TECHNICAL NOTE

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The Effects of Fingermark Enhancement Light Sources on Subsequent PCR-STR DNA Analysis of Fresh Bloodstains

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ABSTRACT: This paper describes a study designed to investigate the effects of light sources used to enhance fingermarks on the subsequent polymerase chain reaction (PCR)-short tandem repeat (STR) analysis of bloodstains. Dried bloodstains on glass were exposed for up to 30 min to five different light sources: Argon ion laser, Polilight UV, Polilight green, Superlite, and shortwave UV. The bloodstains were subsequently analyzed using a quadruplex PCR system. It was found that treating the bloodstains with four of the five light sources had no appreciable effect on the results obtained from subsequent PCR analysis. However, exposure of the bloodstains to shortwave UV light for more than 30 s precluded the acquisition of results from PCR testing. Therefore, under casework conditions, it would be preferable to avoid exposing bloodstains, on which PCR typing is to be performed, to shortwave UV.

KEYWORDS: forensic science, DNA, fingerprints, laser, ultraviolet, PCR

Significant evidence can be obtained from fingermarks, particularly when a fingermark in blood is found. Swabbing of such a blood mark for DNA testing may result in the loss of some, if not all, of the ridge detail. Consequently it is preferable to enhance a fingermark and image it prior to carrying out DNA analysis. In this laboratory, initial enhancement of fingermarks would usually involve the use of powerful light sources with a variety of wavelength outputs. However, if such enhancement procedures were to affect adversely the DNA in the bloodstain, then subsequent tests carried out on the DNA might not yield results. In order that the full evidential value of such stains are realized it is necessary to ensure compatibility between any fingermark enhancement and DNA profiling methods used.

A recent advance in DNA profiling technology has been the development of polymerase chain reaction (PCR)-based tests for typing very small amounts of blood, such as might be associated with a fingermark. A PCR-based method of DNA analysis has recently been introduced into casework in this laboratory, namely the quadruplex system developed by Kimpton et al. (1). The basis of this test is the simultaneous amplification of DNA at four short tandem repeat [STR] loci in a multiplex reaction followed by fluorescent detection of the products using an automated DNA sequencer. The four loci involved are HUMvWAFA31/A (VWA) (2), HUMTHO1 (THO) (3), HUMF13A1 (F13) (4), and HUMFES/FPS (FES) (5). Sequencing studies (6) have shown that the variation in allelic size at these loci is generally due to the variation in numbers of four base pair repeat units. The quadruplex STR system has been extensively validated for use in forensic casework (1,7,8), and this work demonstrated that it is possible to obtain reliable results from as little as 1 ng of DNA.

The aim of this study was to investigate whether the use of fingermark enhancement light sources according to the procedures set in this laboratory would adversely affect subsequent STR quadruplex typing of blood stains under typical casework conditions.

Materials and Methods

Preparation of Bloodsmears

Typically, it is weaker marks in blood that are enhanced at crime scenes, thus thin bloodsmears were used as samples. All the bloodsmears used originated from the same laboratory donor. Freshly-drawn human blood (2 μL) was aliquoted onto a glass microscope slide (51 by 76 mm) cleaned with absolute alcohol. A thin bloodsmear was produced by dragging the edge of a second slide through the blood drop. The samples were stored in the dark at room temperature for a maximum of 48 h before treatment.

Light Sources and Exposure Details

Four separate light sources were used: An Argon-ion laser (Model 2045, Spectra Physics), a Polilight (Rofin Sinar, Australia), a Superlite (Ultrafine Technology, UK), and a shortwave UV source (Cole Palmer, Chicago). Details of the wavelengths, output powers, and operating distances are given in Table 1. The illumination conditions, including exposure times, were chosen to be representative of those in operational use in this laboratory.

After exposure, the samples were stored in the dark under ambient laboratory conditions prior to DNA extraction, which was carried out 2 to 24 h following treatment.

