Evaluation and In-House Validation of Five DNA Extraction Methods for PCR-based STR Analysis of Bloodstained Denims

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ABSTRACT

One type of crime scene evidence commonly submitted for analysis is bloodstain on denim. However, chemicals (e.g., indigo) used to produce denim materials may co-purify with DNA and hence, affect subsequent DNA analysis. The present study compared five methods (e.g., standard organic, organic with hydrogen peroxide (H_2O_2), modified FTA™, organic/Chelex®-Centricon®, and QIAamp® DNA Mini Kit-based procedures) for the isolation of blood DNA from denim. A Short Tandem Repeat (STR)-based analysis across two to nine STR markers, namely, HUMvWA, HUMTH01, D8S306, HUMFES/FPS, HUMDHFRP2, HUMF13A01, HUMFGA, HUMTPOX, and HUMCSF1PO, was used to evaluate successful amplification of blood DNA extracted from light indigo, dark indigo, indigo-sulfur, pure indigo, sulfur-top, and sulfur-bottom denim materials. The results of the present study support the utility of organic/Chelex®-Centricon® and QIAamp® Kit procedures in extracting PCR-amplifiable DNA from five different types of denim materials for STR analysis. Furthermore, a solid-based method using FTA™ classic cards was modified to provide a simple, rapid, safe, and cost-effective procedure for extracting blood DNA from light, dark indigo and pure indigo denim materials. However, DNA eluted from bloodstained sulfur-dyed denims (e.g., sulfur-top and sulfur-bottom) using FTA™ procedure was not readily amplifiable.

Keywords: DNA extraction, bloodstained denims, PCR, short tandem repeat, FTA™ classic card, inhibitors

INTRODUCTION

For successful DNA analysis, sufficient DNA template of good quality and purity must first be recovered from samples. However, selection of DNA isolation procedures is affected by the type of sample and the matrix to which a sample adheres. Biological fluids such as saliva, semen, sweat, and blood on denim are frequently submitted as evidentiary material that were recovered from crime scenes. However, chemical substances in denim are known to inhibit the activity of restriction enzymes (Prinz & Berghaus, 1990) and DNA polymerases (Larkin & Harbison, 1999; Jung et al., 1991; Del Rio et al., 1996) during different typing procedures.

Denim is a tight twill cloth made up of cotton, sometimes combined with polyester, where the characteristic blue color is determined by the level of

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indigo dye absorbed in the material after dyeing and washing (Larkin & Harbison, 1999). For example, material that was dyed with indigo followed by sulfur is known as *sulfur-top denim*. If the dyeing procedure is reversed, the material produced is classified as *sulfur-bottom denim* (Alviar, personal communications, 2002). The resulting denim material is subsequently processed to make branded clothing (e.g., jeans).

Several extraction methods are available to isolate DNA from bloodstained denim. These methods include the use of Chelex® 100 (Bio-Rad Laboratories, Hercules, CA, USA) (Walsh et al., 1991), Chelex® in tandem with Centricon® 100 (Amicon, Beverly, MA, USA) (Jung et al., 1991), and QIAamp® spin columns (Qiagen Inc., CA, USA) (Greenspoon et al., 1998). However, most materials (i.e., reagents, columns, filters) involved in these procedures are expensive, and many laboratories continue to use phenol and chloroform in conventional organic solvent-based extraction procedures. These procedures unfortunately introduce the problem of organic waste disposal. There is thus a need to develop an affordable, non-phenol based procedure for extracting DNA from bloodstained denim.

In the present study, a modified FTA™ Classic Card procedure for extracting PCR-ready DNA from bloodstained denim was validated and compared with known methods, e.g., organic extraction (Kirby, 1992), organic method with hydrogen peroxide (Merck, Germany) (Akane, 1996), organic/Chelex®-Centricon® (Jung et al., 1991), and the QIAamp® DNA Mini Kit (Qiagen Inc., Chatsworth, CA, USA) procedures. A PCR-based Short Tandem Repeat (STR) analysis using two to nine markers was used to evaluate successful amplification of blood DNA extracted from six types of denims.

MATERIALS AND METHODS

Sources of samples

Denim samples were collected from two denim factories at different times. The first set of samples consisting of light indigo, dark indigo, and dark indigo-sulfur denims was obtained in 1999, while another set of samples consisting of pure indigo, sulfur-top, and sulfur-bottom denims was obtained in 2002. Ten (10) bloodstains were prepared by depositing 100 µL of fresh blood from a male volunteer on each of the six types of denim material, and left at room temperature (27°C-32°C) away from direct sunlight for 8-16 days. Bloodstained portions of each denim were cut into small pieces using sterile scissors and placed in sterile 1.5 mL microfuge tubes.

DNA extraction

Standard organic extraction

Samples were washed with 1X SSC, followed by brief vortexing and centrifugation at 14,000 rpm for 3 minutes. Pellets were lysed using a cell lysis solution consisting of 375 µL of 0.2 M NaOAc (pH 5.2), 50 µL 10% SDS, and 7.5 µL 20 mg/mL Proteinase K and incubated for 18-24 hours at 56°C (Kirby, 1992). Cell lysate was purified using Phenol:Chloroform:Isoamyl alcohol or PCI (25:24:1). DNA was subjected to ethanol precipitation as described in Sambrook et al. (1989). The resulting pellet was resuspended in 40 µl TE^{-4} buffer.

