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TESTING MINIMUM ULTRAVIOLET LIGHT EXPOSURES TO EFFECTIVELY REMOVE CONTAMINATING DNA FOR USE IN FORENSICS

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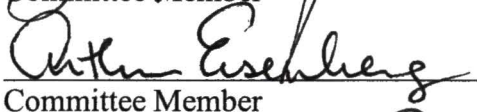

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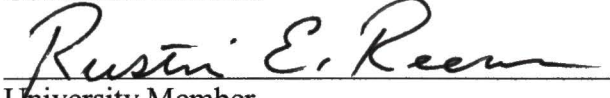

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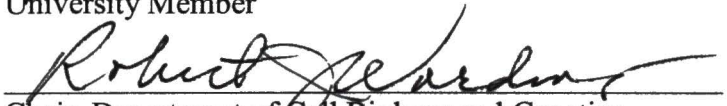

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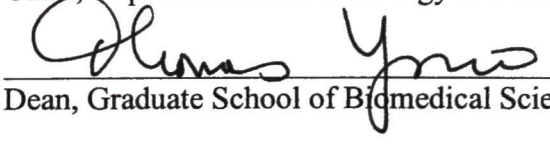

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TESTING MINIMUM ULTRAVIOLET LIGHT EXPOSURES TO EFFECTIVELY REMOVE CONTAMINATING DNA FOR USE IN FORENSICS

INTERNSHIP PRACTICUM REPORT

Presented to the Graduate Council of the

Graduate School of Biomedical Science

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MASTER OF SCIENCE

By

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CHAPTER I: INTRODUCTION

Forensic DNA testing allows the assignment of virtually individualized genetic profiles to DNA sources, playing an unequivocal role in numerous judicial and non-judicial settings, such as linking evidence to suspect, parent to child, or human remains to family. An enormous responsibility exists for forensic scientists and field experts to ensure the reliability of forensic DNA testing, rooted in the extensive criminal or civil implications test results carry. Widespread regulation within the field of forensic DNA testing addresses compliance with all standards set forth to certify the competency of DNA testing laboratories, their scientists, and their methods. These standards, issued by the Director of the FBI in 1998, serve as national requirements for laboratories to institute their individual policies. Standard 6.1.4 includes direct mention of laboratory cleanliness, 'The laboratory follows written procedures for monitoring, cleaning and decontaminating facilities and equipment' (7). From this standard, laboratories are able to employ differing, subjective methods of decontamination, of which few studies have been conducted demonstrating specific means for effective laboratory sterilization.

Mandated decontamination of forensic DNA testing sites works to remove the risk of compromising DNA test results by any contaminating DNA present in the laboratory. Contamination can occur when biological material such as sloughed skin cell, hair, blood, saliva, or semen, deposited in the laboratory or on testing materials is

incorporated into the unknown sample. Another major source of contamination in the forensic laboratory arises from amplified polymerase chain reaction (PCR) products that find their way into pre-PCR samples. These purified PCR fragments, described as naked DNA, have a high affinity for PCR reagents, capable of multiplying and altering DNA test results. Extreme care is necessary to keep PCR products from coming in contact with pre-PCR samples, as the standards instruct in 6.1.3, 'Amplified DNA product is generated, processed and maintained in a room(s) separate from the evidence examination, DNA extractions and PCR setup areas' (7).

Throughout the forensic DNA field, maintenance of equipment and materials used in testing usually includes cleaning benches and equipment with bleach and exposure of materials to ultraviolet (UV) radiation. The action of these techniques expectantly degrades any DNA present, destroying the contamination sources. Studies show limited success and varied results for differing amounts of UV exposure, based on time length, distance from exposure, and energy/wavelength of the radiation.

This study introduces a solar lamp UV light source, for the purpose of removing contaminating DNA in direct relation to forensic testing. The study attempts to demonstrate what level of decontamination occurs from sun lamp exposure at given time intervals of exposure, set at specific distances from the lamp, and for different types of biological samples.

A FS-40 solar lamp was used to irradiate samples of amplified DNA and cellular samples at distances of 5 cm, 10 cm, and 60 cm from the source, with varied exposure

times of 15 min, 30 min, 3 hrs, 6 hrs, 12 hrs and 24 hrs. Common forensic DNA typing concerns include contamination by previously amplified DNA products or from transfer of cellular material onto testing materials. Samples exposed included dried PCR products amplified by AmpF*l*STR® COfiler® kit, dried whole blood, and dried saliva. An organic extraction of the blood and saliva samples isolated any remaining genomic DNA. Control blood and saliva samples were quantitated for accurate DNA concentration. All samples were then amplified by AmpF*l*STR® COfiler® kit and analyzed on an ABI® 310 Genetic Analyzer, along with the necessary controls. Samples of each designated distance, time, and type were prepared in duplicate, along with an unexposed control PCR product, blood, and saliva sample, reagent blank run alongside each PCR product, blood, and saliva series, and positive and negative PCR controls. Fragment analysis data was analyzed by GeneScan® and Genotyper® software to obtain any detectable genetic profile from the samples.

This experimental design mimics a true forensic casework scenario by following a routine chain of procedures used widely throughout the field. The current standard in forensic DNA testing measures short tandem repeats (STRs), which vary significantly in length between individuals. There are thirteen loci used by the Combined DNA Index System (CODIS), the national DNA index managed by the FBI Laboratory. All thirteen loci are typed in a typical DNA test, with the AmpF*l*STR® COfiler® kit amplifying seven of these loci (Table 1-1).

Locus Designation	Chromosome Location	Common Sequence Motif	Size Range (bp)[a]	Dye Label
D3S1358	3p	TCTA $(TCTG)_{1-3}$ $(TCTA)_n$	114–142	5-FAM
D16S539	16q24–qter	$(AGAT)_n$	234–274	5-FAM
Amelogenin	X: p22.1–22.3 Y: p11.2	– –	107 113	JOE
TH01	11p15.5	$(AATG)_n$	169–189	JOE
TPOX	2p23–2per	$(AATG)_n$	218–242	JOE
CSF1PO	5q33.3–34	$(AGAT)_n$	281–317	JOE
D7S820	7q11.21–22	$(GATA)_n$	258–294	NED

Table 1-1. AmpF*l*STR® COfiler® loci, repeat sequence, and size range (1).

For the purposes of this study, successfully decontaminated PCR products, blood, and saliva samples would show no detectable genotype at any of the seven loci. Other DNA testing, such as mitochondrial DNA analysis from hair, bone or teeth, or very low copy number DNA from a small number of cells, require extreme caution to avoid contamination, as these tests have increased sensitivity over standard STR testing. The level of decontamination detected through UV exposure in this study would not provide sufficient information for application to the more sensitive techniques.

CHAPTER II: BACKGROUND

UV radiation. The use of ultraviolet (UV) light was introduced as a PCR decontamination tool in 1990, and as subsequent publications followed, laboratories incorporated UV exposure into their protocols (5, 9, 18, 24, 26). UV light is a form of electromagnetic radiation having wavelengths, measured in nanometers - nm (10^{-9}m), longer than X-rays and shorter than visible light on the electromagnetic spectrum (Table 2-1). Electromagnetic radiation can be equally characterized in terms of wavelength (λ), frequency (υ) and photon energy (E) through the relationship of these three in the following equations:

$\lambda = c/\upsilon$ (c, speed of light = 3×10^8 m/s) and $E = h\upsilon$ (*h*, Plank's constant = 6.6×10^{-34} J*s).

