TECHNICAL NOTE

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Effects of Argon Laser Light, Alternate Source Light, and Cyanoacrylate Fuming on DNA Typing of Human Bloodstains

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ABSTRACT: Restriction fragment length polymorphism (RFLP) profile results were obtained from deoxyribonucleic acid (DNA) isolated from human bloodstains that had been subjected to cyanoacrylate ester ("superglue") fuming, argon ion laser light and alternate light sources. All RFLP profile results obtained from treated samples were consistent with the DNA pattern from untreated bloodstains.

KEYWORDS: forensic science, blood, fingerprints, genetic typing, laser, deoxyribonucleic acid (DNA), restriction fragment length polymorphism (RFLP), alternate light source, cyanoacrylate ester, superglue

The application of modern technologies to the field of forensic science has increased at a rapid rate over the past several years resulting in the development of many new techniques for investigations. Two of the areas that have benefitted most from these advancements are the development of latent prints and the analysis of blood. The use of argon ion lasers, alternate light sources and cyanoacrylate (superglue) fuming have greatly enhanced latent fingerprint analysis while restriction fragment length polymorphism (RFLP) has had a significant impact on the processing and analysis of biological evidence [*1–5*]. Lasers and alternate light sources are most commonly used in fingerprint detection but they are also used to detect biological stains. Ideally stains of interest will be removed from evidentiary items requiring fingerprint analysis prior to the actual fingerprint exam which may include "superglue" fuming. This is not always the case. A simple example of this is a bloody fingerprint. In addition, a fingerprint exam may already have been performed prior to DNA analysis.

Little work has been done to determine the degree of compatibility of these methods when used in concert. The purpose of this study was to assess whether the use of cy-

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anoacrylate ester or a high energy light source or both, would affect the results obtained from RFLP DNA analysis.

Materials and Methods

Cyanoacrylate Ester Fuming of Bloodstains

Bloodstains present on pieces of white cotton cloth were exposed to cyanoacrylate ester (that is "superglue" Loctite Corporation, Newington, CT) vapors for various periods of time ranging from 10 min to 40 h (see Table 1).

High Energy Light Treatment of Bloodstains

Bloodstains were exposed to one of three light sources at different wavelengths for varying periods of time (see Tables 2 and 3). In all cases the light source was 17.5 cm from the treated bloodstain. The light sources used were a Model 2010 argon ion laser (7 to 9 watts; Spectra-Physics, Mountain View, CA), a Laser Print 1000 argon ion laser (0.5 watt; Omnichrome, Chino, CA) and an Omniprint 1000 alternate light source (Omnichrome, Chino, CA). Bloodstains were exposed to each light source for time periods ranging from 30 s to 20 min.

TABLE 1—*Exposure of DNA to cyanoacrylate ester.*

Exposure Time[a]	Sample Number
0	ES 0
10 min	ES 1
30 min	ES 2
1 h	ES 3
2 h	ES 4
4 h	ES 5
8 h	ES 6
16 h	ES 7
20 h	ES 8
40 h	ES 9

[a]Bloodstains were treated with cyanoacrylate ester as described in Materials and Methods.

TABLE 2—*Omniprint 1000 alternate light source.*[a]

	450 nm	485 nm	525 nm	530 nm	570 nm	Laser Print 1000 nm
30 sec	L1	L10	L17	L25	L57	L33
1 min	L2	L11	L18	L26	L58	L34
2 min	L3	L12	L19	L27	L59	L35
3 min	L4	L13	L20	L28	L60	L36
4 min	L5	L14	L21	L29	L61	L37
5 min	L6	L15	L22	L30	L62	L38
10 min	L7	L16	L23	L31	L63	L39
20 min	L8	L9	L24	L32	L64	L40

[a]Bloodstains were exposed to the Omniprint 1000 alternate light source as described in Materials and Methods.