Organic/Hydrogen peroxide (H_2O_2) *method*

Samples were incubated in 2 mL freshly-prepared Stain Extraction (SE) Buffer (0.6% N-lauroylsarcosine; 10 mM Tris-HCl; 50 mM EDTA, pH 7.4; 0.4 mg Proteinase K) at 56°C for 18-24 hours. The denim was transferred to a new microfuge tube and 2 mL SE buffer was added. A second incubation at 37°C for 2 hours was performed upon addition of 100 µL 30% H_2O_2 (Merck, Germany) (Akane, 1996). DNA was precipitated following standard alcohol precipitation procedure as described in Sambrook et al. (1989). The resulting pellet was resuspended in 40 µl TE^{-4} buffer.

Modified FTA™ classic card method

Samples were completely immersed in TE^{-4} buffer (300-400 µL) at 56°C for 18-24 hrs and the resulting

stain solution was deposited on one circular area of an FTA™ classic card. The card was air-dried at room temperature for at least 2 hours, punched using a Harris MicroPunch™ apparatus (Life Technologies, Inc., Gaithersburg, MD, USA), and processed according to manufacturer's instructions (Whatman® BioScience, MA, USA).

Organic/Chelex®-Centricon®

Samples were washed with 1X SSC, followed by brief vortexing and centrifugation at 14,000 rpm for 3 minutes (Jung et al., 1991). Pellets were processed following a standard phenol-chloroform extraction procedure, but without the alcohol precipitation step. Instead, the aqueous layer was purified with 5% Chelex® 100 (Bio-Rad Laboratories, CA, USA) and Centricon® 100 columns (Amicon, MA, USA) according to manufacturer's instructions. DNA was eluted using 100-200 µL of TE^{-4} buffer.

QIAamp® DNA mini kit, dried blood spot protocol, Qiagen 2001

Samples were immersed in 360 µL of Buffer ATL and incubated at 85°C for 10 minutes. The mixture was lysed using Proteinase K and Buffer AL following manufacturer's instructions (QIAamp® DNA Mini Kit Handbook, Qiagen, 2001). The lysed mixture was transferred to a QIAamp® spin column (in a 2-mL collection tube) to allow binding of DNA to the spin column followed by several washing steps. DNA was eluted using 150 µL Buffer AE.

DNA quantification

The amount of DNA extracted was quantified using the Quantiblot® Human DNA Quantitation Kit (Applied Biosystems, Forster City, CA, USA). DNA was first immobilized on a Biodyne B® membrane (PALL Life Sciences, MI, USA) using the S & S Minifold® I Slot-Blot system (Schleicher and Schuell, NH, USA) and probed using a biotin-labeled primate-specific alpha satellite D17Z1 probe. Human DNA was detected using a horse-radish peroxidase-streptavidin (HRP-SA) and chromogen 3,3'5,5'-tetramethylbenzidine (TMB) following manufacturer's instructions (Applied Biosystems). DNA concentration was estimated by comparing band intensities with those of K562 DNA (Gibco BRL, Life Technologies, Inc., Gaithersburg, MD, USA) of known concentrations (Fig. 1).

To check for the presence of inhibitors which co-eluted with DNA during extraction, several dilutions of DNA extracts (1/10, 1/100, and 1/1000) in TE^{-4} buffer were prepared and analyzed.

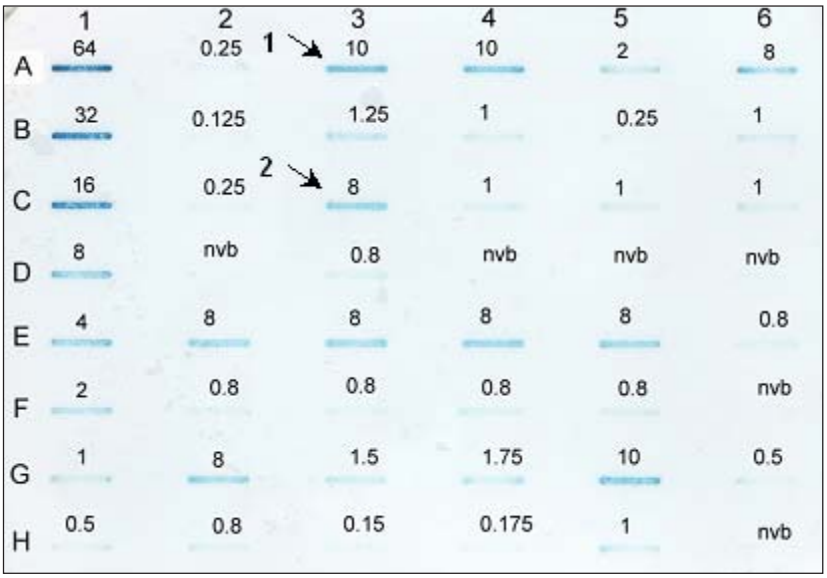

Fig. 1. *Quantification of extracted DNA using the Quantiblot® Kit.* Shown in the blot are: K562 DNA standards at various dilutions (slots 1A-2B), standard DNA solution A (slot 2C), reagent blank (slot 2D), and extracted DNA samples (slots 2E-6H). Numbers above the blue bands denote DNA concentrations in ng/µL. The letters "nvb" refer to no visible bands (unquantifiable samples). Arrows point to samples that were positive for DNA but failed to produce amplification bands after PCR.