Spectrum of Electromagnetic Radiation

Region	Wavelength (Angstroms)	Wavelength (centimeters)	Frequency (Hz)	Energy (eV)
Radio	$> 10^9$	> 10	$< 3 \times 10^9$	$< 10^{-5}$
Microwave	$10^9 - 10^6$	10 - 0.01	$3 \times 10^9 - 3 \times 10^{12}$	10^{-5} - 0.01
Infrared	10^6 - 7000	0.01 - 7×10^{-5}	$3 \times 10^{12} - 4.3 \times 10^{14}$	0.01 - 2
Visible	7000 - 4000	$7 \times 10^{-5} - 4 \times 10^{-5}$	$4.3 \times 10^{14} - 7.5 \times 10^{14}$	2 - 3
Ultraviolet	4000 - 10	$4 \times 10^{-5} - 10^{-7}$	$7.5 \times 10^{14} - 3 \times 10^{17}$	$3 - 10^3$
X-Rays	10 - 0.1	$10^{-7} - 10^{-9}$	$3 \times 10^{17} - 3 \times 10^{19}$	$10^3 - 10^5$
Gamma Rays	< 0.1	$< 10^{-9}$	$> 3 \times 10^{19}$	$> 10^5$

Table 2-1. Spectrum of electromagnetic radiation measured in wavelength, frequency (hertz), and energy (electron-volts).

The UV spectrum consists of a range of wavelengths described as UVA (320-400 nm), UVB (290-320 nm) and UVC (100-290 nm). Different wavelengths produce varied effects in UV exposed substrates. An action spectrum describes the effectiveness for a series of UV doses and the corresponding induced effect by plotting each wavelength against the response. In this study, the induced effect is DNA damage brought about through UV exposure by a sun lamp. The FS-40 sunlamp, used widely in photobiological research, mimics the solar light spectrum, containing both UVB and UVA components, similar to sunlight that reaches the Earth's surface. A study of the three most commonly used solar light sources found the unfiltered FS-40 sunlamp significantly more efficient than the other sources studied at introducing DNA damage, rendering this type of lamp a good UV solar light candidate (30).

Biological responses to UV light in DNA. UV radiation damages DNA through the two kinds of energy absorption mechanisms. Direct excitation of DNA by UV radiation results in the modification of DNA molecules. Damage occurs primarily through dimerization of adjacent pyrimidine bases leading to cyclobutane pyrimidine dimers (CPDs) as the major product, and minor pyrimidine (6-4) pyrimidone photoproducts (6-4 PPs). All other DNA modifications from direct UV absorption, including oxidative base modifications, purine dimers, single-strand breaks (SSB), double-strand breaks (DSB), or apurinic-apyrimidinic sites (AP sites), form at an incidence less than 1:10 with pyrimidine dimer formation (8,13). A comparison of cellular and purified naked DNA found no difference in the prevalence of CPD as the major photoproduct formed following irradiation by simulated sunlight (31).

The second type of DNA damage, indirect, occurs through the interaction of DNA with reactive species excited by the radiation. Endogenous chromophores, such as inorganic ions, molecules, or water, act as photosensitizers, generating reactive oxygen species that cause oxidative base modification through energy transfer to DNA molecules (23). The primary mutagenic effects from UVA exposure occur through indirect reactions, as DNA weakly absorbs UV at wavelengths >320 nm (21). A larger percentage of UV solar light exists as UVA (>95%), but small doses of shorter wavelength UVB carry a greater genotoxic effect than larger doses of longer wavelength UVA (14). A study involving commercial tanning lamps, which emit primarily UVA, estimated that the UVB component represented only 0.8% of the total output, yet accounted for 75% of the induced CPDs and 50% of the oxidative damage to DNA (29). Low levels of CPDs can form indirectly through energy transfer from an excited chromophore; however the exact means of this indirect reaction remains unclear (12).

A comparison of direct and indirect mechanisms for base modifications showed a ratio of 7:1 in mammalian and human melanoma cells after exposure to solar radiation (12, 22). Still, a portion of the mutagenic spectrum generated by solar light is attributed to indirect reaction, and without the action of photosensitizers this type of DNA damage would not be expected, as in the case of purified DNA (amplified STRs for this study). However, one study repeatedly found low levels of oxidative lesions in twice purified plasmid DNA, with no proposed mechanism or explanation (14).

CPD incidence. The most abundant lesions produced by UV radiation are repeatedly found to be CPDs, and under simulated sunlight (SSL) occur at a reported

incidence 20-40 times more frequently than any other photoproduct in purified or cellular DNA (31). Formation of CPDs occurs by dimerization of adjacent pyrimidines through a four-membered ring structure, covalently linked together at the 5 and 6 pyrimidine carbons (10). The pyrimidine composition of either cytosine (C) or thymine (T) influences the rate of CPD formation. The most abundant product results from adjacent thymines, T-T, followed by C-T, T-C, and C-C dimers occurring at a reported ratio of 68:13:16:3 in plasmid DNA (16). The DNA sequence flanking potential dimer sites also influences CPD yield for example a 5'ATTA sequence showed greater incidence than 5'ATTG (11). The predominant isomer formed is the *cis-syn* thymine dimer (Fig 2-1); however other types do exist at low concentrations (10).

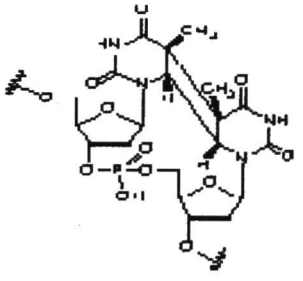

Fig 2-1. *Cis-syn* thymine dimer.

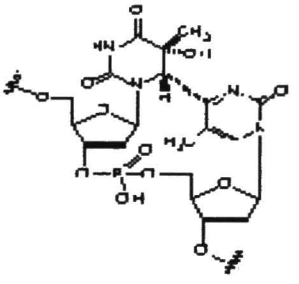

Fig 2-2. (6-4) thymine photoproduct.

The (6-4) PPs (Fig 2-2) comprise the majority of remaining lesions, which also occur at differing ratios for base and sequence composition. They form preferentially at 5'TC and 5'CC locations, over 5'CT and 5'TT (31). The introduction of dimer lesions disrupts PCR by inhibiting the DNA polymerase at these sites during extension, which is the basis for decontamination methods using UV light. The dimers reside inside the double helix, and can disrupt duplex formation in double stranded DNA (Fig 2-3), while dimers can form from adjacent and non-adjacent pyrimidines in single stranded DNA. CPDs block

DNA synthesis by terminating polymerase activity primarily one base prior to the lesion (4).