DNA Extraction

Each treated bloodsmear was removed from the glass slide using a piece of clean white cotton (5 mm^2) wetted with sterile distilled

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TABLE 1—*Illumination parameters of light sources used for fingermark enhancement.*

Light Source (see text for details)	Output Wavelength(s) (nm)	Incident Power ($mWcm^{-2}$)	Working Distance (cm)
Argon-ion Laser	514	200	5–20
PoliLight 530	513–555	20	5–20
PoliLight UV	315–385	10	5–20
Superlite	320–400	10	5–20
Shortwave	255	1	25–35

water. Extraction of the DNA was carried out using Chelex® resin, as described by Walsh et al. (9). The extracts were stored at −20°C.

Quantification of Extracted DNA

DNA extracts were quantified using the dot-blot hybridization method described by Walsh et al. (10). This method involves the hybridization of biotinylated primate specific probe to 'unknown' and 'reference' DNA samples fixed to a nylon membrane and detection by enzyme driven chemiluminescence. The amount of DNA in the sample extracts was estimated by visual comparison of sample 'dots' with reference 'dots' on the resulting lumigraph.

Amplification of Sample DNA

Amplification conditions were those previously described (7,8). PCR reactions contained 20 μL 'master-mix' (Support Unit, FSS, Birmingham) comprising PCR buffer, dNTPs, and primers, and ≤3 ng template DNA, in a total volume of 50 μL. 'Master-mix' sufficient for a reaction batch was boiled for 3 min and 'snap cooled' on ice before adding 1.25 U Amplitaq (Perkin Elmer) per reaction and distributing into thin-walled Geneamp reaction tubes (Perkin Elmer). Amplification was carried out using a Geneamp PCR System 9600 (Perkin Elmer) using the following conditions: 95°C for 1 min, 2 min cooling ramp, 54°C for 1 min, 72°C for 1 min, for 28 cycles, followed by a 'soak' at 72°C for 10 min before cooling to 4°C. One positive control sample (1 ng of DNA, of known type) and one negative control sample (no DNA added) were included in each amplification batch.

Detection of Amplified DNA Product

1.5 μL of amplified product were added to 2.5 μL of loading buffer (formamide, 2% (w/v) dextran blue, 2.4 nM GS25000-ROX internal lane standard (ABI)). The samples were then heat denatured at 90°C for 2 min and 'snap-cooled' on ice prior to loading on a 6% polyacrylamide, 8 M urea, TBE sequencing gel (12-cm well-to-read). Electrophoresis was carried out for 3 h at 1500 V, 30 mA, 28 W on an automated DNA sequencer (Applied Biosystems, model 373). Sizing of DNA fragments was carried out using Genescan 672 software version 1.2.2-1 (ABI) with reference to the internal size standards, by the method of Elder and Southern (11).

Analysis of Electropherogram (EPG) Results

The use of fluorescence detection technology enables the yield of amplified product to be estimated, when compared with a suitable reference. Allelic product is represented in EPG results as a peak, the area of which (in arbitrary units) is calculated by the software. The peak areas obtained from amplifying the treated samples were compared with those obtained from amplifying the untreated control samples, in order to assess whether any variation in PCR yield had occurred. The laboratory donor who provided blood samples for these experiments has an STR profile which is homozygous at the VWA locus, but heterozygous at the 3 other loci included in the quadruplex system. Therefore, 7 peak area figures were obtained from each sample amplification.

Experimental Design

The bloodsmears were exposed to the five light sources under typical fingermark searching/imaging conditions (as outlined in Table 1). Two experiments were carried out, as detailed below and each one was repeated three times. A mean peak area value for each allelic peak was then calculated.

Experiment 1

Light sources—Argon-ion laser, Polilight-green, Polilight-UV, Superlite, and shortwave UV. Exposure times—30 s, 15, and 30 min.

