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Evaluation of Deoxyribonucleic Acid (DNA) Isolated from Human Bloodstains Exposed to Ultraviolet Light, Heat, Humidity, and Soil Contamination

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ABSTRACT: This study was designed to analyze the effects of common environmental insults on the ability to obtain deoxyribonucleic acid (DNA) restriction fragment-length polymorphisms (RFLP) patterns from laboratory prepared specimens. The environmental conditions studied include the exposure of dried bloodstains to varying amounts of relative humidity (0, 33, 67, and 98%), heat (37°C), and ultraviolet light for periods of up to five days. In addition, the effect of drying over a four-day period in whole blood collected with and without ethylenediaminetetraacetate (EDTA) was examined.

The results of the study showed that, under the conditions studied, the integrity of DNA is not altered such that false RFLP patterns are obtained. The only effect observed was that the overall RFLP pattern becomes weaker, but individual RFLP fragments are neither created nor destroyed.

KEYWORDS: criminalistics, serology, deoxyribonucleic acid, restriction fragment length polymorphisms, bloodstains

Since the demonstration of the first highly polymorphic region of deoxyribonucleic acid (DNA) by Wyman and White [1], there have been over 200 polymorphic DNA markers reported [2] that have been shown to exhibit Mendelian inheritance [3]. We and others have demonstrated that DNA restriction fragment length polymorphisms (RFLPSs) can be reliably identified in dried blood, semen stains, and biological evidentiary specimens [4-8].

The purpose of this study was to investigate the effect of environmental conditions on the quantity and quality of DNA isolated from prepared human bloodstains. Although DNA has been shown to be stable in dried semen and blood, it is important to demonstrate that frequently encountered environmental conditions will not adversely affect the RFLP patterns produced. Experiments were designed to study the effects of ultraviolet (UV) light, heat, humidity, and soil contamination on the integrity of DNA in dried specimens and to deter-

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mine if the RFLP pattern produced under the experimental condition varied from those of control samples.

Materials and Methods

Preparation of Samples

Bloodstains were prepared for the ultraviolet, heat, and humidity studies from fresh unclotted whole blood, which had been collected in ethylenediaminetetraacetate (EDTA, sodium salt) vacutainers. The blood was dispensed in 175- μ L aliquots with a hypodermic needle onto 100% cotton fabric swatches. The prepared stains were dried at room temperature (24°C) for four days before exposure conditions were introduced.

Exposure of Samples

Exposure to Soil—Aliquots of 400 μ L of human blood were applied directly onto various soil samples and allowed to dry for four days. The blood was dispensed onto five different soil types, each done in duplicate: beach sand, soil containing clay, commercial potting soil, soil containing organic matter, and a gravel/sand mixture.

Ultraviolet Irradiated Samples—Duplicate bloodstained samples exposed to ultraviolet light were divided into five exposure groups and processed separately. The samples were placed on a flat surface 10 cm (4 in.) below the UV light source (BLE Spectroline Model XX-15) and irradiated from one to five days.

Heat-Treated Samples—Duplicate bloodstain samples were placed in paper envelopes and exposed in a 37°C incubator for one to five days.

Samples Exposed to Varying Degrees of Humidity—Humidity chambers were prepared using saturated aqueous solutions maintained at 20°C inside closed vessels. Dried, duplicate bloodstain samples were suspended in the vessels and exposed to 0, 33, 67, and 98% humidity conditions for up to five days. The 0% humidity samples were exposed for five days in a sealed vessel containing the desiccant Drierite®. The 33% humidity chamber was prepared with a saturated solution of calcium chloride ($\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$) with sample exposures for one, two, and five days. The 67 and 98% humidity conditions were prepared with sodium nitrite (NaNO_2) and copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) respectively, with sample exposures for one, two, and five days [9]. Each exposure was run in duplicate.

Drying Time Samples

Stains prepared from fresh blood collected in EDTA (sodium salt) vacutainers and stains prepared from blood collected in tubes free of preservative were aliquoted in 5-, 3-, 2-, 1-, 0.5-, and 0.25-mL volumes. The stains were covered and permitted to dry onto clean glass plates. The drying time of the blood samples deposited on the same substrata and exposed to the same environmental conditions was directly related to its volume and took place over a period of four days. Samples (0.2 g) were used for analysis from each of the aliquoted specimens. In two instances in the EDTA blood (volumes 0.5 and 0.25 mL) and in one instance in the nonanticoagulated blood (0.25 mL), 0.2 g was not available for analysis.