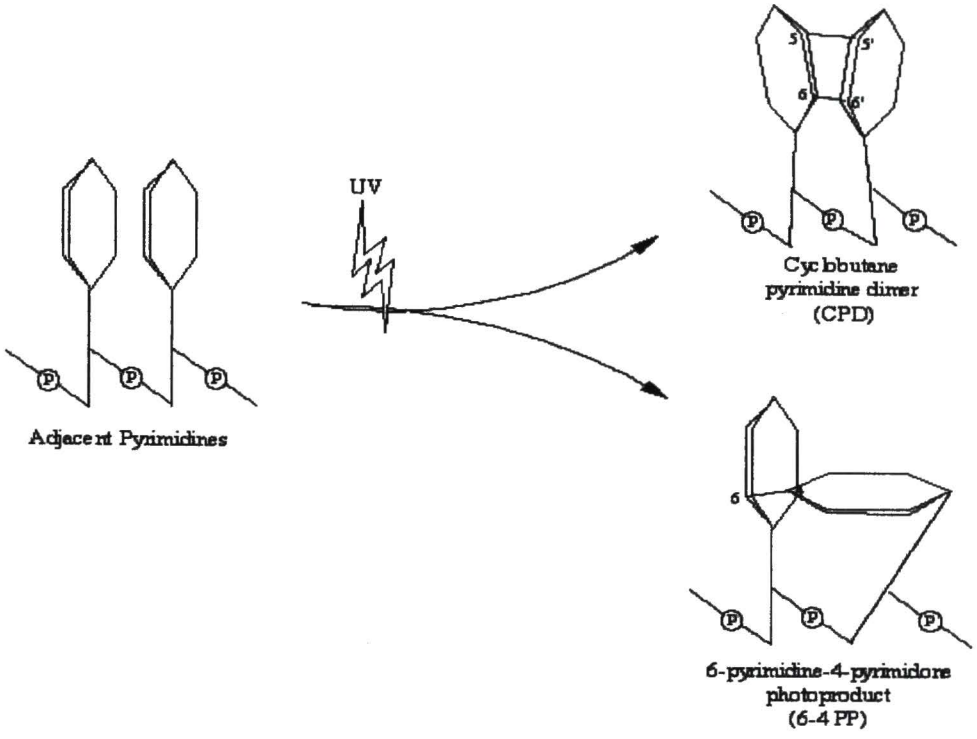

Fig 2-3. Cyclobutane pyrimidine dimer and pyrimidine (6-4) pyrimidone depiction along the sugar-phosphate backbone.

UV products are wavelength/dose dependent. UV exposure at the absorption maximum of DNA (λ=260 nm) generates the most significant pyrimidine dimer production. The high, direct absorption in the UVC range correlates with findings that exposure at 254 nm induces an estimated 20-100 folder higher incidence of CPDs than UVB (16) and a considerably higher induction rate (approximately 10^5 fold) when compared to UVA (28). A comparison of UVC, UVB, UVA and SSL showed (6-4) PPs readily formed under all treatments except for UVA exposure (20).

The administered radiation dose also plays a role in the action spectra of UV light on DNA. Exposure doses for UV radiation measure the incident energy per unit area on the target material, measured in units of joules (J) per square meter (m^2). Dimer formation reaches a steady-state maximum for high doses of UV light, which varies between dimer forms. For a known 117 bp segment, CPDs reached a plateau at around 500 J/m^2 for CC dimers, while TT dimers leveled at a dose of 2,000 J/m^2 (11). Overall CPD induction showed an increasing dose-response for UV doses of 0.26, 0.52 and 0.78 J/cm^2 in UVB (295-320 nm), and of 216, 432, and 648 J/cm^2 in UVA (340-400 nm) treated genomic DNA (Fig 2-4) (3).

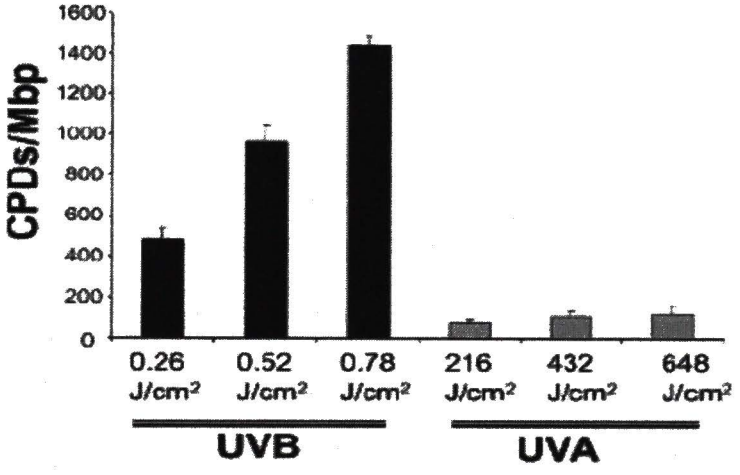

Fig 2-4. Dose dependence of cyclobutane pyrimidine dimer formation in human genomic fibroblast DNA for UVB and UVA exposure (3).

Specification of both wavelength and dose emissions are important for radiation response studies, as differences in these parameters produce varied UV effects in DNA. The approximate spectral output reported for the FS-40 sun lamp ranges from >310 nm through the visible spectrum to 750 nm, with a fluence rate of measured in a previous

study using the same lamp of 2.5-1.7 J/m^2 (without and with petri dish lids) at 24 cm (15).

Previous studies in UV decontamination. Sarkar and Sommer's first study on UV light and PCR contamination appeared in early 1990 (26). The study used a combination of 254 and 300 nm bulbs to expose a 6 kb plasmid at concentrations ranging from 3-30,000 pg in PCR reaction mix, at time intervals of 5 or 20 min. No PCR product was detected for a target 750 bp region after exposure, while a control plasmid DNA, added after irradiation was efficiently amplified.

In response to this study, Cimino *et. al.* pointed out that only a fraction of pyrimidines form dimers, as they reach a steady-state level dependent upon irradiation wavelength, and pyrimidine base composition (previously discussed) (5). The study theorized that for a 100 bp fragment, there existed an average of 6 modified sites capable of terminating synthesis, but for a large pool of 100 bp fragments, as in the case of PCR products, a statistically large number of fragments would contain no modified bases, and thus not be sterilized. The original Sarkar and Sommer study (26) was repeated by Cimino *et. al.* (5) using 115 and 500 bp fragments under the same conditions, with an added 30 min exposure. The 115 bp fragment was readily detected after exposure, while the 500 bp fragment was only sterilized after 30 min exposure. These two studies demonstrated the efficiency of UV decontamination had limitations for small DNA fragments, but to what extent had not been established.

Sarkar and Sommer replied by acknowledging thymine dimer formation to be sequence and size dependent, and reported a further study that found 5 different DNA

segments >700 bp to be susceptible to UV light, but only 1 of 4 different DNA segments <250 bp showed no detectable level (24). According to these results, approximately 25% of DNA fragments <250 bp would be inhibited during PCR using this technique. The following year, three more studies advocated the use of UV exposure to reduce false positives in PCR reactions as a worthwhile step, while also admitting limitations due to segment size and sequence (9, 18, 25).

A study by Padua *et. al.* demonstrated that for 1 *u*g of genomic DNA in water, 15 min of UV exposure was sufficient to inhibit amplification of a 530 bp fragment at a distance of 60 cm in the absence of dNTPs, and 45 min of UV exposure was needed at a distance of 10 cm in the presence of dNTPs (19). For target products of 186, 218 and 253 bp, exposure time to eliminate was 15, 45, and 60 min, respectively at a distance of 10 cm. Padua *et. al.* also reported that 45 min exposure at a distance of 10 cm was necessary to eliminate previously amplified DNA products (length of 218 bp). The study showed complete success in decontaminating fragments between 186-253 bp, but did not give the wavelength or power output for the OMNI workstation employed in their study. The importance of a clean air laminar flow hood in preventing contamination was also stressed when preparing PCR reactions.