TABLE 3—*Omniprint alternate light source set to white light.*

Exposure Time[a]	Sample Number
30 s	L49
1 min	L50
2 min	L51
3 min	L52
4 min	L53
5 min	L54
10 min	L55
20 min	L56

[a]Bloodstains were exposed to the Omniprint alternate light source set to white light as described in Materials and Methods.

Extraction and Restriction Enzyme Cleavage of DNA

DNA was extracted and restricted according to the FBI protocol with some modification. Briefly, the stain was cut into small pieces and placed into a 1.5 mL microcentrifuge tube at which time the bloodstain was incubated at 56°C overnight in the presence of stain extraction buffer and Proteinase K. After collection by centrifugation the supernatant was extracted with phenol/chloroform/isoamyl alcohol and the DNA was collected by ethanol precipitation followed by resuspension in TE buffer. An estimate of the quality and quantity of the recovered DNA was made by use of a yield gel. Restriction enzyme cleavage was carried out by incubation of the DNA with Hae III (40 U/μg DNA) at 37°C overnight. The restricted DNA was collected by ammonium acetate/ethanol precipitation and resuspended in TE buffer (1 mM EDTA; 10 mM Tris-HCl, pH 7.5). The completeness of digestion was determined by the use of a restriction gel.

Gel Electrophoresis and Southern Blotting of DNA

In all cases the analytical gels were composed of 1% Seakem LE agarose (FMC BioProducts, Rockland, ME) in 1X TAE buffer. Electrophoresis was carried out at 40 V for 17 h using a 20 by 22 cm Bios (Bios Corporation, New Haven, CT) electrophoresis system. In addition, an allelic control DNA lane (i.e. K562) and lanes containing Lifecodes 23Kb molecular weight markers (Lifecodes Corporation, Valhalla, NY) were present on all analytical gels. The DNA was transferred to Zetaprobe membrane according to the manufacturer's instructions.

Hybridization and Autoradiography of DNA

Hybridization was carried out according to the FBI RFLP protocol. Probes used for hybridization were labeled with ^{32}P-dCTP (New England Nuclear, Boston, MA) using the Prime-a-gene system (Promega Corp, Madison, WI). Autoradiography was performed at −70°C using X-Omat AR film (Eastman Kodak, Rochester NY) and Quanta-III intensifying screens (DuPont, Boston, MA).

Measurement of Allele Sizes

The determination of band sizes was accomplished by the "FBI DNA DATA IMAGE ANALYSIS SYSTEM" program that was used on MS-DOS-based computer outfitted

with a real time variable lens camera and a color television monitor. The computer program enabled an objective determination of the sizes of the DNA fragments in the allelic control lane and in each test specimen lane. The sizing program was used to calculate the fragment sizes in base pairs for each of the DNA bands.

Results

RFLP Analysis of DNA Exposed to Cyanoacrylate Ester (Superglue)

The potential effect of cyanoacrylate ester on RFLP analysis of DNA was determined by exposure of dried human bloodstains to superglue vapors. As shown in Table 1 individual bloodstains were treated with cyanoacrylate vapors for periods of time ranging from 10 min to 40 h. An untreated bloodstain was used as a control. Exposure of the samples to superglue vapor produced no apparent effects either on the relative amounts of DNA or on the susceptibility DNA to Hae III digestion (data not shown). In addition, no alteration in the expected allelic patterns of treated DNA was seen following hybridization with the polymorphic probe MS1 (locus D1S7; see Fig. 1).