Extraction of Samples

Bloodstains on cotton swatches were cut into small pieces and DNA lysis buffer (DLB) (10mM Tris hydrochloric acid [HCl], pH 7.4, 10mM EDTA salt) was added to saturate the material. Proteinase K (final concentration 150 μ g/mL) and sodium dodecyl sulfate (SDS)

(final concentration 1% w/v) were added to the DLB, and the sample was incubated at 37°C overnight on a rocker platform. The fabric was removed and the solution containing the isolated DNA was extracted with an equal volume of liquid phenol equilibrated with Tris HCl. The upper layer organic phase was removed with a wide-bore pipette and replaced with an equal volume of phenol-chloroform-isoamyl alcohol solution [phenol: (chloroform-isoamyl alcohol (1:1)]. (The chloroform:isoamyl alcohol was prepared in a 24:1 stock solution prior to addition of phenol.) After discarding the lower organic layer, we added an equal volume of chloroform:isoamyl alcohol (24:1) and repeated the procedure.

Dialysis of Samples

After removal of the last organic layer, we transferred the samples into dialysis membrane tubings (Spectrapor-membrane tubing [molecular weight cutoff 12 000 to 14 000], Spectrum Medical Industries Inc.) and dialyzed overnight against four changes of $\times 1$ TE (10mM Tris HCl, pH 7.4, 1mM EDTA) at 4°C.

Yield Gel Analysis of Samples

One percent of the total DNA was loaded onto a 0.9% agarose gel. The tank buffer contained ethidium bromide (0.5 mg/mL) to stain the DNA to determine if high molecular weight (HMW) DNA was present. Electrophoresis was carried out for 1 h at 50 V. Serially diluted Lambda DNA was present in 500-, 300-, 200-, 100-, 50-, 25-, and 10-ng aliquots, to give an approximation of the concentration of HMW DNA present.

RFLP Analysis of Samples

RFLP analysis of the samples was done according to previously described methods [8].

DNA Probes

Three human DNA probes that recognize single-locus polymorphisms in Pst I digested DNA over a wide size range were used for the analysis. The probe pAC 225 contains short tandem repeats of a chemically synthesized consensus sequence that recognizes a polymorphism at the D2S44 locus [10]. The probe pAC 255, isolated by Cook [11], recognizes a polymorphism at the DXYS14 locus. The probe pAC 256, isolated at Lifecodes Corp., recognizes a polymorphism at the D17S79 locus. A probe containing the Escherichia coli ribosomal gene, pAC 267, including cloning vectors, was used to detect the presence of conserved sequences of bacterial DNA.

Results and Discussion

Exposure to Heat

Incubation at 37°C for up to five days did not adversely affect the ability to obtain consistent RFLP patterns in any of the exposure conditions (Fig. 1). These results are consistent with the findings of Madisen et al. Their results indicated that HMW DNA could be extracted from samples incubated for up to 26 weeks at 37°C followed by subsequent RFLP identification [12]. In one of the duplicate bloodstains exposed for 5 days, three additional bands were observed that were not found in the control samples Fig. 1 (arrows). These three bands were observed to hybridize to the bacterial probe pAC 267, indicating that these extra bands were contributed by nonhuman DNA (Fig. 2, arrows).

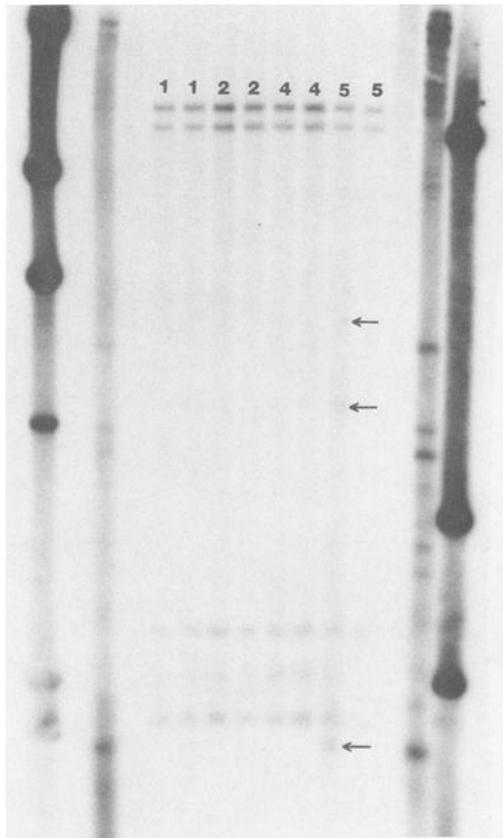


FIG. 1—Autoradiograph of duplicate samples exposed to 37°C for one, two, four, and five days. Numbers above lanes reflect number of days sample was exposed to 37°C heat. Arrows indicate spurious bands identified in one of the five-day exposure samples.