A less accessible, but highly effective method using γ irradiation reported using doses of 150-400 krad to successfully prevent amplification of 294, 280, and 717 bp fragments present at 10^5, 10^4, and 10^4 copies, respectively (6). Control samples remained capable of amplification when added after irradiation, with reduced PCR efficiency at doses greater than 400 krad.

In the forensic field, a UV crosslinker is a commonly used UV source for decontamination. The Stratalinker® UV crosslinker instruction manual reports use of the equipment to irradiate PCR reaction mixes as a secondary, or miscellaneous function (27). The crosslinker emits 254nm UV at a recommended dose of 200,000-300,000 uJ/cm^2 for removing contaminating DNA in PCR reaction buffer, with no reported effectiveness level.

An effective UV light exposure for decontaminating the amplified PCR products, and genomic DNA isolated from whole blood and saliva in this study would result in no profile across the seven COfiler® loci, presumably after increasing exposure time.

CHAPTER III: MATERIALS AND METHODS

Sample preparation. Previously phenol-chloroform isolated and quantified human genomic DNA from a single source buccal swab was amplified using AmpF*l*STR® COfiler® PCR amplification kit according to the UNT-HSC DNA Identity Laboratory standard. Fifteen microliters (*u*l) of a reaction mix made containing 10.5 *u*l AmpF*l*STR® PCR Reaction Mix, 5.5 *u*l AmpF*l*STR® COfiler® primer pair mix and 0.5 *u*l AmpliTaq Gold® DNA Polymerase was combined with a genomic DNA volume of 1ng/10*u*l sterile ddH_2O. Five identical PCR reactions were prepared using the isolated genomic DNA, along with a positive (9947A) PCR control reaction and a negative (no DNA) PCR control reaction. Amplification was performed on a GeneAmp® 9700 Thermal Cycler at conditions of a 95°C hold for 11 minutes, 28 cycles of 94°C for 1 minute, 59°C for 1 minute, and 72°C for 1 minute, followed by a 60°C hold for 45 minutes. The five genomic DNA reaction samples were pooled together and used for fragment analysis on an ABI Prism® 310 Genetic Analyzer. A STR profile obtained through GeneScan® and Genotyper® software confirmed the presence of amplified STRs from a single, known source.

The pooled PCR products (referred to as STR samples from this point) were stored at 4°C until UV light exposure. Whole blood was provided by a single donor,

different from the STR source, and stored at 4°C until UV exposure. Saliva, provided by the same donor as the STR source, was also stored at 4°C until UV exposure.

Ultraviolet light exposure of samples. A fluorescent solar lamp (FS-40) sunlamp provided simulated solar light in an effort to measure sterilization of DNA samples for use in a forensic setting. Prior to exposure, 2 *u*l amplified STR product, 1 *u*l whole blood, or 2 *u*l saliva were added to sterile 1.5 ml microcentrifuge tubes, allowed to dry, and the caps sealed. Samples were prepared in duplicate for three exposure distances of 5 cm, 10 cm, and 60 cm, and six time intervals of 15 min, 30 min, 3 hrs, 6 hrs, 12 hrs, and 24 hrs exposure for a total of 36 samples per source. The tubes were placed on their side with the bottom of the tube towards the lamp. Following exposure, each sample was held at room temperature and placed away from any light until exposure was completed for all samples in the series. The temperature within the exposure area was monitored, and remained constant around 26.5°C over the 24 hr time period.

DNA extraction and isolation. Immediately after exposure to UV light was finished, an organic phenol-chloroform extraction was performed on the blood and saliva. Simultaneously, a reagent blank and a non-exposed biological sample were also extracted for both the blood and saliva series. Samples were resuspended in 300 *u*l Stain Extraction Buffer, combined with 5 *u*l of Proteinase K and incubated on a 56°C heat block for 6 hrs. An equal volume of phenol: chloroform: isoamyl alcohol (PCIA) was added to each sample, vortexed for 15 sec, and centrifuged at 10,000 rpm for 3 min. An estimated ninety-percent of each aqueous layer was pipetted to a sterile, labeled microcentrifuge

tube for each sample. Samples were ethanol precipitated with 1 ml of 100% cold ethanol, incubated at -20°C for 90 min, and centrifuged at 10,000 rpm for 20 min. The ethanol was decanted, and each sample was washed with 1 ml of 70% ethanol. Samples were then centrifuged at 10,000 rpm for 10 min, and the ethanol was pipetted off. Samples were dried by incubation on a 56°C heat block, resuspended in 100 *u*l of TE^{-4}, and stored at 4°C. Following exposure of the STR samples, 40 *u*l sterile ddH_2O was used to rinse the walls of each tube, as well as a reagent blank and an unexposed STR control, and all samples were stored at 4°C until amplification.

DNA quantitation of blood and saliva. Accurate measures of sample DNA concentration provide optimal PCR product quantities for use in forensic DNA fragment analysis. During forensic casework, a DNA-specific quantitation step precedes amplification to determine the optimal sample volume added to the PCR reaction mixture. A real-time PCR method was applied to the control blood and control saliva samples to determine the quantity of DNA present in the non-exposed samples. Using the ABI Prism® 7000 Sequence Detection System, a Quantifiler™ Human DNA Quantification Kit yielded concentrations of 0.462 ng/*u*l (blood control), and 0.451 ng/*u*l (saliva control). No sufficient means were available to the researcher to accurately quantify the control STR sample.

PCR amplification. All blood, saliva, and STR samples were amplified using an AmpF*l*STR® COfiler® PCR amplification kit. Fifteen microliters of a reaction mix made containing 10.5 *u*l AmpF*l*STR® PCR Reaction Mix, 5.5 *u*l AmpF*l*STR® primer pair mix

and 0.5 *u*l AmpliTaq Gold® DNA Polymerase per sample was combined with a DNA volume of 1ng/10*u*l sterile ddH_2O (based on the control sample concentration) for the blood and saliva series samples. One microliter from each STR series sample, plus 9 *u*l sterile ddH_2O, was added to the PCR reaction mix. A positive (9947A) PCR control and negative (no DNA) PCR control were also prepared. Amplification was performed on a GeneAmp® 9700 Thermal Cycler at conditions of a 95°C hold for 11 minutes, 28 cycles of 94°C for 1 minute, 59°C for l minute, and 72°C for 1 minute, followed by a 60°C hold for 45 minutes. Samples were stored at 4°C.

Fragment analysis on the ABI PRISM® 310. Capillary electrophoresis (CE) is the primary method utilized in forensic DNA testing for analyzing DNA samples. An ABI Prism® 310 Genetic Analyzer conducted CE for fragment analysis and data collection. The genetic analyzer was set-up according to ABI Prism® 310 Genetic Analyzer User's Manual. All samples were prepared by adding 10 *u*l of a mix containing 10 *u*l deionized formamide and 0.5 *u*l ROX internal lane standard per sample number, with 1 *u*l PCR product for each sample, and a ladder sample using 1.5 *u*l COfiler ladder. All samples were heat denatured for 3 min at 95°C on the GeneAmp® 9700 Thermal Cycler and placed on ice for 3 min prior to electrophoresis.

Software analysis. GeneScan® software was applied to the fragment analysis data collected by the ABI Prism® 310, which uses the ROX internal lane standard to assign precise lengths to fragment peaks. Genotyper® software was then used to provide allele

designation based on the COfiler® ladder, and also made available peak heights in relative fluorescent units (RFU), and peak locations in base pairs (bp).

