to the accuracy and reproducibility of both the technical aspect of deriving RFLP patterns as well as the computer-assisted procedures of measuring allele sizes.

Loci D17S79 and D10S28—All data reported in the preceding sections for the locus D2S44 were similarly observed and evaluated for the loci D17S79 and D10S28. Equivalent trends of reproducibility were found for these probes when analyzed for the same parameters of gel conditions and extraction methods.

Lifecodes Nonorganic Bloodstain DNA Extraction Results—The DNA from the extracts obtained from four of the laboratories that performed the Lifecodes nonorganic bloodstain DNA extraction procedure (50%) gave noticeable band shifts when compared with the allele sizes of the liquid standard. The DNA extracts that gave rise to these band shifts were discolored, whereas the DNA extracts that did not give rise to band shifts were clear. This indicates that the cause of the DNA band shifts was most likely due to a lack of procedural experience rather than to the procedure itself. An example of this band shifting is shown in Fig. 3A. The occurrence of band shifting in the samples submitted by these four laboratories was noted for all three probes. The band shift was observed on the LE agarose gels without ethidium bromide but not on the ME agarose gels with ethidium bromide. The possible cause of the band shift/nonshift occurrence is currently under investigation. The Minnesota laboratory has extracted DNA from several sets of bloodstains with the Lifecodes nonorganic procedure and has been unable to obtain a band shift. This suggests that the procedure was performed differently by those laboratories whose extracted DNA gave rise to band shifts. According to Lifecodes, a

TABLE 5—*D2S44 band No. 1 sizing data (in base pairs) for blood and semen stains extracted by various methods and electrophoresed on 20-cm LE agarose gels without ethidium bromide.*

Extraction Method			Participating Laboratory									Average	% Difference Mean	% Difference (High and Low)
			A	B	C	D	E	F	G	H	I			
CM		blood	4130	4133	4107	4124	4122	. . .	4089	4098	4133	4117	0.2	1.1
CM		semen	4125	4102	4094	4136	. . .	. . .	4087	4096	4110	4107	0.05	1.2
FBI	DIFF	semen	4125	. . .	. .	4121	. . .	. . .	4109	4093	. . .	4112	0.07	0.8
FBI	REG	blood	4128	. . .	4097	4120	4108	. . .	4107	4118	. . .	4113	0.1	0.8
FBI	REG	semen	4130	. . .	4102	4094	4107	. . .	4108	4092	. . .	4105	0.1	0.9
LC	OR	blood	4089	4132	4100	4129	. . .	4108	4101	. . .	. . .	4110	0.02	1.0
LC	OR	semen	4071	4128	4071	4113	. . .	4096	4096	4130	. . .	4101	0.2	1.4
LC	NO	blood	4102	4296	4115	4151	4226	4333	4171	4298	. . .	4211	2.4	5.5
LC	NO	semen	4063	4125	4080	4080	4089	4096	4086	4085	. . .	4088	0.5	1.5

TABLE 6—*D2S44 band No. 2 sizing data (in base pairs) for blood and semen stains extracted by various methods and electrophoresed on 20-cm LE agarose gels without ethidium bromide.*

Extraction Method			Participating Laboratory									Average	% Difference Mean	% Difference (High and Low)
			A	B	C	D	E	F	G	H	I			
CM		blood	1828	1827	1812	1824	1820	. . .	1820	1820	1824	1822	0.0	0.9
CM		semen	1826	1804	1811	1832	. . .	. . .	1819	1830	1813	1819	0.2	1.5
FBI	DIFF	semen	1810	. . .	. . .	1830	. . .	. . .	1826	1823	. . .	1822	0.0	1.1
FBI	REG	blood	1817	. . .	1827	1851	1821	. . .	1831	1816	. . .	1827	0.3	1.9
FBI	REG	semen	1826	. . .	1830	1841	1822	. . .	1827	1819	. . .	1827	0.3	1.2
LC	OR	blood	1830	1822	1804	1832	. . .	1823	1815	. . .	. . .	1821	0.05	1.5
LC	OR	semen	1817	1821	1809	1838	. . .	1823	1809	1815	. . .	1819	0.2	1.6
LC	NO	blood	1835	2017	1825	1846	1884	2185	1847	1942	. . .	1923	5.3	18.7
LC	NO	semen	1822	1841	1817	1834	1824	1813	1818	1809	. . .	1822	0.0	1.8

TABLE 7—*D2S44 band No. 1 sizing data (in base pairs) for blood and semen stains extracted by various methods and electrophoresed on 20-cm (A, B, C, D) and 16-cm (E, F, G, H, I) ME agarose gels with ethidium bromide.*

