TECHNICAL NOTE

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QIAamp Spin Columns as a Method of DNA Isolation for Forensic Casework*

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ABSTRACT: The Detroit Police Crime Lab has historically used Chelex as a method to isolate DNA for amplification and typing of bloodstains at the HLADQA1, PM and D1S80 loci. However, preliminary validation of several STR systems for casework has demonstrated that the Chelex procedure is not the best method of DNA isolation for STR amplifications for our purposes. Long term storage at −20°C in the presence of unbuffered Chelex beads (approximately 1 year), combined with multiple freeze thaws, resulted in signal loss at a locus for many database samples. Therefore, we have employed the QIAamp spin column as an alternative method of DNA isolation for amplification and typing of STR loci currently being validated for use in the laboratory. Moreover, we determined that QIAamp isolated DNA is also suitable for HLADQA1, PM and D1S80 typing. A matrix study was performed to determine if the QIAamp DNA procedure would give better results on bloodstains deposited on ''problem surfaces'' such as leather, dirt and various dyed fabrics. Again, QIAamp isolated DNA was more readily typeable than Chelex isolated DNA.

We successfully replaced the phenol/chloroform extraction steps utilized in our laboratory for differential extractions, a commonly used method for separating sperm and non-sperm fractions of sexual assault evidence, with the QIAamp spin columns. The QIAamp extracted DNA performed as well in all PCR amplification and typing procedures tested (PM, HLADQA1, D1S80, and STR (PowerPlex)) as the phenol/chloroform Centricon isolated or EtOH precipitated DNAs. Thus we concluded that QIAamp spin columns are a superior method for isolating DNA to be typed for a variety of loci.

KEYWORDS: forensic science, DNA typing, Polymarker, HLADQA1, short tandem repeats, D1S80, DNA extraction, differential extraction, PowerPlex, LDLR, GYPA, HBGG, DF58, GC

Materials and Methods

Sample Collection

Blood samples were obtained from previously prepared dried blood database samples (provided by the Red Cross) that had been stored at −20°C. Matrix study samples were prepared by depositing liquid blood (preserved with EDTA) on the various surfaces, air drying and storing at 4°C for several days prior to use. Sexual assault samples used for the differential extraction experiments were obtained from adjudicated casework samples which had been stored at −70°C.

Purification of DNA

Dried Bloodstain Samples Using Chelex—DNA was isolated from dried bloodstains using Chelex (BioRad) according to the manufacturer's recommendations. DNA samples designated ''new'' were isolated using fresh Chelex, stored at 4°C and amplified within 48 h. ''Old'' DNA samples had been isolated with fresh Chelex and stored at −20°C for approximately one year and had been thawed for use several times. DNA was quantitated using the QuantiBlot kit (Perkin Elmer) according to the manufacturer's recommendations.

Dried Bloodstain Samples Using the QIAamp Spin Columns—All samples extracted using the QIAamp spin columns employed the QIAamp Tissue Kit and the extractions were performed according to the manufacturer's recommendations for dried bloodstains.

Differential Extraction Using the QIAamp Spin Columns—DNA from sexual assault samples containing a mixture of sperm and epithelial (non-sperm) cells was prepared following the commonly utilized differential extraction procedure (1–4), which initially lyses the epithelial cells and separates their DNA from the sperm cell fraction. After the 6 h to overnight lysis of the sperm cell fractions, both the sperm cell fractions and the epithelial cell fractions were split into three samples of equal volume. The tubes designated for the QIAamp extraction had an equal volume of AL Buffer (provided with QIAamp kits) added to the cell lysates for both the sperm and non-sperm fractions, were vortexed gently and incubated at 70°C for 10 min. After incubation, a 105% volume of 100% EtOH was added to each tube, the samples vortexed vigorously and then loaded onto appropriately labeled QIAamp spin columns. The QIAamp columns have a maximum capacity of 600 μL, so if the digest buffer volume combined with the AL Buffer and EtOH volumes was greater than 600 μL, the column was loaded with 600 μL sample, centrifuged at 10,000 × g for 1 min,

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the effluent discarded, then 600 μL more of the sample loaded and the process repeated until the entire digest sample was passed through the column. All the remaining steps were carried out according to the manufacturer's recommendations for dried bloodstains.

Differential Extraction Using Other Methods—Two other methods of DNA extraction were employed: a differential extraction procedure using phenol/chloroform extraction followed by Centricon column concentration as described (above references) and a differential extraction procedure using phenol/chloroform extraction followed by precipitation with 1/10 volume 3M sodium acetate (Ph 5.5), 2 volumes 100% EtOH, and 1 μL of glycogen at −70°C for 20 min (5).