Results analysis. Detected peaks were recorded according to their assigned genotype and relative fluorescent unit (RFU) value. The percent decrease in RFU values as compared to the control sample for that series was reported for certain loci. Also, the percent of correctly called alleles (excluding stutter peaks) relative to the control profiles were reported for each sample as an average between the duplicate samples.

CHAPTER IV: RESULTS

All sample RFU and allele call data assigned by Genotyper® is reported in Tables A-1 to A-12 located in the appendix. Samples are named first by their distance as 5, 10 or 60 cm from the UV source, second by the time exposed, with 1=15 min, 2=30 min, 3=3 hrs, 4=6hrs, 5=12 hrs, and 6=24 hrs, and third by the sample type in duplicate, with a,b=STR, c,d=blood, and e,f=saliva samples.

STR results. The STR reagent blank, and PCR positive and negative controls gave the expected result. The unexposed STR positive showed a complete profile with varied RFU values for different loci. D3S1358 and D16S539 had balanced, heterozygous peaks, with a stutter peak called for each allele. Stutter peaks result from polymerase slippage during replication, creating products usually one repeat unit shorter, and are recognized by their position and peak height relative to the true allele. Amelogenin showed balanced peaks at very high RFU values (> 6000 RFU). TH01 had a homozygous peak at a high RFU value (5700) and two stutter peaks called. TPOX, CSF1PO, and D7S820 showed heterozygous, balanced peaks with RFU levels decreasing with increasing loci fragment length (Table A-1).

For all distances, RFU values generally decreased as exposure time increased, with significantly lower effectiveness as the distance from UV exposure increased. For exposure times of 15 and 30 min, sample results were similar to that of the control at 5 and 10 cm distances. At 3, 6, 12, and 24 hrs, allelic dropout increased and RFU values

decreased with exposure length for both the 5 and 10 cm distances (Table A-4, A-5). Only the shortest loci, D3S1358 and the X allele of amelogenin, survived beyond 6 hrs at 5 cm. D3S1358 and the X allele averaged a decrease of 73% and 94%, respectively, in RFU relative to the control after 24 hrs exposure at 5 cm. At 10 cm, the three shortest loci survived 6-24 hrs exposure, but with RFU average decreases for D3S1358, X and Y amelogenin, and TH01 of 39%, 41%, 96%, and 96%, respectively for 24 hrs exposure. At 60 cm, sample results were similar to that of the control for exposure times of 15 min-6 hrs (Table A-6). The four shortest loci remained after 12 and 24 hrs exposure, with average RFU changes of +25%, -12%, -36%, -58%, and -93% for D3S1358, X and Y amelogenin, TH01, and TPOX, respectively.

Blood results. The blood reagent blank, and PCR positive and negative controls gave the expected result. The unexposed blood positive gave a complete profile with four balanced, heterozygous loci and three homozygous loci (Table A-2). Blood results showed numerous inconsistencies between duplicate samples, increasing time lengths and distances.

At 5 cm (Table A-7), the 15 min samples showed a weak, partial profile, the 30 min samples showed no data, while one of each the 3 and 6 hr samples gave complete profiles with RFU values greater than that of the control. For the 12 and 24 hr exposures at 5 cm, amelogenin remained in 2 of the 4 samples at RFU levels <180, and the heterozygous TPOX and CSF1PO alleles presented RFUs from 160-218 in the same samples. The other two samples for 12 and 24 hrs were negative. Samples at 10 cm (Table A-8) gave partial profiles with decreasing allele numbers and RFU values for

longer exposure time, with one allele called for the 12 hr samples and no alleles called for the 24 hr samples. A complete profile was obtained at 60 cm for 3 and 12 hr exposures, with varied partial profiles seen for other time intervals (Table A-9).

Saliva results. The saliva reagent blank and PCR negative control gave the expected result. The PCR positive control sample had RFU values much larger than the previous STR and blood control, ranging between 1100-5900. The unexposed saliva control provided an incomplete profile with all loci RFU values <430 (Table A-3).

The only evident trend was that an increasing number of alleles were callable as the exposure distance increased. The saliva series from 5 cm (Table A-10) showed only 3 alleles called for all 12 samples, at amelogenin for 15 and 30 min. From 10 cm (Table A-11), D3S1358 and amelogenin had peaks in both of the 24 hr samples. At 60 cm (Table A-12), there were partial profiles through 15 min, 30 min, 3 hrs, and 6 hrs, with no data for the 12 and 24 hr samples. Due to the weak results of the control, the extent of UV decontamination in the saliva samples cannot be determined based on these results.

Using the known genotype of the STR/saliva, and blood donors, the percent of correctly called alleles, averaged between duplicate samples, is represented for each sample type, time interval and exposure distance in Figs 4-1, 4-2, and 4-3. These crude representations show the increased effectiveness for the combination of increased time interval of exposure and increased proximity in reducing the number of alleles present in exposed samples of all three types.

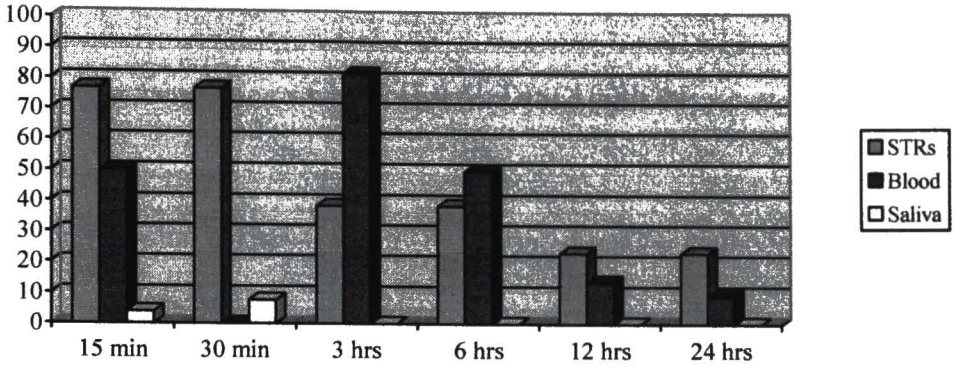

Fig 4-1. Percent of total alleles called for STR, blood and saliva samples at 5 cm.

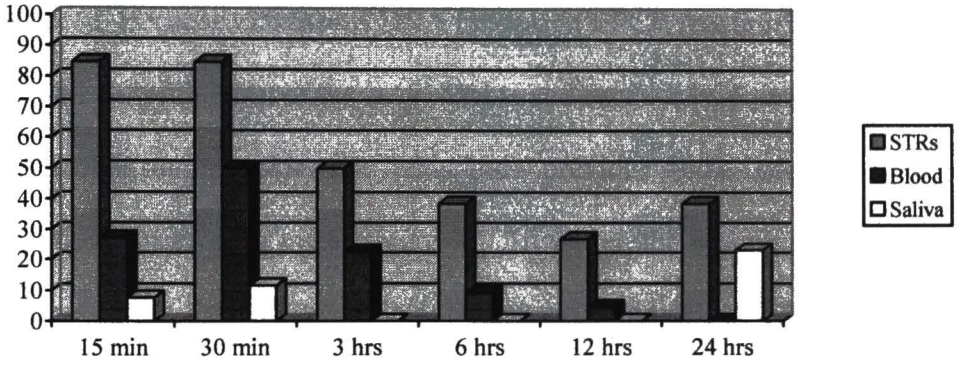

Fig 4-2. Percent of total alleles called for STR, blood and saliva samples at 10 cm.