Experiment 2

Light source—shortwave UV. Exposure times—30 s, 1, 3, 5, 10, and 15 min. Control samples were processed and analyzed as part of each experiment as follows:

a) Positive control—untreated bloodsmear, b) Negative control—clean glass slide. Tests were carried out 'blind,' i.e., the extraction, amplification, and subsequent analysis of the EPG

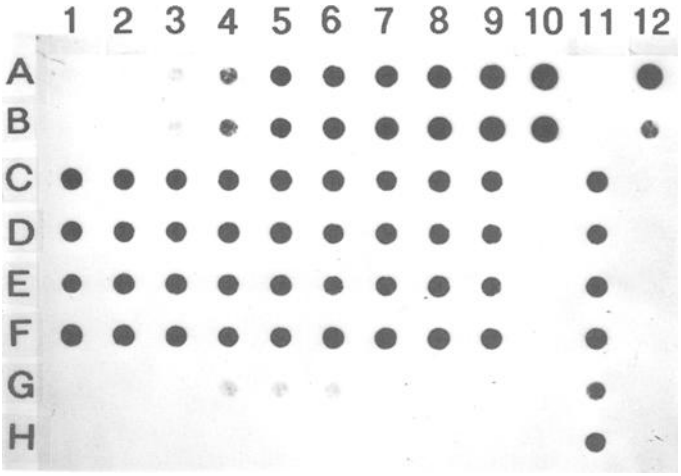

FIG. 1—*Lumigraph produced from the quantification of DNA samples extracted from bloodstains exposed to the light sources under test. 7 μL of each sample and control extract were loaded. Rows A and B, 1–10 contain the quantification standards: A, B 1:0.1 ng; A, B 2:0.25 ng; A, B 3:0.5 ng; A, B 4:1.0 ng; A, B 5:2.0 ng; A, B 6:3.0 ng; A, B 7:4.0 ng; A, B 8:5.0 ng; A, B 9:7.0 ng; A, B 10:10.0 ng. Row A, position 11 contains 10 μL of water only (quantification negative control). Rows A and B, position 12 contain quantification positive control samples: A12:10.0 ng; B12:1.0 ng. Sample extracts were loaded into rows C to G, corresponding to the following light sources and exposure times: Row C: Argon ion laser; 1–3:30 s, 4–6:15 min, 7–9:30 min. Row D: Polilight green; 1–3:15 min, 4–6:30 s, 7–9:30 min. Row E: Polilight UV; 1–3:15 min, 4–6:30 min, 7–9:30 s. Row F: Superlite; 1–3:30 s, 4–6:30 min, 7–9:15 min. Row G: Shortwave UV: 1–3:30 min, 4–6:30 s, 7–9:15 min. Rows C–H, position 11 contain aliquots of extracts from untreated bloodstains. Rows C–H, position 10 contain aliquots of negative control samples included in each extraction batch.*

results were carried out with no knowledge of either the light source or exposure times used.

Results

Experiment 1 consisted of exposing the bloodsmears to the five different light sources for periods ranging from 30 s to 30 min. The lumigraph obtained from carrying out the dot-blot hybridization DNA quantification procedure on aliquots of extracts obtained from these samples is shown in Fig. 1. From this, it can be seen that no DNA was detected in aliquots of samples which had been exposed to shortwave UV illumination for longer than 30 s. However, DNA was detected in aliquots taken from all other samples. The level of DNA detected in samples which were exposed to shortwave UV illumination for 30 s appears greatly reduced when compared with the levels detected in samples exposed to all other light sources and the control samples.

Under casework procedure in this laboratory, STR quadruplex analysis is only carried out on extracts in which DNA is detected following the quantification procedure described. This policy was reflected in the study described and amplification of all samples in which DNA was detected was carried out, 3 ng of DNA were added to each PCR reaction, except in the case of samples which had been exposed to shortwave UV light, for which the final reaction volume limited the amount of DNA added to 2.1 ng. The results obtained from amplification of samples containing detectable levels of DNA are given in Fig. 2. In summary, full profiles were obtained from all samples which were amplified. The mean peak area values obtained from treated samples were similar to those obtained from the control samples in all cases, except for the samples exposed to shortwave UV for 30 s, in which a marked decrease in peak area, relative to the control samples, can be seen.