Pointed in arrows are samples which were positive for human DNA, e.g., (1) bloodstained dark indigo-sulfur denim extracted using standard organic procedure labeled as DIS-[SO] with estimated yield of 25 ng and (2) light indigo denim extracted using organic/H_2O_2 method labeled as LI-[OH] (approx. 20 ng), but produced negative amplification (Fig. 3b).

PCR amplification

DNA was amplified at loci HUMFES/FPS, HUMF13A01, HUMvWA, HUMTH01, HUMDHFRP2, D8S306 (FFvTDD), HUMCSF1PO, HUMTPOX, and HUMFGA (CTF) (Table 1) using 2.5 µL of liquid DNA extract or one 2-mm FTA™ extracted disc as template. PCR amplification reaction for each locus was composed of 1X PCR Buffer (50 mM KCl, 20 mM Tris-HCl), 1.5 mM $MgCl_2$, forward (Cy5-labeled) primer (GenSet Oligos, Singapore), reverse (unlabeled) primer (Gibco BRL) (FFvTDD: 0.72 µM, CTF: 0.504 µM), dNTP mix (FFvTDD: 37.5 µM, CTF: 50 µM), 60 ng/µL bovine serum albumin (BSA), and 0.02 U/µL Taq polymerase (Gibco BRL). PCR was carried out in a Biometra Uno II thermocycler (Biometra, Germany) or GeneAmp 9700 thermoycler (Applied Biosystems) following thermocycling conditions: 96°C initial denaturation for 2 min, followed by 30 cycles of 94°C denaturation for 1 min, 56°C annealing for 1 min, 72°C extension for 1.5 min, and a 72°C final extension for 10 min, for the amplification of FFvTDD loci, while the following parameters: 96°C initial denaturation for 2 min, followed by 10 cycles of 94°C for 1 min, 64°C for 1 min, and 70°C for 1.5 min, and 20 cycles of 90° C for 1 min, 60° C for 1 min, and 20° C for 1.5 min were used for the amplification of CTF loci (Halos et al., 1999; Tabbada et al., 2002). K562 DNA (Gibco BRL) and in-house DNA standard NSDNA 3967 were used as positive controls while sterile distilled deionized water was used as negative control. All amplification reactions were done in duplicate. In addition, in-house control NSDNA 3967 and an equal amount of purified DNA (organic-based extraction) were mixed and subsequently amplified to confirm the presence of inhibitors in solution.

DNA fragment analysis

Amplified products were resolved and detected in an automated fluorescence detection system using the ALF Express™ DNA Sequencer and Allelinks™ version 1.01 software (Amersham Pharmacia Biotech, Uppsala, Sweden) according to manufacturer's

Table 1. Characteristics of short tandem repeat (STR) markers used.

Locus name	Locus definition	Chromosome number	Size range (bp)	Repeat Motif (ISFH)	References
HUMvWA	Human von Willebrand factor gene	12	126-170	[TCTG][TCTA]	Kimpton et al., 1992
HUMFES/FPS	Human c-fes/fps	15	206-238	[ATTT]	Polymeropoulos et al., 1991a
HUMF13A01	Human coagulation factor XIII a subunit gene	6	179-235	[GAAA]	Polymeropoulos et al., 1991b
HUMTH01	Human tyrosine hydroxylase gene	11	146-189	[TCAT]	Polymeropoulos et al., 1991c
HUMCSF1PO	Human c-fms proto oncogene for CSF-1 receptor gene	5	291-323	[TAGA]	Hammond et al., 1994
D8S306	not identified	8	249-298	[AAAG]	Nelson et al., 1993
HUMDHFRP2	Human dihydrofolate reductase psi-2 pseudogene	6	157-173	[AAAC]	Polymeropoulos et al., 1991d
HUMFGA	Human alpha fibrinogen gene 3rd intron	4	160-314	[CTTT]	Mills et al., 1992
HUMTPOX	Human thyroid peroxidase	2	106-138	[GAAT]	Anker et al., 1992

instructions. Sizes of PCR products were compared and scored using in-house allelic ladders described previously (Halos et al., 1999; Tabbada et al., 2002). Heterozygous alleles were assigned only when the smaller peak area was at least 60% of the larger peak area (Clayton et al., 1998).

RESULTS

DNA quantity

DNA was successfully extracted from six bloodstained denim samples after 8-16 days of storage at room temperature using five methods included in the present study. Estimated DNA yield varied between the four liquid-based extraction procedures with organic/Chelex®-Centricon® method consistently resulting in the highest DNA yield (Table 2).

Up to 800-1000 ng of DNA per 100 µL of bloodstain was recovered from bloodstained denims except for the dark indigo denim with yield of 100 ng DNA per 100 µL of bloodstain using organic/Chelex®-Centricon® procedure. Similar yields of 320 ng/100 µL bloodstain were obtained from bloodstained denims extracted using standard organic and organic/H_2O_2 methods, except for the dark indigo-sulfur where yield varied between the two methods. On the other hand, low DNA yields (75-300 ng/100 µL of bloodstain) were observed using the QIAamp® Kit procedure.

Table 2. Typical DNA yields (in ng) from different types of bloodstained denim extracted using four procedures.