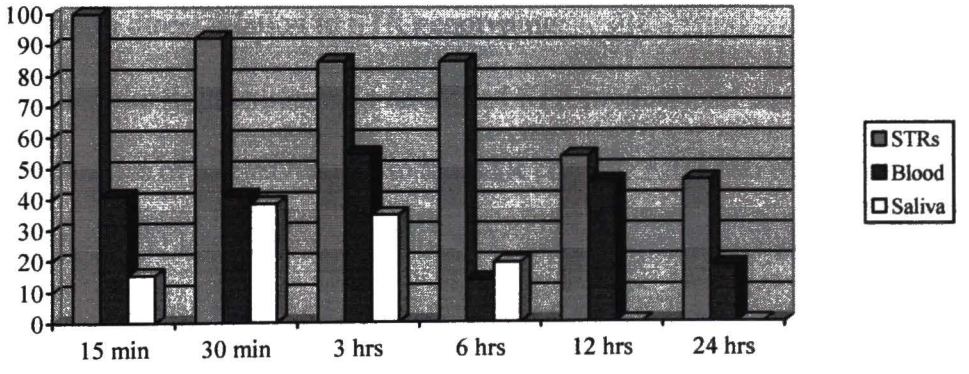

Fig 4-3. Percent of total alleles called for STR, blood and saliva samples at 60 cm.

CHAPTER V: DISCUSSION

Much of what is known about UV decontamination methods relies on studies that specifically tested PCR reaction mixtures. In these tests, contaminating DNA was added to reaction mixtures containing the necessary PCR reagents, with the exception of the polymerase, and the amount of DNA product present following various UV exposures was measured. Because extraneous DNA present in a PCR reaction can dramatically interfere with amplification of the target DNA, and because PCR is such a widely used technique across multiple scientific fields, these previous studies addressed the major contamination concerns for most researchers. However, the decontamination effectiveness of UV light on dried biological material and dried PCR products capable of transfer into a DNA testing system had not been directly approached. This study sought to determine applied solar radiation effects as a method of decontamination on these sample types in direct relation to STR genotyping.

Ultraviolet light exposure to amplified STR products did significantly reduce the signal or presence of alleles relative to the STR control. After two rounds of amplification, RFU levels for the control STR sample were very high for the shorter alleles of amelogenin, D3S1358 and TH01. This trend continued with the shorter fragments exhibiting the greatest longevity in the exposed samples as exposure times increased. UV exposure at distances of 10 and 60 cm resulted in only moderate reductions in alleles present and RFU values relative to the control; and partial profiles

with strong signals remained even after 24 hrs of exposure. A distance of 5 cm and 24 hrs exposure was not sufficient to eliminate all previously amplified STRs. The inability to block synthesis of small fragment lengths, as seen in amelogenin (107 bp), and D3S1358 (114-142 bp), is consistent with previous studies that also reported difficulty in removing small DNA fragments through UV light exposure (5, 24). The smaller fragments theoretically have fewer potential dimerization locations, taken into account with the possibly, unrealistically high STR concentration used, would explain the ineffectiveness of the exposure. Given a normally lower level of extraneous DNA present, UV exposure by a solar lamp at a distance of 5 cm could be a useful decontamination technique.

Analysis of the blood samples shows varied results with a general trend towards allelic dropout and lower RFU values for increasing exposure times and proximity. Numerous inconsistencies between duplicate samples could be accounted for as stochastic effects. A large proportion of the alleles called were <200 RFU, and slight differences between duplicate samples in the initial amount of cells present, extraction efficiency, or PCR setup could result in an allele call versus allelic dropout. After 24 hrs exposure, amelogenin and CSF1PO still remained at a distance of 5 cm in one of the samples, and no data was seen at 10 cm. No minimum effective exposure limit could be established from these results. In general, the homozygous loci showed the greatest resilience for increasing UV exposures in the blood samples.

The saliva control sample gave only a partial profile for 1 ng of DNA used in PCR amplification. The weak profile, considered with a strong positive PCR control result, could indicate PCR inhibition in the saliva samples giving limited results for

fragment analysis. The saliva samples were also delayed several weeks following PCR, from being genotyped due to instrument maintenance, which over time the samples may have further degraded, affecting the overall quality. No data was detected after 3 hrs exposure at 5 cm, whereas two loci were detected after 24 hrs from 10 cm, and up 6 hrs from 60 cm, suggesting a much higher effectiveness for the 5 cm distance.

Contamination surfacing in fragment analysis data could be interpreted as stutter peaks, heterozygous instead of homozygous loci, a mixture, or the true profile depending on the size and location of the contaminating DNA. As forensic analysts, a definitive strategy for removing biological materials and extraneous amplified DNA before testing applications seems crucial. The use of a solar radiation consistently reduced the amount of contaminating DNA present at a distance of 5 cm and minimum of 6 hrs exposure in all sample types; however, the UV exposure did not completely inhibit amplification of some smaller fragments isolated from blood and amplified STRs.

Upon review, several factors would have been interesting to address, and beneficial in understanding the proposed question of a minimal UV light exposure for effective decontamination. First, the UV dose administered at the three distances was not measured due to lack of instrumentation. As discussed previously, differing doses create specific action spectrums of induced DNA damage, based on the total energy of the UV light exposure. An exact dose reading allows assignment of a quantitative value to a definite cut-off point for successful decontamination. Even though a clear threshold was not determined through this study, knowledge of the exact UV dose used is relevant for reproducibility, comparative analysis, and further direction in subsequent studies.

Also, in relation to the amount of incident energy on the samples, exposure occurred through closed polypropylene microcentrifuge tubes, which are known to prevent penetration of shorter wavelengths. A range of wavelengths emitted by the sunlamp was penetrable to the tubes, based on the level of decontamination seen with increasing exposure time in close proximity to the lamp. The exact range of penetrable wavelengths, and how that affected the overall incident energy within the tubes is unknown, and would have been useful to measure. However, the sunlamp did provide a spectral output capable of inhibiting amplification of purified and cellular DNA within closed microcentrifuge tubes. The ability of the sunlamp spectrum to penetrate these tubes indicates a useful advantage over shorter wavelength exposure for instances when the entire interior surface area is not available to direct exposure, and could be useful for decontaminating other plastic products.

The method presented in this study showed consistent reduction in the amount of contamination present following sunlamp exposure, with only minimal amounts of DNA detected in the STR and blood samples after 12 and 24 hrs exposure, 5 cm from the source. Further investigation involving the addition of the mentioned samples to known, biological samples, such as a buccal swab or blood sample, and determining the mixed sample profiles would indicate how evident the minimal amounts of post-exposure DNA are when incorporated. In the presence of a larger concentration of intact genomic DNA, the loci surviving from exposure may not be able to amplify to a detectable level, and would therefore be considered successfully decontaminated.