Analysis of DNA

Amplification and typing of DNA at HLADQA1, PM™, and D1S80 loci were accomplished utilizing the Perkin Elmer kits as recommended by the manufacturer. Routinely, 5 ng of input DNA were used for the amplification reactions for HLADQA1 and PM™ loci and 2.0 to 2.5 ng were used for D1S80. Acrylamide gels used to analyze the D1S80 amplification products were composed of GeneAmp Detection Gel concentrate (Roche, Perkin Elmer). After electrophoresis, D1S80 gels were stained with a 1:10,000 dilution of SYBR Green I (FMC BioProducts) for 10 min in the dark, at room temperature and visualized using a Hitachi FMBIO fluorescence imaging system with a 505 nm filter.

STR loci were typed using the PowerPlex Beta Test kit (Promega) which allowed for DNA amplification at 8 loci simultaneously. Routinely 5 ng of input DNA was used for amplification in a 9600 thermocycler (Perkin Elmer), using the manufacturer's recommended amplification parameters. The amplification products were electrophoresed in Marathon Gel-Mix 4 or Gel-Mix 6 (Gibco BRL) acrylamide and the gels scanned using a Hitachi FMBIO fluorescence imaging system. Gels containing the PowerPlex loci were scanned using a 605 nm filter for the CSF1PO, TPOX, HUMTHO1, and vWA loci and a 505 nm filter for the D16S539, D7S820, D13S317, and D5S818 loci.

Results and Discussion

QIAamp Spin Column DNA Extraction versus Chelex

STR loci are highly informative genetic markers and are becoming the loci of choice for DNA typing in forensics (6–9). The use of PCR amplification (10) on minute amounts of DNA isolated from evidentiary samples, paired with the ability to type multiple loci in a single amplification reaction, make STR analysis a very powerful system for use in human identification. However, in the context of the forensic laboratory, the amplification and typing of the DNA samples may be delayed many months or even years after DNA isolation, so samples should be able to be stored for long periods of time without compromising the DNA. Chelex (11) is a very commonly used DNA isolation reagent in forensic science. Cells are gently removed from the evidentiary sample into a microfuge tube, the cells lysed in the presence of the alkaline Chelex bead suspension, and the DNA freed in the presence of chelators such that it is ready for PCR amplification. This is a rapid and useful method of DNA isolation for many currently utilized PCR-based DNA typing procedures (e.g., HLADQA1, PM and D1S80) (12–18). However, when we attempted to type our database samples for STR loci, using DNA that had been isolated using Chelex and stored in the −20°C freezer, some problems arose. The DNAs from our database samples were routinely extracted using Chelex, amplified and typed successfully for D1S80 (Perkin Elmer). When these same extracted database samples, which had been frozen for approximately one year at −20°C and had undergone several freeze thaws, were used for STR amplification reactions (PowerPlex, Promega), signal loss at a locus was observed in approximately 30% of the samples analyzed (Fig. 1). The database samples were then freshly isolated with Chelex and amplified for STR loci and no signal loss at a locus was observed.

We attempted to resolve the signal loss at a locus observed with the frozen Chelex DNA samples by boiling the samples, then vortexing and centrifuging them instead of simply vortexing and centrifuging them prior to amplification. However, this greatly exacerbated the problem of signal loss at a locus, so that frequently the entire amplification reaction became unsuitable for STR typing (Fig. 2). Typically, those reactions where signal loss at a locus was observed when the Chelex was not boiled showed the loss of all alleles at all loci when that same Chelex DNA preparation was boiled. Conversely, in the STR reactions where QIAamp columns were used to isolate the DNA, no signal loss at a locus was observed (Figs. 1 and 2).

QIAamp spin columns (QIAgen Inc.), supplied a quick and easy DNA isolation method that provided a solution to the problem of signal loss at a locus. We have extracted over 500 database samples using the QIAamp columns and have stored the samples frozen at −20°C. Some of these samples have been stored frozen for over 1 1/2 years, where they have undergone multiple freeze-thaws. Ninety-eight of these same samples have been reliably typed for the PowerPlex STRs without a single instance of signal loss at a locus (data not shown, personal observations). With some Qiagen sample preparations (Fig. 1, AC11, AC12, AC13, and AC14), the vWA locus showed a somewhat reduced signal. The reason for this difference is not clear. However, the reduced signal at the vWA locus observed in Fig. 1 did not appear to be a trend among over 150 QIAamp extracted database samples which have been typed for the PowerPlex loci and recently reviewed (data not shown, personal observations). Ninety-eight of the 150 samples had been frozen for over 1 1/2 years.