Isolation of DNA from Bloodstains Following Exposure to Laser or High Energy Light

Exposure to various forms of high level energy such as ultaviolet light and radioactive particles has been shown to have deleterious effects on DNA. Such consequences could result in problems associated with successful genetic typing of DNA isolated from forensic biological samples (for example, bloodstains) following exposure to a high energy detection source. Therefore, to determine the possible effect on the yield of DNA, bloodstains were exposed to high energy light at a range of wavelengths for various times using an Omniprint 1000 alternate light source (see Table 2). Visual examination of agarose gels revealed that there were no significant differences in the relative amounts of DNA isolated from both treated and untreated bloodstains. In addition, all DNA bands were of a uniform appearance and migrated at the same rate on the agarose gel indicating little or no apparent degradation of DNA in any of the treated samples. Similar studies which exposed bloodstains for various times to white light using the Omniprint alternate light source (Table 3) and the Spectra Physics Argon Ion Laser (data not shown) likewise indicated no adverse effects on DNA samples with respect to quality and quantity.

Analysis of DNA After Exposure to a Laser or High Energy Light

Although exposure to laser or high energy light had no apparent adverse effects on DNA samples with respect to quality and quantity it was unclear whether treated DNA would be suitable for RFLP analysis. Therefore, a selection of human bloodstains that were exposed to three different high energy sources; 1) an Omniprint 1000 alternate light source, 2) an Omniprint 1000 0.5 Watt portable argon ion laser, and, 3) a Spectra Physics argon ion laser (see Tables 4, 5 and 6) were processed further in the DNA analysis scheme. Results obtained by hybridization using the polymorphic probe YNH24 (locus D2S44) revealed no apparent differences in allelic migration patterns between DNA from treated and control bloodstains (see Figures 2 and 3).

Discussion

The rapid advancement of laboratory technology and its practical application to forensic science has resulted in more efficient and informative methods of evidence analysis. Two