This was the first study to apply UV light decontamination directly to forensic STR analysis. Several other UV light sources offer advantages over the sunlamp, and would be worth investigating under similar conditions. A germicidal lamp emits UV at 254 nm, which is exceptionally effective for damaging DNA, but the wavelengths cannot penetrate glass or plastic, and are extremely mutagenic and carcinogenic to humans. The combination of 254/300 nm bulbs described by Sarker and Sommer (26) introduce a wider range of damage types and a longer wavelength component, but showed limitations for small DNA fragments. The DNA crosslinker also uses 254 nm wavelengths, but in a closed environment, while providing quick and easy exposure. Due to the lengthy exposure time needed (at least 12 hrs) when using a sunlamp, other, higher UV dose sources, could be considered for increased efficiency in UV decontamination methods. Comparison of these three sources for UV decontamination of amplified STRs, isolated DNA, and biological samples could provide very useful and necessary information to forensic DNA analysts.

APPENDIX

	Loci: Allele and RFU value																	
Sample	D3S1358		D16S539		Amelogenin		TH01		TPOX		CSF1PO		D7S820	
STR	15	2521	11	931	X	6170	9	5697	8	1915	11	297	10	175
control	16	2487	12	853	Y	6257			11	1329	12	318	11	188
reagent blank	NR		NR		NR		NR		NR		NR		NR	
PCR – control	NR		NR		NR		NR		NR		NR		NR	
PCR +	14	420	11	416	X	890	8	340	8	763	10	292	10	186
control	15	392	12	375			9.3	296			12	378	11	152

Table A-1. STR control sample results with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value																	
Sample	D3S1358		D16S539		Amelogenin		TH01		TPOX		CSF1PO		D7S820	
blood	15	330	11	377	X	312	8	298	8	462	11	598	9	167
control	16	411	12	345	Y	254	9.3	259						
reagent blank	NR		NR		NR		NR		NR		NR		NR	
PCR – control	NR		NR		NR		NR		NR		NR		NR	
PCR +	14	420	11	416	X	890	8	340	8	763	10	292	10	186
control	15	392	12	375			9.3	296			12	378	11	152

Table A-2. Blood control sample results with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value																	
Sample	D3S1358		D16S539		Amelogenin		TH01		TPOX		CSF1PO		D7S820	
saliva	15	197	11		X	292	9	285	8	166	11		10	
control	16	179	12	163	Y	429			11		12		11	
reagent blank	NR		NR		NR		NR		NR		NR		NR	
PCR – control	NR		NR		NR		NR		NR		NR		NR	
PCR +	14	3944	11	2603	X	5915	8	1729	8	4288	10	1894	10	1175
control	15	2723	12	2570			9.3	2157			12	2258	11	1147

Table A-3. Saliva control sample results with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value															
Sample	D3S1358		D16S539		Amelogenin		TH01		TPOX		CSF1PO		D7S820			
5-1a	15	3380	11	459	X	6584	9	5017	8	1090	NR		NR			
	16	3419	12	418	Y	4950			11	790						
5-1b	15	3646	11	563	X	6886	9	6412	8	1381	11	238	NR			
	16	3630	12	521	Y	4950			11	1030	12	241				
5-2a	15	3765	11	324	X	6204	9	5077	8	815	11	158	NR			
	16	3821	12	319	Y	4096			11	618	12	188				
5-2b	15	2966	11	265	X	6580	9	3679	8	601	NR		NR			
	16	2972	12	235	Y	6971			11	460						
5-3a	15	2546	NR		X	6891	9	921	NR		NR		NR			
	16	2550			Y	982										
5-3b	15	2917	NR		X	6826	9	976	NR		NR		NR			
	16	2813			Y	998										
5-4a	15	1649	NR		X	3722	9	200	NR		NR		NR			
	16	1633														
5-4b	15	1621	NR		X	4002	9	225	NR		NR		NR			
	16	1629														
5-5a	15	746	NR		X	918	NR		NR		NR		NR			
	16	736														
5-5b	15	1032	NR		X	1208	NR		NR		NR		NR			
	16	1023														
5-6a	15	452	NR		X	339	NR		NR		NR		NR			
	16	435														
5-6b	15	929	NR		X	473	NR		NR		NR		NR			
	16	938														

Table A-4. STR sample results from exposure at 5 cm with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value																	
Sample	D3S1358			D16S539			Amelogenin			TH01			TPOX			CSF1PO			D7S820
10-1a	15	3418		11	681		X	6745		9	6987		8	1686		11	268		NR
	16	3481		12	633		Y	6663					11	1183		12	260		
10-1b	15	3025		11	611		X	6824		9	6292		8	1508		11	238		NR
	16	3046		12	565		Y	4473					11	1135		12	238		
10-2a	15	3244		11	419		X	6466		9	5132		8	998		11	166		NR
	16	3300		12	369		Y	4072					11	768		12	170		
10-2b	15	3708		11	529		X	5992		9	6229		8	1304		11	209		NR
	16	3777		12	507		Y	4688					11	941		12	236		
10-3a	15	2693		NR			X	3864		9	1597		NR			NR			NR
	16	2595					Y	2559											
10-3b	15	3171		12	154		X	6599		9	2596		8	309		NR			NR
	16	3045					Y	5746					11	238					
10-4a	15	2265		NR			X	6620		9	832		NR			NR			NR
	16	2266					Y	885											
10-4b	15	2187		NR			X	7010		9	879		NR			NR			NR
	16	2226					Y	964											
10-5a	15	1578		NR			X	3058		NR			NR			NR			NR
	16	1577																	
10-5b	15	1510		NR			X	3112		9	155		NR			NR			NR
	16	1522																	
10-6a	15	1553		NR			X	4120		9	282		NR			NR			NR
	16	1578					Y	242											
10-6b	15	1483		NR			X	3227		9	234		NR			NR			NR
	16	1491					Y	252											

Table A-5. STR sample results from exposure at 10 cm with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value																	
Sample	D3S1358		D16S539		Amelogenin		TH01		TPOX		CSF1PO		D7S820	
60-1a	15	3707	11	1327	X	5554	9	3402	8	3418	11	415	10	229
	16	3863	12	1275	Y	6478			11	2512	12	441	11	227
60-1b	15	4515	11	1578	X	6350	9	6157	8	4079	11	505	10	278
	16	4576	12	1500	Y	6109			11	2925	12	528	11	281
60-2a	15	2906	11	809	X	6585	9	7056	8	2112	11	289	NR	
	16	3071	12	767	Y	5404			11	1561	12	285		
60-2b	15	3757	11	1239	X	5289	9	3390	8	3189	11	408	10	225
	16	3921	12	1192	Y	6691			11	2357	12	430	11	206
60-3a	15	3987	11	466	X	6701	9	5998	8	1149	11	198	NR	
	16	4058	12	442	Y	5171			11	860	12	193		
60-3b	15	3113	11	562	X	6842	9	5507	8	1343	11	223	NR	
	16	3205	12	516	Y	4599			11	989	12	213		
60-4a	15	4548	11	436	X	6635	9	5852	8	1051	11	188	NR	
	16	4500	12	410	Y	6795			11	757	12	185		
60-4b	15	3278	11	335	X	6877	9	4409	8	788	11	150	NR	
	16	3272	12	301	Y	6323			11	596	12	157		
60-5a	15	3633	NR		X	6184	9	2774	8	224	NR		NR	
	16	3573			Y	4841			11	165				
60-5b	15	2390	NR		X	6256	9	2200	8	233	NR		NR	
	16	2330			Y	4452			11	189				
60-6a	15	3033	NR		X	4481	9	2013	NR		NR		NR	
	16	3010			Y	3028								
60-6b	15	3265	NR		X	6417	9	2746	8	229	NR		NR	
	16	3201			Y	4959			11	210				