Since QIAamp spin columns appeared to improve the quality of the DNA used for STR amplification, the next questions addressed were whether QIAamp purified DNA could be amplified and typed for HLADQA1, Polymarker and the AmpliFLP D1S80 loci as successfully and accurately as DNA isolated using Chelex. In Panels A, B and C (Fig. 3), HLADQA1 and PM™ loci, as well as D1S80 analyses were performed on DNA extracted from database samples isolated using either Chelex or QIAamp techniques. The quality of the DNA typing results was virtually identical for all three typing procedures and from either DNA isolation method.

A matrix study was performed in which blood was deposited on a variety of surfaces, many of which can inhibit PCR amplification or cause degradation of the DNA so that it is difficult to obtain the necessary PCR products. Liquid blood was deposited on the various surfaces (Table 1) and allowed to dry overnight. The blood sample was removed from the surface, extracted using either Chelex or QIAamp, amplified, and then typed using the PM/HLADQA1 kit. The results were graded as to the quality of the typing. QIAamp purified DNA supplied a larger number of high quality typing results for the PM and HLADQA1 loci than did the Chelex extracted DNA. Moreover, there were a variety of

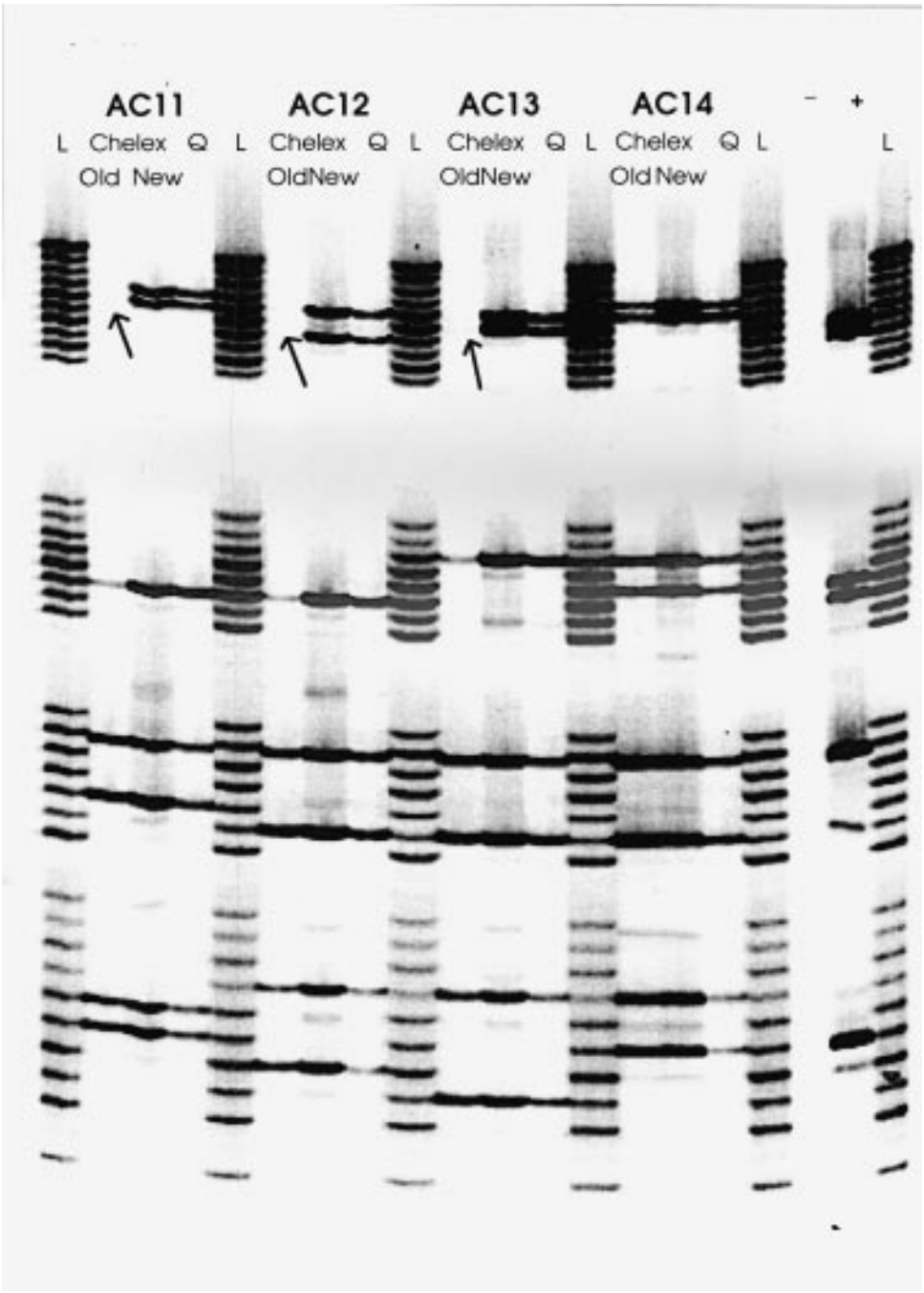

FIG. 1—*Comparison of DNA isolated from bloodstains by Chelex, old and new, and the QIAamp procedure. The CTTV quadriplex is shown (605 nm scan). 5 ng of DNA were used for the STR reactions using the PowerPlex kit (Promega). The amplified DNAs were electrophoresed in a Marathon Gel-Mix 4 acrylamide gel and visualized using the FMBIO-100 (Hitachi) fluorescence imaging system. AC = American Caucasian database sample; Q = QIAamp isolated DNA; + = positive control (kit); − = negative control; and L = ladder. Arrows indicate signal loss at a locus.*