Extraction Method			Participating Laboratory									Average	% Difference Mean	% Difference (High and Low)
			A	B	C	D	E	F	G	H	I			
CM		blood	4110	4095	4109	4116	4102	. . .	4076	4113	4098	4102	0.4	1.0
CM		semen	4109	. . .	4072	4109	. . .	. . .	4103	4098	4064	4093	0.2	1.1
FBI	DIFF	semen	4094	. . .	. . .	4079	. . .	. . .	4071	4073	. . .	4079	0.1	0.6
FBI	REG	blood	4079	. . .	4129	4056	4082	. . .	4063	4088	. . .	4083	0.05	1.8
FBI	REG	semen	4016	. . .	4075	4061	4045	. . .	4048	4046	. . .	4049	0.9	1.5
LC	OR	blood	4111	4065	4137	4111	. . .	4093	4104	. . .	. . .	4104	0.5	1.8
LC	OR	semen	4122	4038	4098	4064	. . .	4075	4090	4086	. . .	4082	0.1	2.1
LC	NO	blood	4135	4067	4112	4077	4079	4114	4106	4059	. . .	4094	0.2	1.9
LC	NO	semen	4097	4039	4047	4029	4066	4045	4041	4032	. . .	4050	0.9	1.7

TABLE 8—*D2S44 band No. 2 sizing data (in base pairs) for blood and semen stains extracted by various methods and electrophoresed on 20-cm (A, B, C, D) and 16-cm (E, F, G, H, I) ME agarose gels with ethidium bromide.*

Extraction Method			Participating Laboratory									Average	% Difference Mean	% Difference (High and Low)
			A	B	C	D	E	F	G	H	I			
CM		blood	1825	1818	1831	1825	1829	. . .	1822	1835	1826	1826	0.3	0.9
CM		semen	1827	. . .	1811	1822	. . .	. . .	1829	1830	1813	1822	0.1	1.0
FBI	DIFF	semen	1827	. . .	. . .	1812	. . .	. . .	1815	1821	. . .	1819	0.1	0.8
FBI	REG	blood	1817	. . .	1823	1805	1821	. . .	1820	1826	. . .	1819	0.1	1.2
FBI	REG	semen	1799	. . .	1810	1807	1801	. . .	1818	1812	. . .	1808	0.7	1.1
LC	OR	blood	1837	1813	1849	1823	. . .	1834	1837	. . .	. . .	1832	0.7	2.0
LC	OR	semen	1827	1804	1825	1807	. . .	1810	1823	1820	. . .	1817	0.2	1.3
LC	NO	blood	1834	1816	1836	1812	1822	1839	1835	1821	. . .	1827	0.4	1.5
LC	NO	semen	1807	1807	1805	1805	1806	1796	1821	1805	. . .	1806	0.8	1.4

TABLE 9—*Example of one laboratory's data comparing the sizing values between K562 cell line control and sample stains with their corresponding mean standard values; the samples were run on a 16-cm ME agarose gel with ethidium bromide and probed with YNH24 (locus D2S44).*

			Lane	Band	Cell Line	K562 Std	Percent Difference
K562	Cell	Line	2	1	2914	2910	0.14
			2	2	1799	1793	0.33
Extraction Method			Lane	Band	Stains	Liquid Std	Percent Difference
CM		blood	3	1	4110	4055	1.36
			3	2	1825	1824	0.05
CM		semen	4	1	4109	4055	1.33
			4	2	1827	1824	0.16
FBI	DIFF	semen	6	1	4094	4055	0.96
			6	2	1827	1824	0.16
FBI	REG	blood	7	1	4079	4055	0.59
			7	2	1817	1824	0.38
FBI	REG	semen	8	1	4016	4055	0.96
			8	2	1799	1824	1.37
LC	OR	blood	10	1	4111	4055	1.38
			10	2	1837	1824	0.71
LC	OR	semen	11	1	4122	4055	1.65
			11	2	1827	1824	0.16
LC	NC	blood	12	1	4135	4055	1.97
			12	2	1834	1824	0.55
LC	NC	semen	13	1	4097	4055	1.04
			13	2	1807	1824	0.93

second possible cause for the band shift was the necessity to follow restriction digestion with a lithium chloride (LiCl) precipitation step. This step is used to remove excess blood proteins that could cause migration shifts in electrophoresis. This step was not performed in this study. Additional studies were attempted at the Minnesota laboratory using the Lifecodes nonorganic DNA extracts that had previously shown band shifts; however, these extracts lacked sufficient DNA to generate the necessary RFLP patterns to confirm that a LiCl step would rid these samples of band shifting. DNA from several additional bloodstains was extracted by the Lifecodes nonorganic procedure in the Minnesota laboratory to study the band shift occurrence. Each sample was digested with and without the LiCl precipitation step. Since none of these samples showed band shifting, it was not possible to assess the value of the LiCl step in eliminating band shifts. Another area under investigation with respect to the band shift/nonshift occurrence is why significant shifting was not observed in ME agarose gels containing ethidium bromide (Fig. 3) and whether the ME agarose or the ethidium bromide was responsible for eliminating the band shift.