Table A-6. STR sample results from exposure at 60 cm with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value																	
Sample	D3S1358		D16S539		Amelogenin		TH01		TPOX		CSF1PO		D7S820	
5-1c	15	163	NR		X	150	NR		8	211	11	167	NR	
					Y	178								
5-1d	15	169	11	153	X	214	NR		8	175	11	153	NR	
					Y	177								
5-2c	NR		NR		NR		NR		NR		NR		NR	
5-2d	NR		NR		NR		NR		NR		NR		NR	
5-3c	15	275	11	171	X	491	9	248	8	255	NR		NR	
	16	212	12	<150	Y	351			11	171				
5-3d	15	409	11	<150	X	217	8	195	8	371	11	364	9	204
	16	447	12	186	Y	247	9.3	166						
5-4c	15	749	11	458	X	699	8	573	8	878	11	741	9	584
	16	711	12	448	Y	663	9.3	620						
5-4d	NR		NR		NR		NR		NR		NR		NR	
5-5c	NR		NR		NR		NR		NR		NR		NR	
5-5d	NR		NR		X	<150	NR		8	218	11	192	NR	
					Y	150								
5-6c	NR		NR		X	176	NR		NR		11	160	NR	
					Y	<150								
5-6d	NR		NR		NR		NR		NR		NR		NR	

Table A-7. Blood sample results from exposure at 5 cm with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value																				
Sample	D3S1358				D16S539				Amelogenin				TH01				TPOX				CSF1PO				D7S820			
10-1c	15	172			11	211			X	<150			NR				8	299			11	288			9	171		
	16	<150			12	<150			Y	175																		
10-1d	NR				NR				NR				NR				NR				NR				NR			
10-2c	15	215			NR				X	154			8	156			8	241			11	183			NR			
	16	199							Y	<150			9.3	<150														
10-2d	15	181			NR				X	<150			NR				8	205			11	174			NR			
	16	191							Y	151																		
10-3c	NR				NR				NR				NR				NR				NR				NR			
10-3d	15	331			NR				X	171			NR				8	197			11	190			NR			
									Y	209																		
10-4c	NR				NR				X	181			NR				8	>150			11	160			NR			
									Y	>150																		
10-4d	NR				NR				NR				NR				NR				NR				NR			
10-5c	NR				NR				NR				NR				NR				11	169			NR			
10-5d	NR				NR				NR				NR				NR				NR				NR			
10-6c	NR				NR				NR				NR				NR				NR				NR			
10-6d	NR				NR				NR				NR				NR				NR				NR			

Table A-8. Blood sample results from exposure at 10 cm with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value																	
Sample	D3S1358		D16S539		Amelogenin		TH01		TPOX		CSF1PO		D7S820	
60-1c	15	157	11	<150	X	238	NR		8	167	11	203	NR	
	16	170	12	150	Y	<150								
60-1d	NR		NR		X	168	NR		8	237	11	187	NR	
					Y	<150								
60-2c	15	189	11	189	X	248	9.3	230	8	264	11	167	9	162
	16	216	12	<150	Y	222								
60-2d	NR		NR		NR		NR		NR		NR		NR	
60-3c	15	394	11	253	X	811	9	409	8	318	11	276	NR	
	16	459	12	259	Y	699								
60-3d	NR		11	193	NR		NR		8	197	11	210	NR	
			12	<150										
60-4c	NR		NR		NR		NR		8	152	NR		NR	
60-4d	NR		NR		X	<150	NR		NR		11	198	NR	
					Y	186								
60-5c	15	324	11	<150	X	249	8	187	8	299	11	238	9	<150
	16	258	12	187	Y	211	9.3	<150						
60-5d	NR		NR		X	215	NR		NR		11	196	NR	
					Y	<150								
60-6c	NR		NR		NR		NR		8	162	11	245	NR	
60-6d	15	159	NR		NR		NR		8	170	NR		NR	

Table A-9. Blood sample results from exposure at 60 cm with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value						
Sample	D3S1358	D16S539	Amelogenin	TH01	TPOX	CSF1PO	D7S820
5-1e	NR	NR	Y 204	NR	NR	NR	NR
5-1f	NR	NR	NR	NR	NR	NR	NR
5-2e	NR	NR	NR	NR	NR	NR	NR
5-2f	NR	NR	X 378 Y 280	NR	NR	NR	NR
5-3e	NR	NR	NR	NR	NR	NR	NR
5-3f	NR	NR	NR	NR	NR	NR	NR
5-4e	NR	NR	NR	NR	NR	NR	NR
5-4f	NR	NR	NR	NR	NR	NR	NR
5-5e	NR	NR	NR	NR	NR	NR	NR
5-5f	NR	NR	NR	NR	NR	NR	NR
5-6e	NR	NR	NR	NR	NR	NR	NR
5-6f	NR	NR	NR	NR	NR	NR	NR

Table A-10. Saliva sample results from exposure at 5 cm with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value						
Sample	D3S1358	D16S539	Amelogenin	TH01	TPOX	CSF1PO	D7S820
10-1e	NR	NR	NR	NR	NR	NR	NR
10-1f	15 154	NR	X 186	NR	NR	NR	NR
10-2e	NR	NR	NR	NR	NR	NR	NR
10-2f	15 168	NR	X 195	9 172	NR	NR	NR
10-3e	NR	NR	NR	NR	NR	NR	NR
10-3f	NR	NR	NR	NR	NR	NR	NR
10-4e	NR	NR	NR	NR	NR	NR	NR
10-4f	NR	NR	NR	NR	NR	NR	NR
10-5e	NR	NR	NR	NR	NR	NR	NR
10-5f	NR	NR	NR	NR	NR	NR	NR
10-6e	16 157	NR	X 487 Y 298	NR	NR	NR	NR
10-6f	15 161	NR	X 608 Y 501	NR	NR	NR	NR

Table A-11. Saliva sample results from exposure at 10 cm with allele call and RFU value (NR=no result).

	Loci: Allele and RFU value													
Sample	D3S1358		D16S539	Amelogenin		TH01		TPOX			CSF1PO	D7S820		
60-1e	15	187	NR	X	272	9	229	NR			NR	NR		
				Y	245									
60-1f	NR		NR	NR		NR		NR			NR	NR		
60-2e	NR		NR	X	265	9	290	8	184		NR	NR		
				Y	167			11	164					
60-2f	15	267	NR	X	508	9	386	NR			NR	NR		
	16	251		Y	513									
60-3e	15	192	NR	X	430	9	168	NR			NR	NR		
				Y	224									
60-3f	15	206	NR	X	423	9	191	NR			NR	NR		
	16	183		Y	226									
60-4e	16	169	NR	X	189	NR		NR			NR	NR		
60-4f	NR		NR	X	162	9	162	NR			NR	NR		
				Y	192									
60-5e	NR		NR	NR		NR		NR			NR	NR		
60-5f	NR		NR	NR		NR		NR			NR	NR		
60-6e	NR		NR	NR		NR		NR			NR	NR		
60-6f	NR		NR	NR		NR		NR			NR	NR		

Table A-12. Saliva sample results from exposure at 60 cm with allele call and RFU value (NR=no result).

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