''problem'' surfaces, for example grass, which provided an uninterpretable result (no discernable C or S dot) for Chelex extracted DNA, but provided an interpretable result when the same sample was extracted using QIAamp. Therefore, we concluded that the QIAamp isolated DNA typed more consistently than the Chelex isolated DNA on a variety of problem surfaces.

Differential Extractions and the QIAamp Spin Columns

The most commonly used differential extraction method employs cumbersome phenol/chloroform extractions followed by Centricon column concentration of the DNA sample (1–3). We wished to improve upon this technique by simply replacing these steps with the QIAamp spin column procedure. This was accomplished by integrating the QIAamp spin column procedure for tissue and dried blood samples into the differential extraction process, after cell lysis and separation, thereby removing the organic extraction/concentration steps. Vaginal swab samples from three independent, adjudicated cases were extracted using the phenol/chloroform Centricon (PC/Centricon), the phenol/chloroform 3M NaOAC/EtOH precipitation (PC/EtOH) and the

QIAamp spin column procedures (described in Materials and Methods). The DNA was typed for PM, HLADQA1, D1S80, and STR loci by two different analysts. The epithelial (non-sperm) and sperm cell fractions were isolated and were each divided into three equal volume cell suspensions prior to DNA extraction so that approximately an equal numbers of cells would be extracted in parallel using the three techniques listed above.

All of the DNA typing methods, PM, HLADQA1, D1S80 and STR PowerPlex provided virtually identical results for the three independent vaginal swab samples (Fig. 4, only D1S80 shown, PM, HLADQA1 and STR data not shown). The D1S80 gel shown (Fig. 4) demonstrates that all three DNA isolation procedures produced qualitatively similar DNA typing results. Table 2 displays the estimated yields generated from the three sexual assault samples using the three different isolation techniques. All yields for the isolated DNA were determined using the Quanti-Blot kits (Perkin