Denim	Average DNA Yield (ng) per 100 µL of bloodstain			
	Standard Organic	Organic/ H_2O_2	Organic/Chelex® Centricon®	QIAamp® Kit (Qiagen, Inc.)
Phase I				
Light indigo	320	320	1000	262.5
Dark indigo	320	320	100	300
Dark indigo-sulfur	400	60	800	150
Phase II				
Pure indigo	ND	ND	800	150
Sulfur-top	ND	ND	1000	120
Sulfur-bottom	ND	ND	800	75

ND: Not done

DNA quality

The success rates of PCR amplification of template DNA from bloodstained light indigo, dark indigo, and dark indigo sulfur (Phase I) using five DNA extraction methods are summarized in Table 3. Liquid and bound DNA prepared from the three bloodstained denim samples using the modified FTA™, organic/Chelex®-Centricon®, and QIAamp® Kit procedures were successfully amplified in two STR markers (100%). Peak signals generated at two loci (HUMvWA and HUMTH01) by the modified FTA™-prepared DNA were distinct and comparable with those obtained using organic/Chelex®-Centricon® and QIAamp® Kit procedures. Fig. 2 shows an electropherogram of DNA from bloodstained light indigo denim amplified at HUMvWA locus. On the other hand, low success rates of amplification in two STR markers of 33.3%-66.7% and 33.3% were observed in liquid DNA samples prepared using the standard organic and organic/H_2O_2 procedures, respectively.

The co-elution of inhibitors with DNA extracted by standard organic and organic/H_2O_2 was confirmed when control NSDNA 3967 failed to amplify when an equal volume of DNA extracted via organic procedures was added to the solution (Fig. 3A). Subsequent dilution of the same DNA preparation led to successful amplification (Fig. 3B).

Further comparison between the modified FTA™, organic/Chelex®-Centricon® and QIAamp® Kit procedures was performed by analyzing another set of bloodstained denim samples, e.g., pure indigo, sulfur-top, sulfur-bottom denims (Phase II). Results are summarized in Table 3. Liquid DNA prepared using organic/Chelex®-Centricon® and QIAamp® Kit procedures were successfully amplified in two of three samples (66.7%) in all three markers. On the other hand, lower success rates (33.3%-66.7%) were obtained in amplifying modified

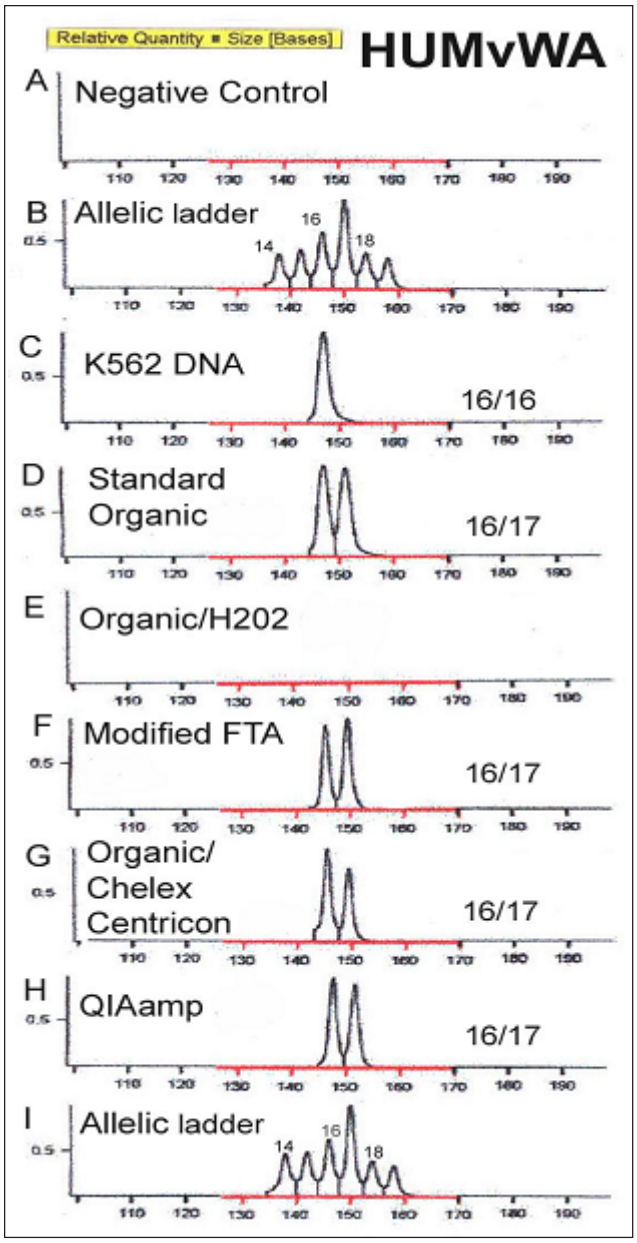

Fig. 2. *Electropherogram of allele peak signals at HUMvWA resulting from amplification of DNA from bloodstained light indigo prepared using five different extraction procedures.*

Shown in figure are in-house generated allelic ladders (B & I), negative (A), and K562 DNA (positive) controls (C) used in the PCR reaction and amplicons from DNA extracted from bloodstained light indigo denim from a male volunteer using standard organic (D), organic with H_2O_2 (E), modified FTA™ procedure (F), organic/Chelex®-Centricon® (G), and QIAamp™ DNA Mini Kit (I) procedures. Clear and typable allele peak signals produced as a result of successful DNA amplification using modified FTA™ procedure were comparable to those extracted via Organic/Chelex®-Centricon® and QIAamp® DNA Mini Kit procedures. Absence of DNA amplification is denoted by the absence of any detectable peak. X-axis represents the size range of alleles (in bp). The Y-axis is a quantitative scale based on % peak area reflecting peak signal with the largest peak area given automatically a value of 100%, while other peaks within the same lane are given values that are a percentage of the largest peak (Pharmacia Biotech).