Exposure to Ultraviolet Light

The intensity of the RFLP patterns produced from samples exposed to UV light over a five-day period decreased slightly as the exposure period increased, Fig. 3. Although UV light is known to affect the molecular structure of DNA [13], these data show that consistent RFLP patterns are obtained under the conditions of this experiment without the occurrence of anomalous results. The numbers above the lanes reflect the number of days the sample was exposed to UV light.

Exposure to Varying Degrees of Humidity

Exposure of samples for up to five days at 98% humidity revealed that consistent band patterns were obtainable for all exposure conditions. In addition, the quantity and quality of extracted DNA was consistent in the 0, 33, and 66% humidity conditions (Fig. 4). The numbers in parentheses included under each of the exposure conditions reflect the number of days each sample was exposed to the specific humidity.

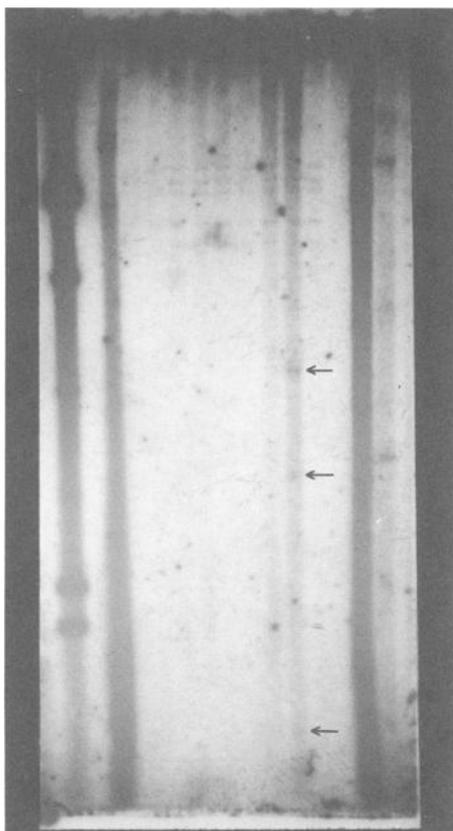


FIG. 2—Autoradiograph of heat samples hybridized to bacterial/vector probe pAC 267. Arrows show hybridization to bands in duplicate five-day exposure lane.

Effect of Drying Time

The drying time study revealed that there were differences in DNA yield when bloodstains were prepared from blood that was collected in EDTA versus those prepared from blood without anticoagulant. This is reflected by the decreased intensity of the hybridized DNA pattern in the latter Lanes 1, 2, 3, 4, and 5 in brackets under "R" (Fig. 5).

In the EDTA specimens taken from the smaller volumes aliquoted (0.5 and 0.25 mL) and in the smaller volume (0.25 mL) for the nonanticoagulated blood, the intensity of the bands obtained was decreased, Fig. 5, Lanes 5 and 6 under bracket "E" and Lane 5 under bracket "R." The decreased banding intensity in these specimens results from smaller sample sizes of these specimens available relative to the other specimens analyzed (0.2 g).

We have previously shown using actual casework specimens that longer exposures to the environment during drying may affect the integrity of DNA [8]. The results of this study have shown that it is not the drying time per se that affects DNA adversely. Indeed, EDTA-preserved (included to inhibit nucleases) whole blood and whole blood without EDTA kept in a protected environment showed no loss in RFLP signal over a four-day drying time.

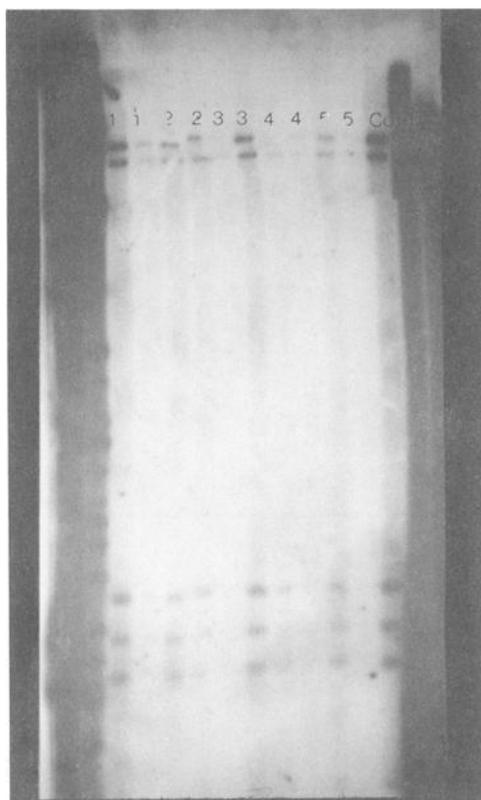


FIG. 3—Autoradiograph of samples exposed to UV light for one, two, three, four, and five days hybridized to probes pAC 255 and pAC 225. Numbers above lane reflect number of days the sample was exposed to UV light. Distortion in gel (top) in first sample exposed for two days is due to a broken gel.