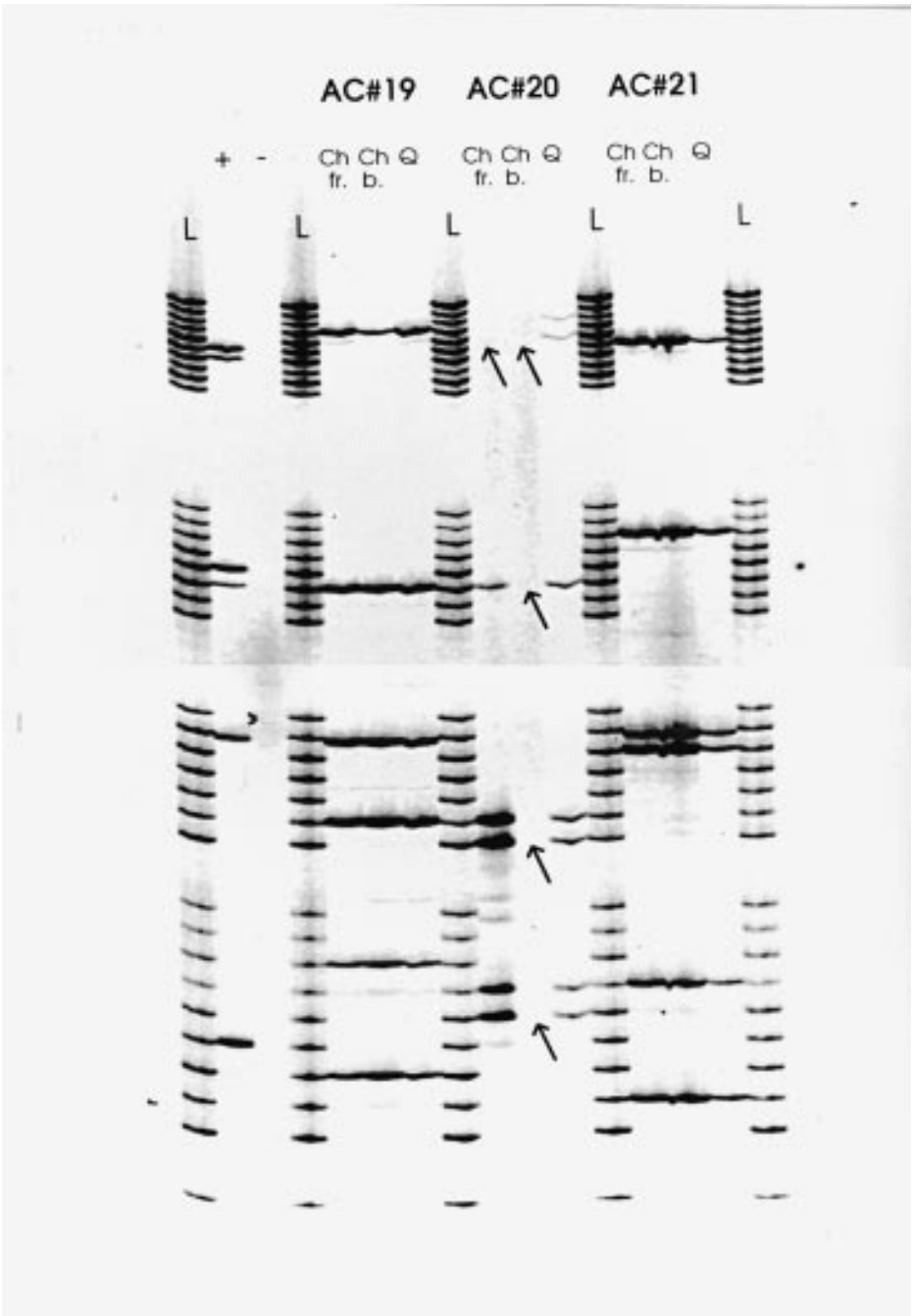

FIG. 2—*Comparison of CTTV STR amplification products using previously frozen Chelex isolated DNAs and frozen QIAamp isolated DNAs. Previously frozen Chelex isolated DNA (Ch.fr.), the same Chelex preparation, but boiled, vortexed and centrifuged prior to amplification (Ch.b.), and DNA isolated from the same dried bloodstain, but using QIAamp columns (Q), were amplified and analyzed in parallel for comparison purposes. Three Caucasion database bloodstain samples were used for the comparisons (AC#19, AC#20 and AC#21). Samples were electrophoresed in a Gel-Mix 6 acrylamide gel and analyzed using a Hitachi FMBIO (605 nm scan). AC = American Caucasian; + = positive control; − = negative control; and L = ladder. Arrows indicate signal loss at loci.*