Conclusions

This study demonstrates that (1) consistent and high yields of high-molecular-weight DNA can be recovered from liquid blood, bloodstains and semen stains by several different extraction methods and (2) that reproducible and reliable RFLP patterns can be produced from the DNA recovered by several different gel conditions and extraction protocols. The results obtained using the Lifecodes nonorganic bloodstain extraction procedure suggest that anomalous electrophoretic migration may occur if the procedure is not performed properly. The reasons for this are currently being investigated. This

TABLE 10—*Example of one laboratory's data comparing the sizing values between two readers of the same autoradiograph. The samples were run on a 16-cm ME agarose gel with ethidium bromide and probed with YNH24 (locus D2S44).*

			Lane	Band	RDR-1	K562 Std	Percent Difference
K562	Cell	Line	2	1	2921	2910	0.38
			2	2	1807	1793	0.78
			Lane	Band	RDR-2	K562 Std	Percent Difference
K562	Cell	Line	2	1	2928	2910	0.62
			2	2	1807	1793	0.78
Extraction Method			Lane	Band	RDR-1	RDR-2	Percent Difference
(K562	CELL	LINE)	2	1	2921	2928	0.24
			2	2	1807	1807	0.00
CM		blood	3	1	4116	4108	0.19
			3	2	1825	1825	0.00
CM		semen	4	1	4109	4094	0.37
			4	2	1822	1823	0.05
FBI	DIFF	semen	6	1	4079	4034	1.12
			6	2	1812	1819	0.39
FBI	REG	blood	7	1	4056	4054	0.05
			7	2	1805	1810	0.28
FBI	REG	semen	8	1	4061	4054	0.17
			8	2	1807	1809	0.11
LC	OR	blood	10	1	4111	4097	0.34
			10	2	1823	1821	0.11
LC	OR	semen	11	1	4064	4052	0.30
			11	2	1807	1812	0.28
LC	NC	blood	12	1	4077	4083	0.15
			12	2	1812	1815	0.17
LC	NC	semen	13	1	4029	4057	0.69
			13	2	1805	1805	0.00

substantiates the need for laboratories to carry out thorough validation studies when considering the implementation of any new procedures.

The findings described in this paper demonstrate clearly that consistent and reliable DNA RFLP patterns can be obtained and that VNTR derived DNA profiles can be reliably compared from laboratory to laboratory, even if variations in extraction methods or electrophoretic conditions are used. The extent of variation tolerable for RFLP data to be shared in the form of population data bases, profiling data bases, and so forth, should be determined from validation studies by each laboratory when different techniques vary from tested and established procedures.

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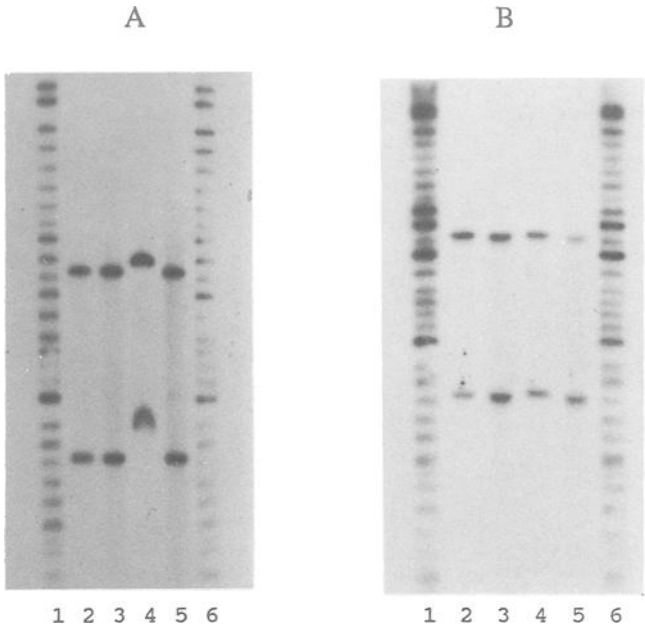

FIG. 3—*Two autoradiographs illustrating the difference between RFLP banding patterns from one laboratory's DNA extracts using the Lifecodes organic (LCOR) extraction procedure and Lifecodes nonorganic (LCNO) extraction procedure on a 20-cm LE agarose gel without ethidium bromide (A) and a 16-cm ME agarose gel with ethidium bromide (B). Lanes 1 and 6 contain molecular weight markers, Lanes 2 and 3 contain LCOR blood and semen stain DNA extracts, respectively, and Lanes 4 and 5 contain LCNO blood and semen stain DNA extracts, respectively.*

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