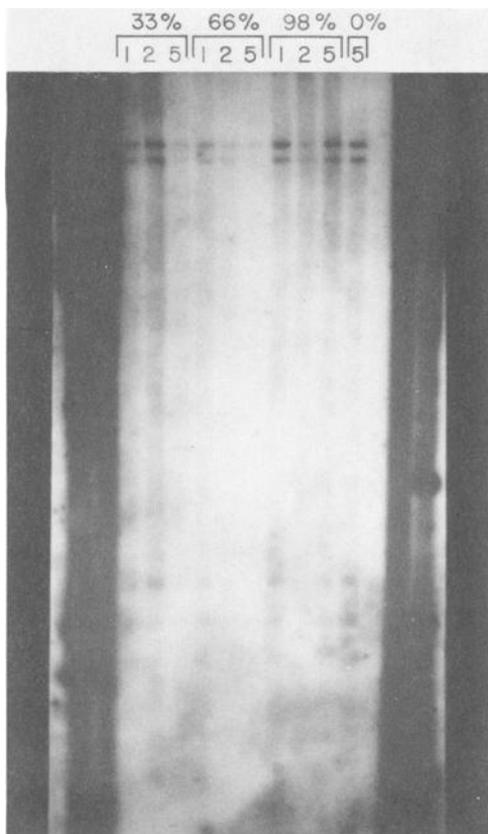


FIG. 4—Autoradiograph of samples exposed to variable humidity conditions and hybridized to probes pAC255 and pAC225. Numbers above lanes reflect number of days that sample was exposed to humidity level indicated.

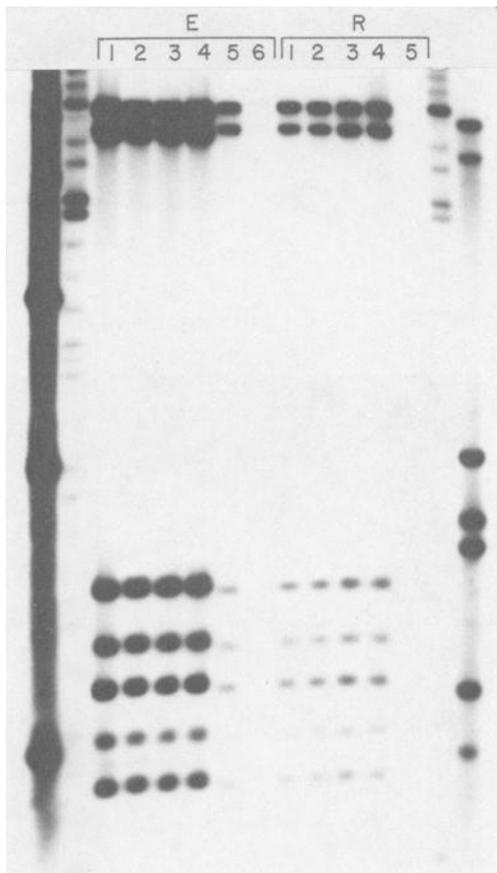


FIG. 5—Autoradiograph of drying time samples isolated from EDTA blood and blood without preservative. Lanes 1E through 4E represent DNA samples obtained from 0.2-g dried EDTA blood isolated from 5-, 3-, 2-, and 1-mL volume bloodstains. Lanes 1R through 4R represent DNA isolated under same conditions from preservative-free blood. DNA in Lanes 5E and 6E was isolated from 0.08- and 0.02-g EDTA blood recovered from 0.5- and 0.25-mL stains. No DNA print was obtained in Lane 5R from the 0.010-g sample isolated from preservative-free blood.

Exposure to Soil

It was not possible to produce human specific RFLP patterns in any of the samples isolated from the soil contamination study (Fig. 6). Hybridization with the bacterial probe pAC 267 revealed bacterial and/or vector contamination in several samples (Fig. 7, Lanes 2, 5, 7, and 8). These samples corresponded to beach sand, soil containing clay, and soil samples with organic matter (collected from a road). Contamination was not found in all the soil samples analyzed. For example, only one of the clay containing soil sample duplicates showed contamination, whereas both of the samples containing organic matter exhibited hybridizations with the pAC 267 probe. The lack of human signal indicates that sufficient intact human DNA was not present to give a hybridization signal.