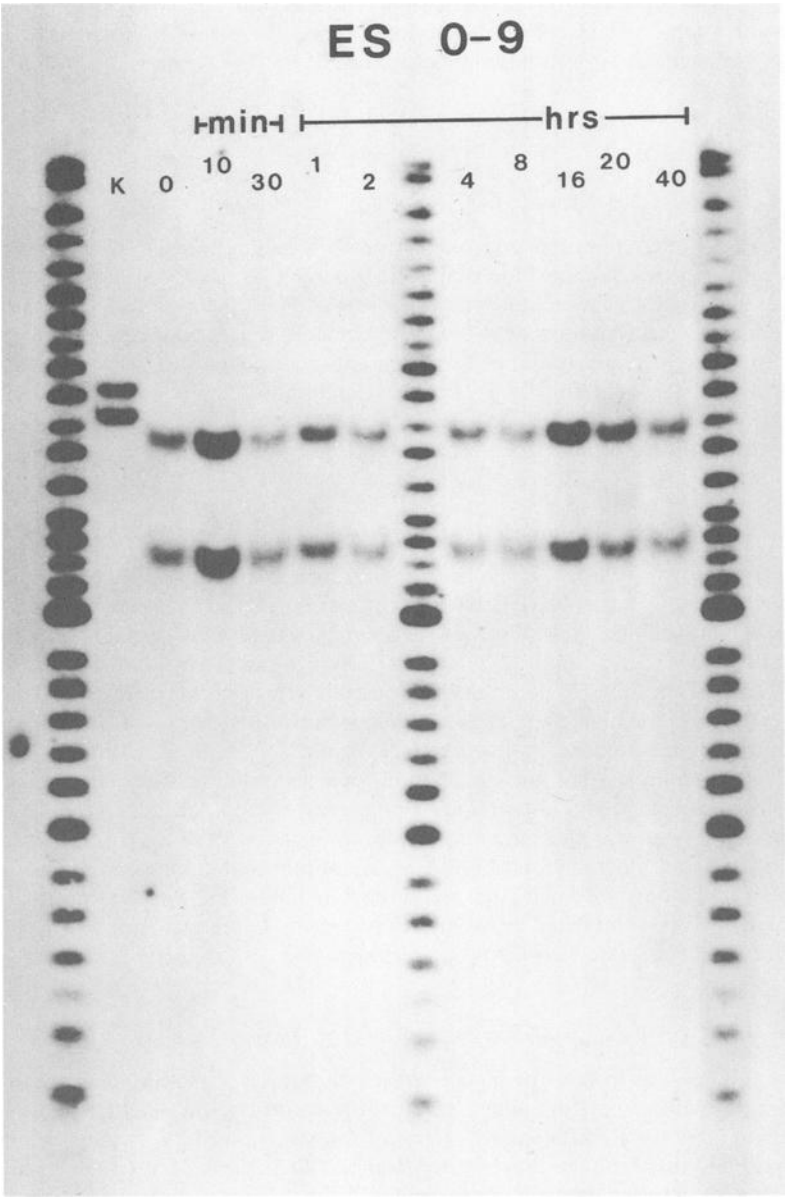

FIG. 1—*DNA was extracted from bloodstains that had been exposed to cyanoacrylate ester as described in Materials and Methods. DNA was extracted, restricted with Hae III, separated by electrophoresis, transferred to Zetaprobe membrane and hybridized with polymorphic probe MS1 (locus D1S7) as described in Materials and Methods. Lane 1 (K) contains control DNA from K562 cells. Lane 2 (0) contains DNA from untreated bloodstain. Lanes 3 and 4 contain DNA from bloodstains that had been exposed to cyanoacrylate ester for 10 min and 30 min respectively. The remaining lanes contain in order DNA from bloodstains that were exposed to cyanoacrylate ester fumes for 1, 2, 4, 8, 16, 20, and 40 h.*

TABLE 4—*Omniprint 1000 alternate light source.*[a]

	450 nm	485 nm	525 nm	530 nm	570 nm	Laser Print 1000 nm
30 s	L1	L10	L17	L25	L57	L33
4 min	L5	L14	L21	L29	L61	L37
10 min	L7	L16	L23	L31	L63	L39
20 min	L8	L9	L24	L32	L64	L40

[a]Bloodstains were exposed to the Omniprint alternate light source as described in Materials and Methods.

TABLE 5—*Omniprint alternate light source set to white light.*

Exposure Time[a]	Sample Number
30 s	L49
4 min	L53
10 min	L55
20 min	L56

[a]Bloodstains were to the Omniprint alternate light source set to white light as described in Materials and Methods.

TABLE 6—*Spectra Physics argon ion laser.*

Exposure Time[a]	Sample Number
30 s	L41
4 min	L45
10 min	L47
20 min	L48

[a]Bloodstains were exposed to the Spectra Physics argon ion laser as described in Materials and Methods.

new and powerful technologies, laser or alternate light source, or both, and DNA profiling, have focused on the detection and analysis of biological material.

It is becoming common in crime scene investigation to use laser light or alternate light sources, or both, for the detection of blood or other biological materials. Use of these tools greatly enhances the probability that important evidence will not be overlooked. A second common technique employed in the laboratory is the use of cyanoacrylate ester (that is, "superglue") fuming in order to visualize fingerprints. Likewise, the use of DNA profiling by RFLP analysis is being more widely used in the analysis of biological evidence. Even though these techniques have proven successful there is little information concerning their use in concert.

This work was initiated in response to the question of whether blood could be analyzed successfully by RFLP analysis after exposure to cyanoacrylate ester ("superglue") fuming and/or to an argon ion laser or alternate light source.

Analysis of human bloodstains revealed that an RFLP profile using the polymorphic probe MS1 (locus D1S7) could be successfully generated after exposure of the bloodstains to cyanoacrylate ester. This observation is important since it may be necessary to examine