FTA®-prepared DNA due to non-amplification of two (sulfur-top and sulfur-bottom) out of three samples in two STR markers (HUMvWA and D8S306: 33.3%) and one (sulfur-bottom) out of three samples at HUMTH01 marker (66.7%).

DNA eluted from bloodstained pure indigo denim using the modified FTA™ procedure was successfully amplified across the three STR markers with peak signals that were clear and distinct to be comparable with those obtained using organic/Chelex®-Centricon® and QIAamp® Kit procedures (Fig. 4, panels D-F). In contrast, variable amplification results were obtained from modified FTA™-prepared DNA from bloodstained sulfur-top denim, while negative amplification results were evident from DNA prepared from bloodstained sulfur-bottom denim using either of the three DNA extraction procedures (Fig. 4, panels G-L).

To determine the overall success rate of amplification of DNA extracted using the modified FTA™ procedure in the remaining six STR markers routinely used in our laboratory, DNA from bloodstained pure indigo, sulfur-top, and sulfur-bottom denims were amplified in six additional STR markers—HUMTPOX, HUMDHFRP2, HUMFGA, HUMF13A01, HUMFES/FPS, and HUMCSF1PO. DNA typing of bloodstained pure indigo denim yielded a full STR profile across the six markers, while partial and null profiles were generated from bloodstained sulfur-top and sulfur-bottom denims, respectively (Table 4).

DISCUSSION

To find the most suitable procedure for isolating DNA from bloodstained denims for forensic applications, five DNA extraction procedures were evaluated and compared in terms of DNA quantity and quality. Sufficient DNA was recovered from bloodstained

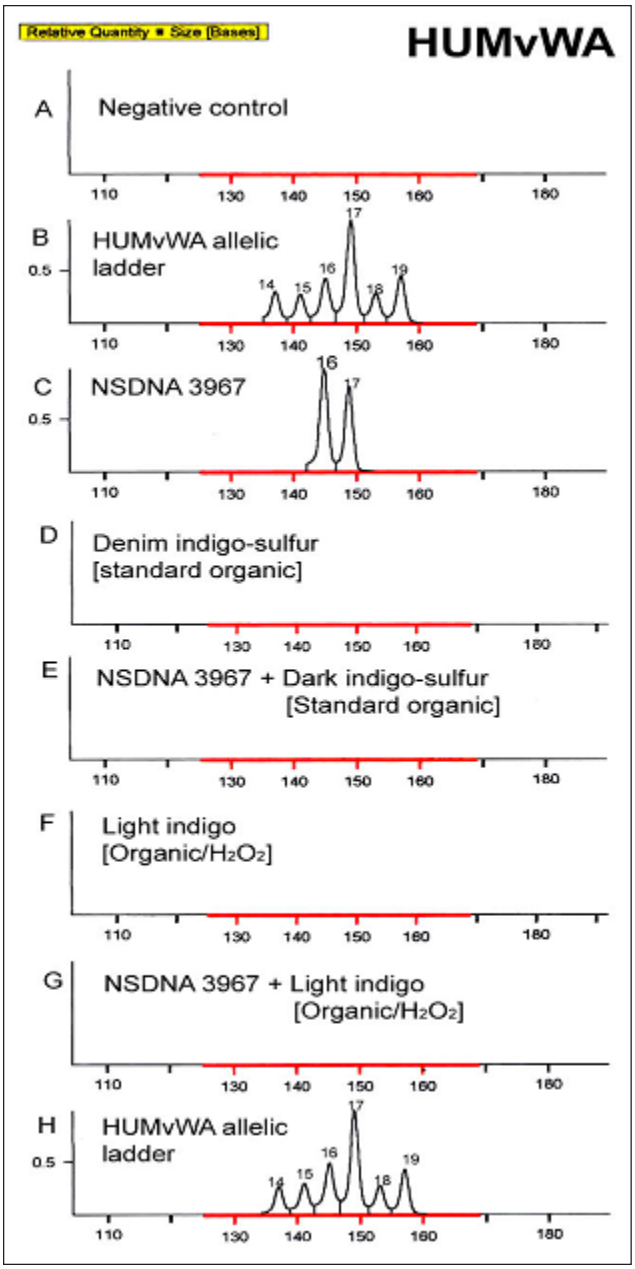

Fig. 3A. *Electrophoregram of allele peak signals at HUMvWA resulting from amplification of DNA eluted from bloodstained dark indigo-sulfur denim and light indigo denim using organic-based methods.*

Shown in the figure are two distinct peaks of control NSDNA 3967 resulting from positive DNA amplification (panel C). Adding an equal volume of DNA extracts from bloodstained dark indigo-sulfur denim extracted using standard organic procedure (panel E) and light indigo denim extracted using organic/H_2O_2 method (panel G) in the PCR mix resulted in negative amplification which indicate the presence of inhibitors in organic extracts.