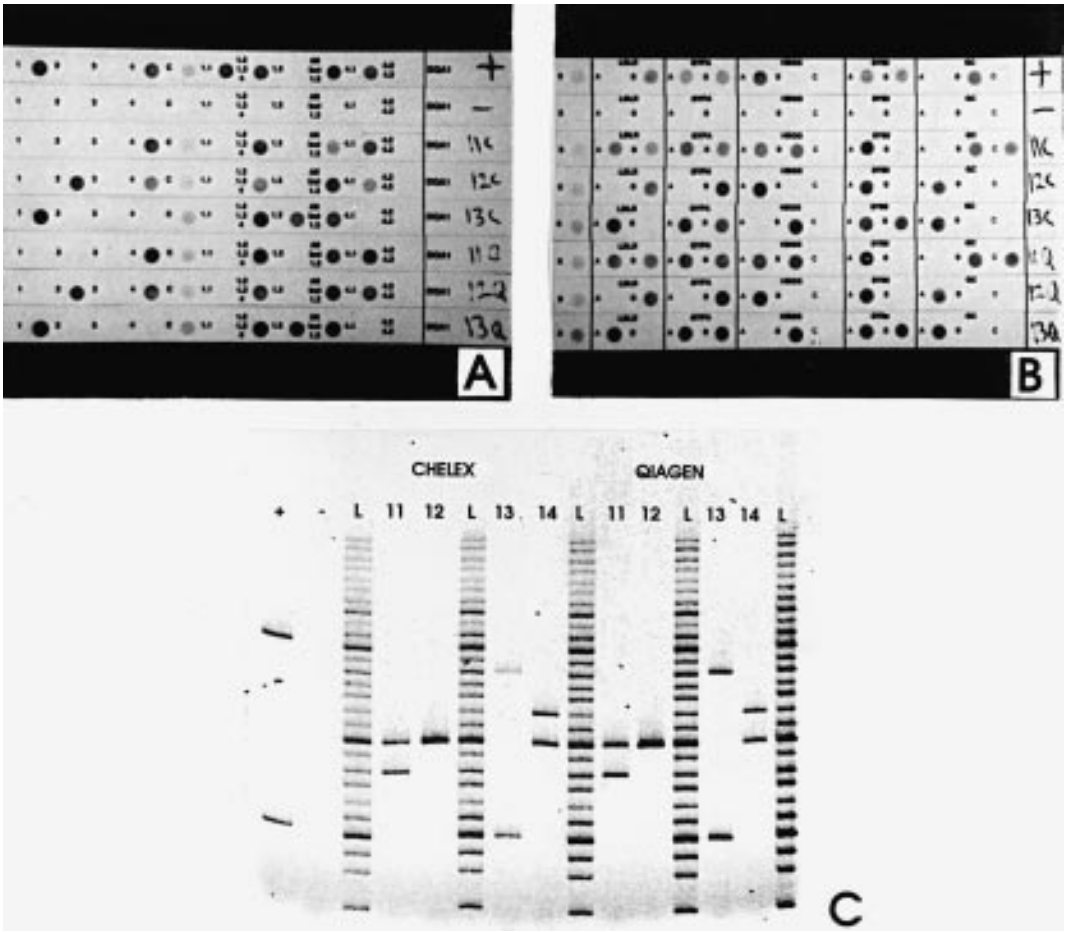

FIG. 3—*QIAamp versus Chelex-HLADQA1, PM and D1S80 typing. Population database samples were typed at the HLADQA1, Polymarker and D1S80 loci using template DNA isolated by the QIAamp and Chelex protocols. Panel A, DQA1. Panel B, PM. Panel C, D1S80. C = Chelex; Q = QIAamp; + = positive control; − = negative control; and L = ladder.*

TABLE 1—*Matrix study. A liquid blood sample (containing EDTA) was deposited on the surfaces listed. The samples were air dried overnight. DNA was isolated using the Chelex and QIAamp methods.*

	DQA1		PM	
Surface	Chelex	QIAamp	CHELEX	QIAamp
Cotton	4	4	4	4
Polyester	3	4	4	4
Hard leather	X	X	X	0
Soft leather	2	4	3	4
Wood	3	3	4	4
Painted wood	3	3	4	4
Vinyl	4	4	4	4
Carpet-home	1	4	1	3
Carpet-car	4	4	3	3
Dirt	X	X	0	X
Blue jeans	1	3	3	3
Grass	0	2	0	1
Wet pavement	4	4	3	4
Floor tile	1	4	0	3

Scores are as follows: X: Typing dots not visible; 0: C/S dot not visible; 1: Barely interpretable result; 2: Interpretable result; 3: Easily interpretable; dark dots; 4: Readily interpretable; very dark dots.

Elmer). Though the Quanti-Blot kits provide an estimate of yield, it cannot accurately discriminate less than a 2-fold difference. Therefore, we can conclude that in one of the three samples (#357), the Qiagen column method produced a greater yield of sperm cell DNA than the PC/Centricon or PC/EtOH methods. However, the non-sperm fraction DNA yield was consistently greater for all samples isolated using the Qiagen and PC/Centricon methods, than for the PC/EtOH isolated preparations. Overall, the DNA isolated from sexual assault samples using the QIAamp spin columns functioned as well as the PC/Centricon and PC/EtOH methods for commonly typed DNA loci used for forensic casework. Moreover, the phenol/chloroform extraction step was eliminated from the differential extraction procedure. The QIAamp column method provided a yield that was at least equivalent and occasionally greater than that generated using either of the other two methods. Additionally, we found the QIAamp differential extraction procedure was simpler and slightly quicker to perform than the other two methods.