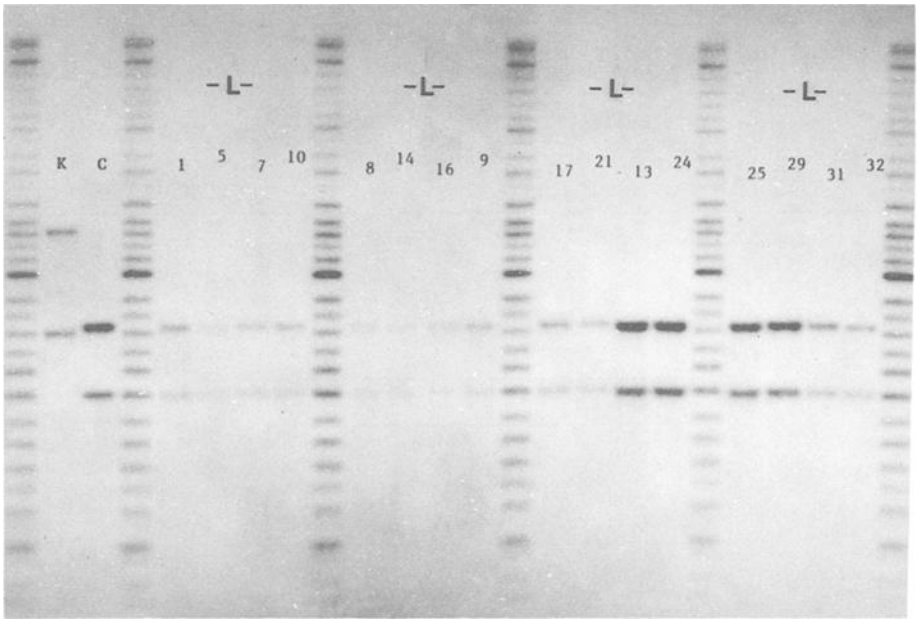

FIG. 2—*DNA was extracted from bloodstains that had been exposed to high energy light treatment as described in Materials and Methods. DNA was extracted, restricted with Hae III, separated by electrophoresis, transferred to Biodyne B membrane and hybridized with polymorphic probe YNH24 (locus D2S44) as described in Materials and Methods. Lane 1 (K) contains control DNA from K562 cells. Lane 2 (C) contains DNA from untreated bloodstain. The remaining lanes contain DNA from bloodstains that were exposed to different light sources for varying times as shown in Table 4.*

objects for fingerprints by this method which also have blood on their surfaces. If deemed necessary we use "superglue" fuming to detect fingerprints before blood is collected for DNA analysis.

Similar experiments used bloodstains which had been exposed to argon laser light or to an alternate light source. It was shown that in none of the conditions used to treat the bloodstains was the DNA pattern generated different from untreated bloodstains following hybridization with the polymorphic probe YNH24 (locus D2S44). As in the case of "superglue" fuming we normally use samples which have been detected by high intensity light of different wavelengths before RFLP analysis.

It should be noted that this study used only bloodstains present on white cloth material and that only a limited number of conditions with respect to exposure times and wavelengths were examined. It is possible that different substrates (for example, metal, plastic, etc.) and/or different conditions might render the DNA unsuitable for RFLP analysis. In addition, other types of biological evidence such as semen or tissue might react differently to exposure to cyanoacrylate ester or high intensity light, or both, resulting in an altered DNA profile. While we do not feel that either of these possibilities is likely we are currently carrying out experiments designed to answer these questions.

References

[*1*] Creer, K. C., "Operational Experience in the Detection and Photography of Latent Fingerprints by Argon Ion Laser," *Forensic Science International*, Vol. 23, 1983, pp. 149–160.