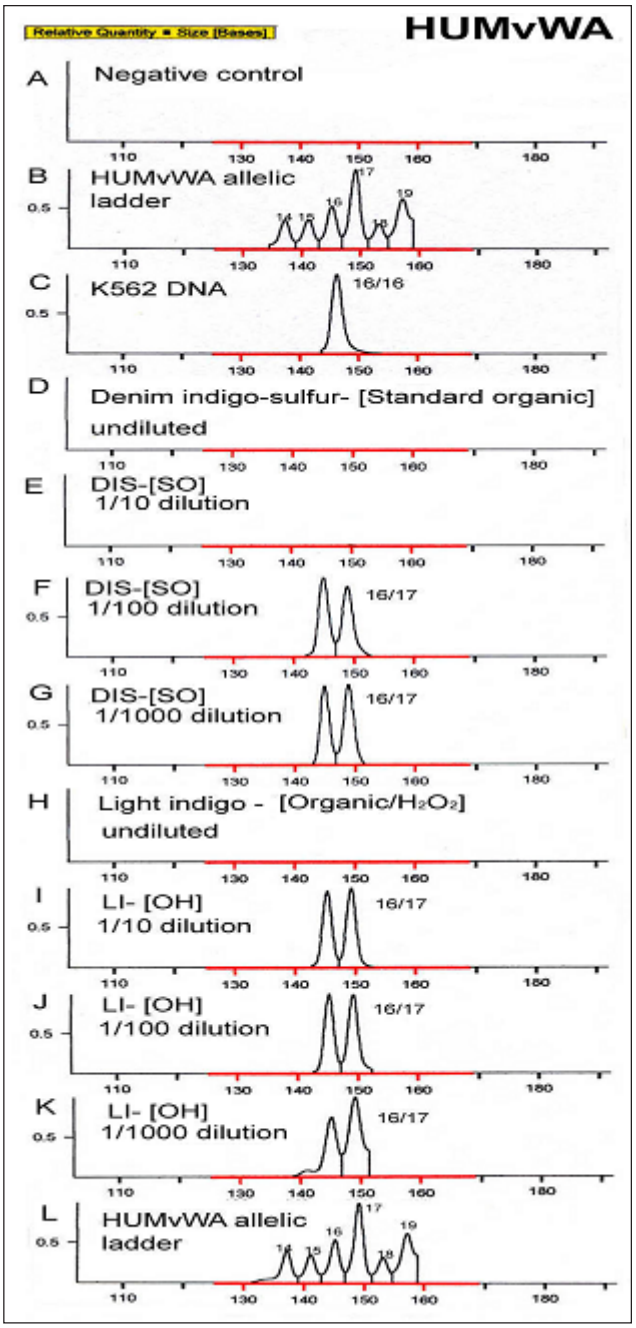

Fig. 3B. *Electrophoregram of allele peak signals at HUMvWA resulting from amplification of serial dilutions of DNA eluted from bloodstained dark indigo-sulfur denim and light indigo denim using organic-based methods.*

Shown in the figure are extracts (described in Figs. 1 & 3A) that previously failed to amplify in the presence or absence of control DNA (panels D & H) but successfully amplified upon serial dilutions with TE^{-4} buffer 10 to 1000 fold (panels E-G & I-K). Dilution significantly reduces the amount of inhibitors in the DNA solution, hence, successful DNA amplification was achieved.

Table 3. Rate of successful amplification of DNA from different types of bloodstained denim extracted using five procedures.

STR Marker	Rate of successful amplification across five extraction methods (%)				
	Standard Organic	Organic/ H_2O_2	Modified FTA™	Organic/Chelex® Centricon®	QIAamp® Kit (Qiagen, Inc.)
Phase I					
HUMvWA	66.7	33.3	100	100	100
HUMTH01	33.3	33.3	100	100	100
Phase II					
HUMvWA	ND	ND	33.3	66.7	66.7
HUMTH01	ND	ND	66.7	66.7	66.7
D8S306	ND	ND	33.3	66.7	66.7

Where: Phase I samples–light indigo, dark indigo and dark-indigo sulfur; Phase II samples–pure indigo, sulfur-top, sulfur-bottom; ND = not done; Success rate is expressed as $(n_{success}/n_t) \times 100$, where $n_{success}$ = number of times DNA successfully amplified and n_t = total number of amplifications made

denim samples (60-1000 ng of human DNA) per 100 µL of bloodstain using the five extraction methods. For the same amount of sample applied onto the denim, variation in the amounts of DNA recovered were observed across procedures and samples indicating that differences exist among the five DNA extraction procedures in terms of extraction efficiency which in turn is affected by the physical/chemical properties of each denim sample. Hence, our results demonstrate the need to validate procedures for analyzing different forensic samples on various types of substrate.

DNA concentration and yield were compared across the four procedures except the modified FTA™ procedure since quantity could not be empirically determined with bound DNA on FTA™ card. Organic/Chelex®-Centricon® produced the highest DNA yield, followed by the standard organic and organic/H_2O_2 and QIAamp® Kit procedures. Theoretically, one 2-mm FTA-extracted punch of blood sample with a typical white blood cell count contains 83-100 ng DNA.