Conclusion

Forensic samples submitted to a crime lab are frequently contaminated with substances which promote degradation of the DNA such as dirt, are found on a variety of different substrates, and may contain mixtures of different cell types. These potential obstacles can sometimes prevent informative DNA analysis. Innovative cell

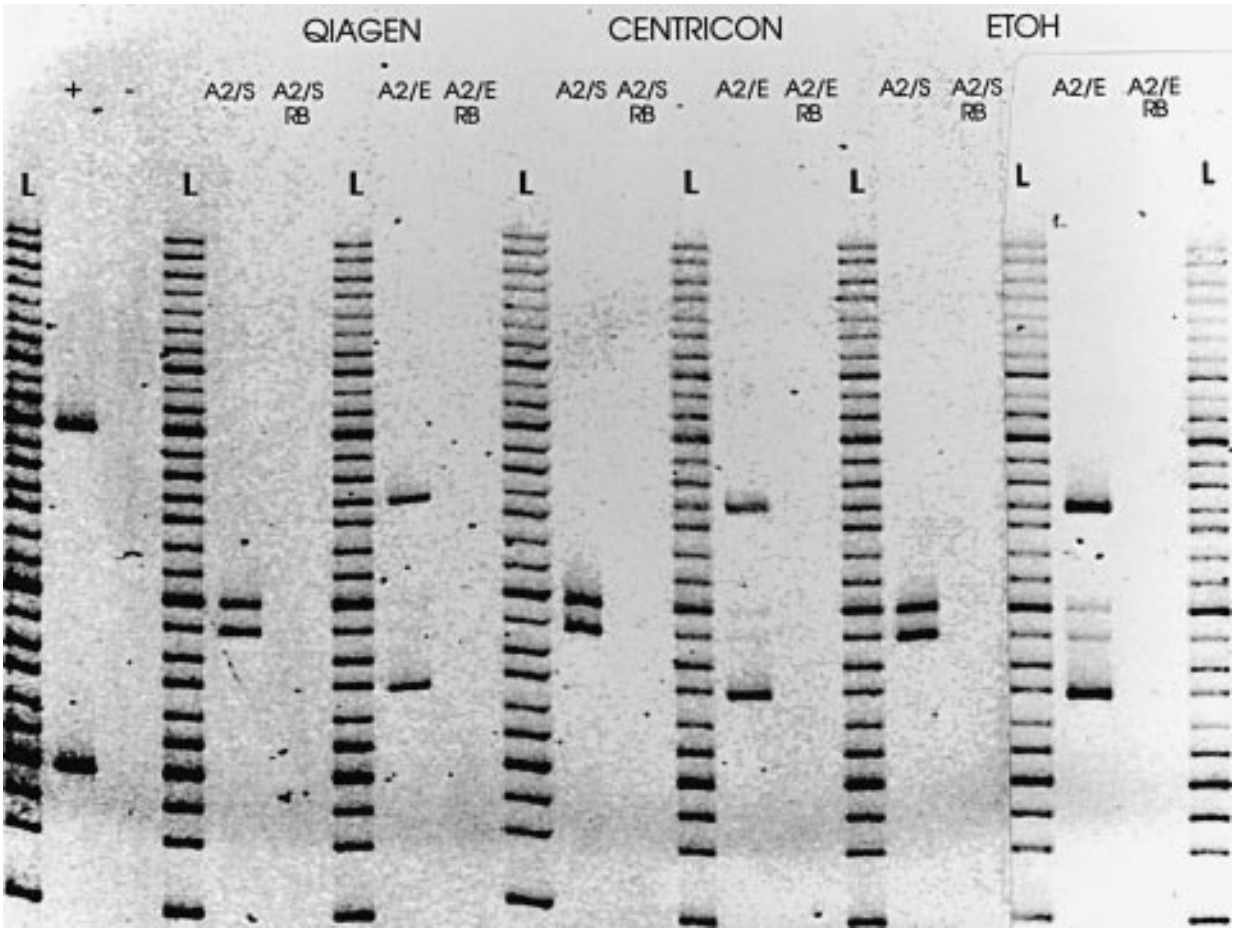

FIG. 4—*D1S80 analysis performed on QIAamp, PC/Centricon and PC/EtOH isolated differential extraction samples. D1S80 analysis was performed on a sexual assault sample (#183) which was divided into three equal volume cell suspensions and the DNA extracted using either QIAamp spin column, PC/Centricon or PC/EtOH methods. A2/S = sperm fraction; A2/E = epithelial (non-sperm) fraction; RB = reagent blank; L = ladder; + = positive control; and − = negative control.*