Experiment 2 was designed to establish a possible exposure limit to shortwave UV between 30 s and 15 min. After carrying out the dot-blot hybridization DNA quantification procedure, it was found that only the untreated control sample extracts and those obtained from the samples exposed for 30 s contained detectable levels of DNA. This confirmed the results obtained from experiment 1. Following amplification of the extracts containing detectable levels of DNA, a reduction in yield of allelic product from the shortwave UV-treated samples compared to the control samples was again observed (data not shown).

In both experiments, amplification of sample DNA extracts using the STR quadruplex system gave allelic peaks at all loci which exceeded the minimum level defined by laboratory policy (50 units) for the reporting of results in a case. All such EPGs obtained gave the same result, in terms of allelic designation and no qualitative differences in profile were observed which would affect the interpretation of a result for casework use.

Discussion

The results obtained from the experiments described here would suggest that the use of shortwave UV for searching items and imaging marks made in blood should be avoided if analysis of the bloodstain using the STR system described is required. The DNA quantification results suggest that prolonged shortwave UV illumination of bloodsmears has the effect of reducing the amount of template DNA available for amplification. In this study, it was shown that quadruplex PCR results could be obtained if the exposure to shortwave UV light lasted only 30 s. However, under normal operating conditions, it would be unusual to limit an exposure time