In this study, the success rates of DNA amplification obtained using the five methods were determined at selected STR markers to evaluate the quality of extracted DNA. The low success rates of amplification consistently obtained when amplifying DNA extracted using standard and organic/H_2O_2 procedures during the Phase I experiment indicate the presence of inhibitors in these extracts which was confirmed in three ways. First, Quantiblot results revealed that some organic DNA solutions showed negative amplification although positive for human DNA with concentrations sufficient for DNA amplification, e.g., bloodstained dark indigo denim (Figs. 1 & 3B). The yield of QIAmp® extraction was lower than that obtained using organic methods and yet QIAamp® extracted DNA consistently amplified, thus, indicating the high quality of DNA in solution. In a second set of experiment, the presence of PCR inhibitors in organic extracts was further demonstrated when amplifications were subsequently achieved upon 1/10 to 1/1000 dilutions of organic extracts in TE^{-4} buffer. Diluting liquid DNA template effectively reduced the amount of inhibitors per given volume, leading to successful PCR amplification (Butler, 2001). In addition, amplification of control DNA (NSDNA 3967) was unsuccessful when an equal volume of organic extract was added in the PCR mixture. Direct observation of blue-colored precipitate in the QIAamp® membrane during purification process also shows that indigo dye co-extracts with DNA in aqueous solution which may have inhibited amplification.

The problem of inhibition was not encountered with the modified FTA™, organic/Chelex®-Centricon®, and QIAamp® Kit procedures. Notably, these procedures are all membrane or filter-based methods which involve cell lysis and selective capture of DNA on a membrane or filter, followed by DNA purification and concentration steps. In contrast, organic methods relied on the cell lysis and release of DNA in the liquid

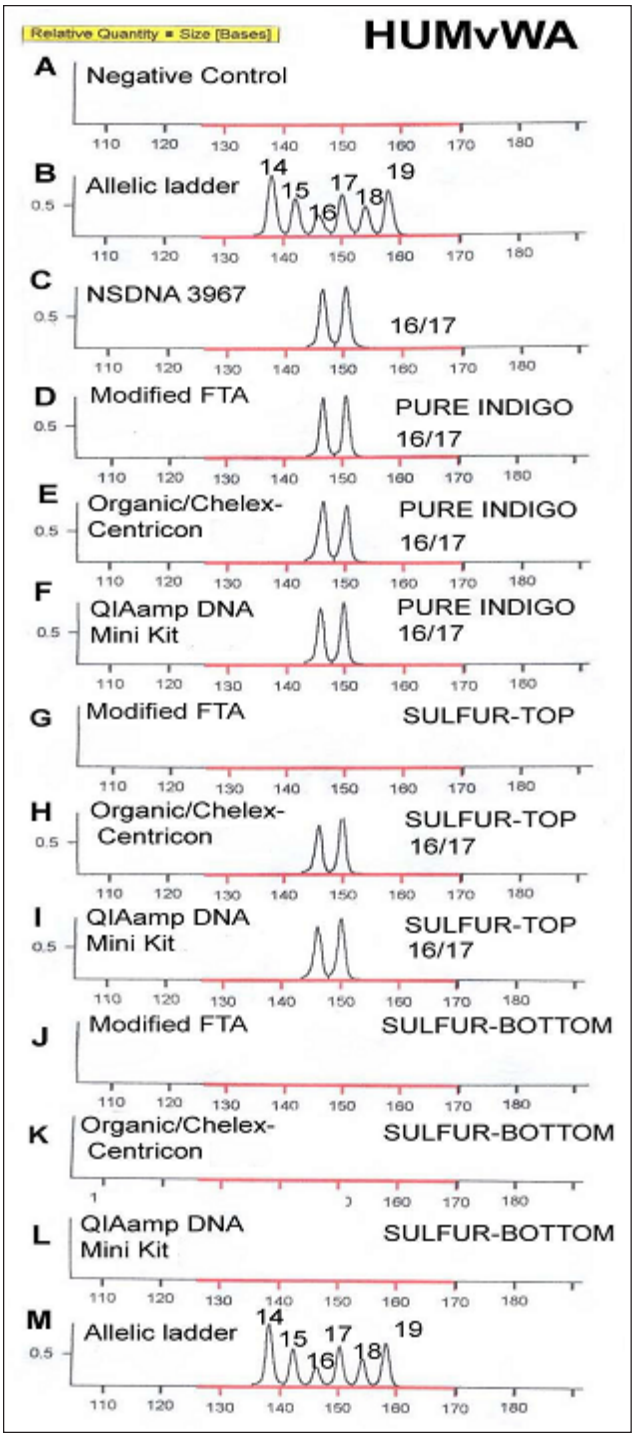

Fig. 4. *Electrophoregram of allele peak signals at HUMvWA resulting from amplification of DNA eluted from bloodstained pure indigo, sulfur-top and sulfur-bottom denims using the modified FTA™, organic/Chelex®-Centricon® and QIAamp® Kit procedures.*

Shown in the figure are allele peaks obtained from modified FTA®-prepared DNA that were distinct and comparable with those of organic/Chelex®-Centricon® and QIAamp® Kit procedures in yielding PCR-amplifiable DNA from bloodstained pure indigo denim (D-F) but not from bloodstained sulfur-top denim (G-I). The absence of amplification in bloodstained sulfur-bottom denim using the three procedures is clearly shown in panels J-L.

suspension without any substrate to bind to, followed by subsequent DNA purification and concentration by serial addition of chemicals. Hence, our data indicate that capturing DNA with a substrate, e.g., column or filter, may be more efficient in purifying inhibitors from bloodstained denim than plain liquid extraction.