TABLE 2—*Comparison of DNA yields using the QIAamp column, PC/Centricon and PC/EtOH precipitation techniques. Sample numbers 183, 274 and 357 refer to different adjudicated casework samples. Concentrations were derived using the Quanti-Blot kit (Perkin Elmer) and volumes were measured using a pipettor (Rainin) in order to estimate total yields. ng = nanogram; μL = microliter.*

Sample No.	Procedure	Sample	Total Yield
183	Qiagen	A2/Sperm fraction	≃100 ng
183	Qiagen	A2/Non-sperm fraction	≃100 ng
183	Centricon	A2/Sperm fraction	≃100 ng
183	Centricon	A2/Non-sperm fraction	≃100 ng
183	Etoh	A2/Sperm fraction	≃100 ng
183	Etoh	A2/Non-sperm fraction	<40 ng
274	Qiagen	A2/Sperm fraction	≤100 ng
274	Qiagen	A2/Non-sperm fraction	≥100 ng
274	Centricon	A2/Sperm fraction	<20 ng
274	Centricon	A2/Non-sperm fraction	≃100 ng
274	Etoh	A2/Sperm fraction	≃100 ng
274	Etoh	A2/Non-sperm fraction	<10 ng
357	Qiagen	Sperm fraction	>200 ng
357	Qiagen	Non-sperm fraction	>100 ng
357	Centricon	Sperm fraction	≃100 ng
357	Centricon	Non-sperm fraction	>100 ng
357	Etoh	Sperm fraction	≃100 ng
357	Etoh	Non-sperm fraction	<100 ng

separation protocols, such as the differential extraction method for sexual assault samples, make the task of discriminating components of DNA mixtures easier. Chelex DNA isolation is a quick and facile method for generating amplifiable DNA from forensic samples. However, we have discovered that there are problems with Chelex isolated DNAs which have been stored for long periods of time and thawed repeatedly, especially when the DNA is to be typed using multiplexed STR loci. Moreover, we discerned that isolation of DNA with Chelex may not be the best method when attempting to type DNA removed from ''problem'' surfaces.

A troublesome problem we confronted when attempting to use previously frozen Chelex isolated DNA for STRs was signal loss at a locus. What causes the signal loss at a locus with some of the Chelex isolated samples is unknown. Long-term storage of frozen DNA samples in the presence of an unbuffered suspension of the Chelex beads, combined with multiple freeze-thaws, could accelerate degradation of the DNA. Alternatively, breakdown of the Chelex itself due to multiple freeze-thaws may adversely affect the multiplex STR reactions we studied. Interestingly, when the Chelex isolated STR amplifications which demonstrated the signal loss at a locus were boiled, all loci were lost. This suggests that boiling the already compromised DNA preparation exacerbated the problem leading to an inability to amplify the DNA. We have observed signal loss at a locus on one other occasion and only

with K562 DNA amplified in the 9600 thermoclycler. K562 DNA contains imbalanced alleles at several loci due to unusual chromosomal content (*GenePrint™ PowerPlex™ Technical Manual*, 1/97) and that is the most likely explanation for the observed signal loss at a locus.

Signal loss at a locus results in the loss of both alleles at a particular locus, while allelic dropout is the loss of one allele at a locus, usually the larger of the two (19). It has been previously demonstrated that too little genomic DNA in the amplification reaction, highly degraded DNA, Taq inhibitors and too low a denaturation temperature can cause allelic dropout (20). It is possible that when any of the above conditions is particularly excessive, it results in complete signal loss at a locus rather than allelic dropout. It has been our observation that the largest locus contained within the multiplex is the first signal lost. By using the QIAamp spin column method of DNA isolation, we have rectified the problem of signal loss at a locus that we encountered when using frozen stored Chelex extracted DNA preparations for STR amplification.

When QIAamp spin columns were incorporated into the differential extraction procedure, the DNA performed as well for commonly typed DNA loci (HLADQA1, PM, D1S80, and PowerPlex STR loci) as the more standard differential extraction methods (PC/Centricon and PC/EtOH) and provided a roughly equivalent and sometimes greater yield. Moreover, the phenol/chloroform extraction step was unnecessary and the QIAamp method took slightly less time to perform. Thus we found the QIAamp column method was a facile and, in some instances, slightly quicker method of DNA extraction, which alleviated the problem of signal loss at a locus and which could be reliably used in place of the more standard procedures.

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