In conclusion, the results of this study have shown that common environmental insults, that is, UV light, humidity, and heat, on DNA in dried biological specimens, under the conditions of these experiments, do not adversely affect the integrity of DNA. Soil or its contaminants does appear to affect the DNA integrity. Drying over a four-day period in a

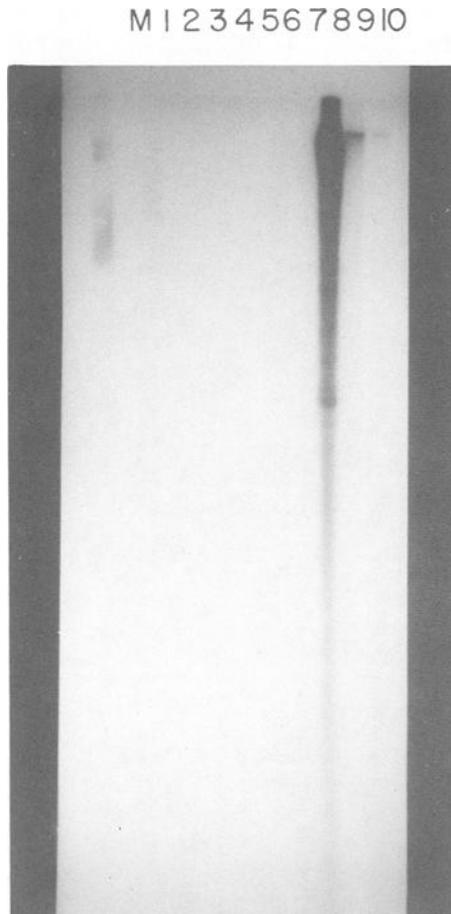


FIG. 6—Autoradiograph of samples dried onto various soil types: Hybridization to pAC 255 and pAC 225. Lane M, molecular weight markers; Lanes 1 and 2, beach sand; Lanes 3 and 4, commercial potting soil; Lanes 5 and 6, soil containing clay; Lanes 7 and 8, soil containing organic matter (collected from a road); Lanes 9 and 10, soil/gravel mixture from a driveway.

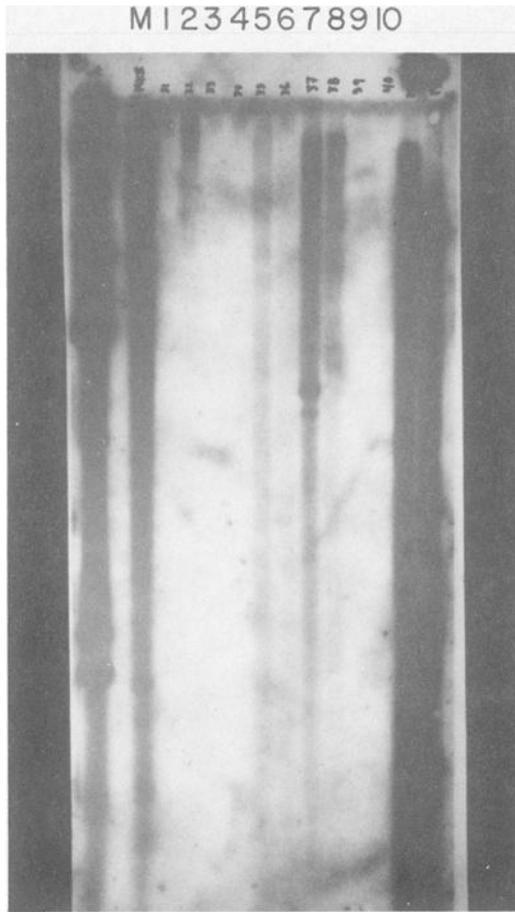


FIG. 7—Autoradiograph of samples dried onto various soil types: hybridization to bacterial/vector probe. Nylon membrane stripped of pAC 255 and pAC 225 signal was hybridized to pAC 267 bacterial/vector probe. Lanes M, molecular weight markers; Lanes 1 and 2, beach sand; Lanes 3 and 4, commercial potting soil; Lanes 5 and 6, soil containing clay; Lanes 7 and 8, soil containing organic matters (collected from a road); Lanes 9 and 10, soil/gravel mixtures from a driveway.

protected environment does not affect DNA integrity in either EDTA-treated blood or in blood not treated with the anticoagulant.

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