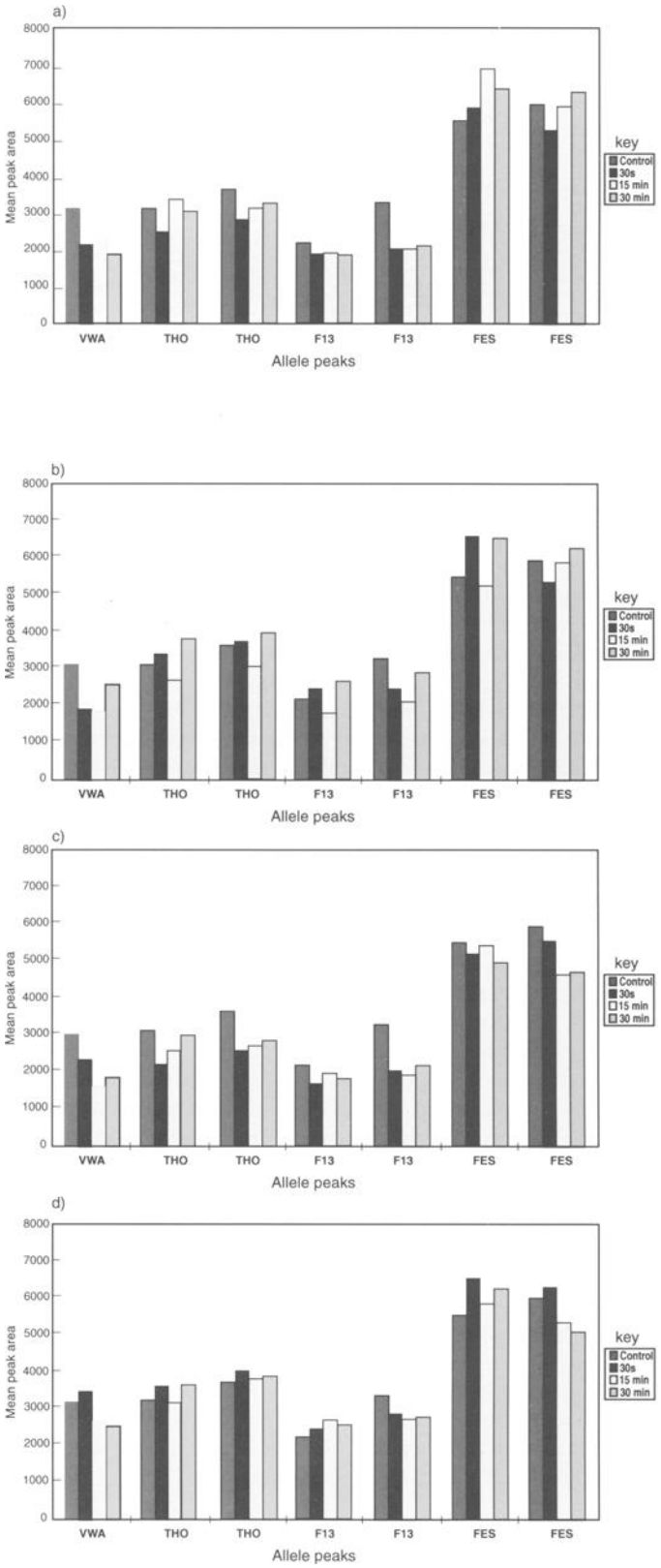

FIG. 2—*Histograms showing the results obtained following amplification of DNA extracts from bloodstains exposed to the light sources tested, compared with the amplification of DNA from untreated bloodstains: a) Argon ion laser, b) Polilight green, c) Polilight UV, d) Superlite, and e) shortwave UV. The area of each allele peak depicted on the EPG is taken as a measure of product yield. The mean value was obtained from the results of amplifying each of the triplicate test samples. For each light source tested, a graph was plotted of mean peak area for each allele present.*

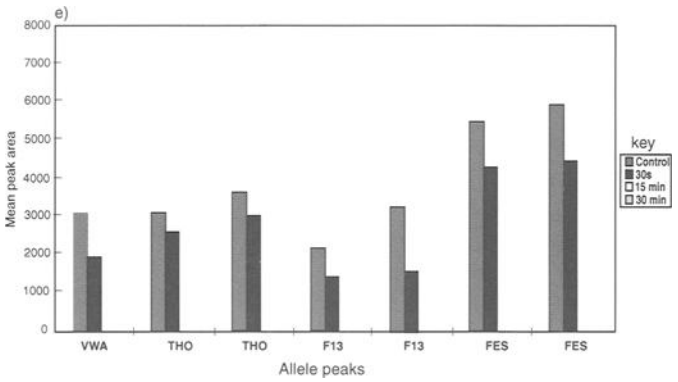

FIG. 2—*Continued.*

to 30 s. The bloodsmears used for these experiments were freshly prepared on a clean substrate, whereas those encountered in casework are often older and subjected to environmental contamination. The results described here were obtained from thin bloodsmears. If thicker bloodsmears were tested, it is possible that different results would be observed depending on the extent to which light penetrated the stain.

The results from this study suggest that illumination of fresh bloodstains with the Argon-ion laser, Polilight-green, Polilight-UV, or Superlite for 30 min has little effect on the results of subsequent STR quadruplex analysis following the methods described. This is supported by the lack of a general decrease in amplification yield observed with increase in exposure time in all cases. Under operational casework conditions, results obtained would be subject to the provisos regarding the condition of the stain described above.

There have been two previous studies on the effects of fingermark enhancement light sources on forensic DNA profiling (12,13), but both studies involved RFLP DNA analysis and not PCR-based testing. The first study (12) investigated the effects of a 5 min exposure to an Argon-ion laser on the yield and quality of DNA extracted from bloodstains—no significant effect on the results obtained was noted. The second study investigated the effects of exposure to white light, an Omniprint light source at several wavelengths between 450–570 nm and an Argon-ion laser on RFLP analysis of bloodstains. It was found that exposure to any of the light sources for times of 30 s to 20 min had no significant effect on the results obtained. The results described here are consistent with those of the previous two studies, although direct comparison of the test conditions is difficult because the experimental systems used were different.

The work described here is a preliminary study. A more intensive project to investigate the effects of increased exposure times and additional light sources on STR typing of a range of body fluids would complement the results obtained so far. The results from additional studies would relate more closely to operational casework scenarios if the effects of environmental contaminants in these experiments were also assessed.

Conclusion

In summary, it would appear that the use of a shortwave UV source to search case items or image marks may be detrimental to any subsequent STR-PCR analysis of bloodstains exposed to the light. In contrast, the use of the other four light sources described does not appear to affect the results obtained from STR testing adversely. Current policy at the MPFSL is not to expose marks that are to be submitted for DNA analysis to shortwave UV light.

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