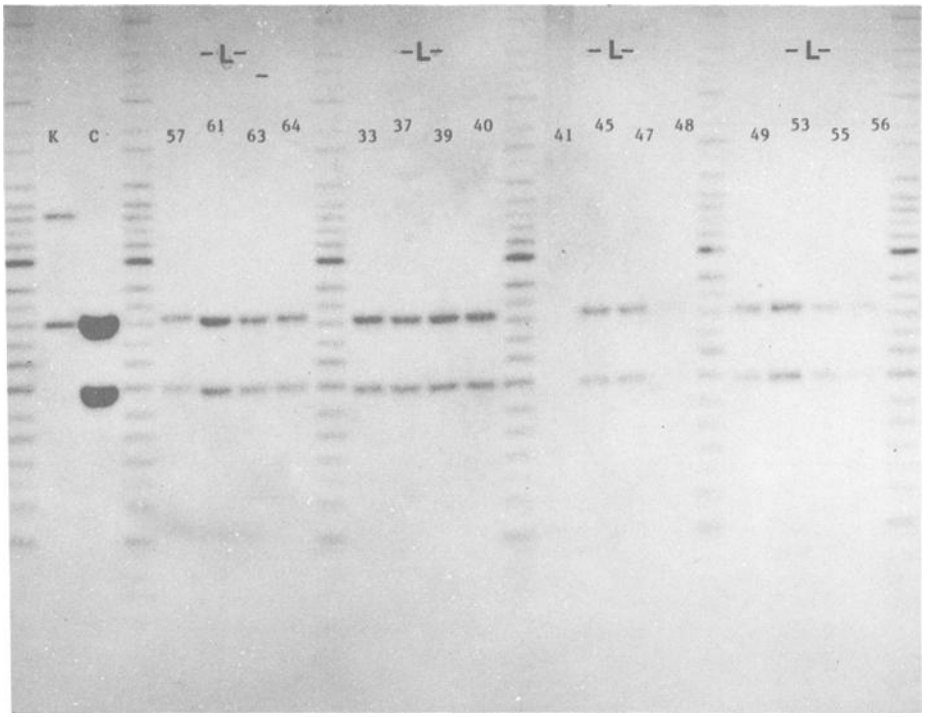

FIG. 3—*DNA was extracted from bloodstains that had been exposed to high energy light treatment as described in Materials and Methods. DNA was extracted, restricted with Hae III, separated by electrophoresis, transferred to Biodyne B membrane and hybridized with polymorphic probe YNH24 (locus D2S44) as described in Materials and Methods. Lane 1 (K) contains control DNA from K562 cells. Lane 2 (C) contains DNA from untreated bloodstain. The remaining lanes contain DNA from bloodstains that were exposed to different light sources for varying times as shown in Tables 4, 5, and 6. The absent (L41) or very light (L48) bands were judged not to be due to the effects of the light source, since all bands became apparent on longer exposures.*

[2] Dalrymple, B. E., Duff, J. M., and Menzel, E. R., "Inherent Fingerprint Luminescence—Detection by Laser," *Journal of Forensic Sciences*, Vol. 22, No. 1, Jan. 1977, pp. 106–115.
[3] Menzel, E. R., "*Fingerprint Detection with Lasers*," Marcel Dekker, New York, 1980.
[4] Gill, P., Jeffreys, A. J., and Werrett, D. J., "Forensic Application of DNA 'Fingerprints'," *Nature*, Vol. 318, Dec. 1985, pp. 577–579.
[5] Kantor, E., Baird, M., Shaler, R., and Balazs, I., "Analysis of Restriction Fragment Length Polymorphisms in Deoxyribonucleic Acid (DNA) Recovered from Dried Bloodstains," *Journal of Forensic Sciences*, Vol. 31, No. 2, April 1986, pp. 403–408.
[6] "Procedures for the Detection of Restriction Fragment Length Polymorphisms in Human DNA," *Protocol of the DNA Analysis Unit*, FBI Laboratory, Washington, DC, 1989.
[7] Southern, E. M., "Gel Electrophoresis of Restriction Fragments," *Methods in Enzymology*, Vol. 68, 1980, pp. 152–176.
[8] Lee, H, et al., "The Effect of Presumptive Test, Latent Fingerprint and Some Other Reagents and Materials on Subsequent Serological Identification, Genetic Marker and DNA Testing in Bloodstains," *Journal of Forensic Identification*, Vol. 39, No. 6, 1989, pp. 339–358.

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