Aside from the problem of inhibition observed in organic-based methods, the disadvantages of using organic-based methods are: (1) the methods are time-consuming; (2) they involve the use of hazardous and toxic chemicals (i.e., phenol and chloroform, which present the additional problem of organic waste disposal); and (3) they involve multiple tube transfer of liquids, which may increase the risk of error or contamination. Thus, standard organic and organic H_2O_2 methods were excluded in the Phase II experiment where the modified FTA™, organic/Chelex®-Centricon®, and QIAamp® Kit procedures were further evaluated using another set of bloodstained denims.

The efficiency of DNA extraction using QIAamp® spin columns is based on the optimal binding of high molecular weight DNA to the silica-gel membrane inside the column as well as the salt and pH conditions in the lysate that ensure proteins and other contaminants which can inhibit PCR and other downstream enzymatic reactions, are not retained in the membrane (QIAamp® DNA Mini Kit Handbook). The use of Chelex® 100 effectively removes PCR inhibitors in crude organic DNA extracts by chelation of polyvalent metal ions such as iron cations (associated with hematin in blood) and magnesium ions (that activate DNA degrading enzymes) with iminodiacetate ions of the styrene divinylbenzene co-polymers (Walsh et al., 1991; Jung et al., 1991; Butler, 2001). Subsequent processing of Chelex-purified DNA by passing through Centricon® filter devices greatly improves DNA quality by providing additional concentration and desalting of DNA solution by ultrafiltration. However, both procedures require multiple liquid transfers and are very expensive.

Table 4. Rate of successful amplification of DNA from different types of bloodstained denims extracted using the modified FTA™ procedure.

Denim	Rate of successful amplification in six STR markers (%)					
	TPOX (n_t=2)	DHFRP2 (n_t=2)	FGA (n_t=2)	F13A01 (n_t=2)	FES/FPS (n_t=2)	CSF1PO (n_t=2)
Pure Indigo	100	100	100	100	100	100
Sulfur-top	100	0	100	50	0	0
Sulfur-bottom	0	0	0	0	0	0

This paper presents a possible alternative procedure for handling bloodstained denims. A modified procedure involving FTA™ cards for DNA isolation described here was successful when bloodstained light indigo, dark indigo, and dark indigo-sulfur denims were analyzed. The effectivity of this method is that DNA is isolated immediately upon contact of the sample on the dry solid matrix of the FTA™ paper impregnated with chelators, denaturants, and free-radical traps which lyse cell membranes, denature proteins, and immobilize the DNA in the FTA™ matrix; thus, DNA is protected against chemical and/or biological degradation (Whatman® BioScience, MA, USA). Differences in the success of DNA amplification between the modified FTA™, organic/Chelex®-Centricon®, and QIAamp® Kit procedures were noted in the analysis of the second set of bloodstained denims particularly sulfur-top and sulfur-bottom denims. Organic/Chelex®-Centricon® and QIAamp® Kit procedures were successful in typing DNA from bloodstained sulfur-top denim in three STR markers (100%), which was not achieved with the modified FTA™ procedure (33.3%). Hence, modified FTA™ procedure may be regarded as secondary or alternative to organic/Chelex®-Centricon and QIAamp® Kit procedures, which both obtained the same results in all markers.

The complete amplification of bloodstained pure indigo denim and the variable amplification across 9 STR markers of modified FTA™-prepared DNA from bloodstained sulfur-top denim and sulfur-bottom denim using either modified FTA™, organic/Chelex®-Centricon®, or QIAamp® Kit procedures support possible co-extraction of sulfur dye with the DNA in solution and subsequently inhibit PCR reaction as reported in previous studies (De Ungria et al., 1999). Sulfur dye is produced by fusing organic chemicals with sulfur; hence, sulfur and/or any organic chemical components may likely to affect the activity of the Taq polymerase. Caution must therefore be taken in handling samples such as bloodstained sulfur-top and sulfur-bottom denims.

Nonetheless, the use of FTA™ cards for isolating DNA from bloodstained pure indigo denim as demonstrated here provided an attractive alternative to the more expensive organic/Chelex®-Centricon® and QIAamp® Kit procedures. One extraction using the modified FTA™ procedure costs less than half of the expenses in purchasing Chelex® resin and Centricon® columns, or QIAamp® kits. In addition, modified FTA™ procedure is a good alternative to organic/Chelex®-Centricon® and QIAamp® Kit procedures since it is (1) *simple and rapid*, involving few extraction steps and no quantitation step as DNA is immobilized in the paper; (2) *health and environment- friendly*, not involving the use of highly toxic chemicals which also introduces the problem of waste disposal, e.g., phenol and chloroform; (3) *not prone to errors and contamination*, requiring only one tube throughout extraction; and (4) *cost-effective*, requiring minimal storage space at room temperature.

CONCLUSION AND RECOMMENDATION

The data presented in this study demonstrate that the success of DNA extraction and subsequent analysis depends on the selection of an appropriate DNA extraction procedure.

Of the five extraction procedures evaluated, organic/ Chelex®-Centricon® or QIAamp® DNA Mini Kit procedures proved to be the most suitable DNA extraction procedures for PCR-based STR analysis of bloodstained denims for forensic applications. Alternatively, the modified FTA™ procedure provides a simple, rapid, less expensive, and health and environment-friendly means for successful DNA typing of bloodstained light indigo, dark indigo, dark indigo-sulfur, and pure indigo denims. Future development of this method to effectively prevent sulfur dye in sulfur-containing denims from co-purifying with DNA is recommended. Hence, further work to optimize procedures that incorporate its use is currently underway.

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