TECHNICAL NOTE

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A Simple Automated Instrument for DNA Extraction in Forensic Casework*

ABSTRACT: The Qiagen BioRobot EZ1 is a small, rapid, and reliable automated DNA extraction instrument capable of extracting DNA from up to six samples in as few as 20 min using magnetic bead technology. The San Diego Police Department Crime Laboratory has validated the BioRobot EZ1 for the DNA extraction of evidence and reference samples in forensic casework. The BioRobot EZ1 was evaluated for use on a variety of different evidence sample types including blood, saliva, and semen evidence. The performance of the BioRobot EZ1 with regard to DNA recovery and potential cross-contamination was also assessed. DNA yields obtained with the BioRobot EZ1 were comparable to those from organic extraction. The BioRobot EZ1 was effective at removing PCR inhibitors, which often co-purify with DNA in organic extractions. The incorporation of the BioRobot EZ1 into forensic casework has streamlined the DNA analysis process by reducing the need for labor-intensive phenol-chloroform extractions.

KEYWORDS: forensic science, forensic biology, forensic casework, deoxyribonucleic acid extraction, Qiagen BioRobot EZ1, paramagnetic beads, automation, robotics

Forensic DNA laboratories are experiencing a demand to process an increasing number of cases and evidence samples due to the successful application of DNA technology to evidence collected from a wide variety of crimes. This demand has often resulted in large case backlogs that forensic laboratories have difficulty managing. In an effort to meet the rising need for DNA analyses, laboratories have sought methods to increase throughput. Robotic sequencers are routinely used to analyze amplified samples and some forensic laboratories have implemented the use of liquid handlers for more efficient sample management (1,2). In addition, the use of large-scale automated DNA extraction instruments is becoming common in databasing laboratories and even some larger casework laboratories (3).

The DNA extraction of forensic evidence samples is a critical step in the analysis process and plays a significant role in the success of downstream applications. The wide range of samples processed in forensic casework, which contain varying amounts of DNA, require a robust DNA extraction method. The traditional DNA extraction process, which is well suited to forensic samples, uses detergent-mediated cell lysis and proteinase treatment followed by purification with organic solvents and DNA concentration through precipitation or microconcentration (4–8). This process requires multiple sample manipulations and can be time-consuming when processing large numbers of samples. This liquid phase method has previously been a challenge to automate due to the reagent volumes employed, the hazardous nature of the solvents, and the complex sample manipulations involved. Current methods of robotic DNA extractions utilize solid-phase DNA extraction techniques, which are more amenable to automation and do not require the use of organic solvents.

The Qiagen Corporation has developed an automated method for DNA extraction involving the BioRobot EZ1 workstation (Fig. 1) and magnetic bead technology. The BioRobot EZ1 workstation is a small, rapid, and reliable extraction instrument that functions using pre-programmed extraction protocol cards and single-use reagent cartridges. The BioRobot EZ1 is capable of extracting high quality DNA from up to six samples in as few as 20 min using a chaotropic extraction with paramagnetic silica bead purification.

DNA extractions on the BioRobot EZ1 employ a guanidine thiocyanate (GuSCN)/guanidine hydrochloride (GuHCl) extraction method. These chaotropic agents lyse cells, denature proteins and inhibit nucleases as well as promote the binding of DNA to the paramagnetic-silica beads (9–12). On the BioRobot EZ1, the binding of DNA to the silica beads and the wash steps occur within a barrier pipette tip. DNA bound to the silica beads is eluted in a solution of low ionic strength.

The San Diego Police Department Crime Laboratory has developed extraction procedures that incorporate the BioRobot EZ1, the EZ1 DNA Tissue Kit and the EZ1 Forensic Card for the wide
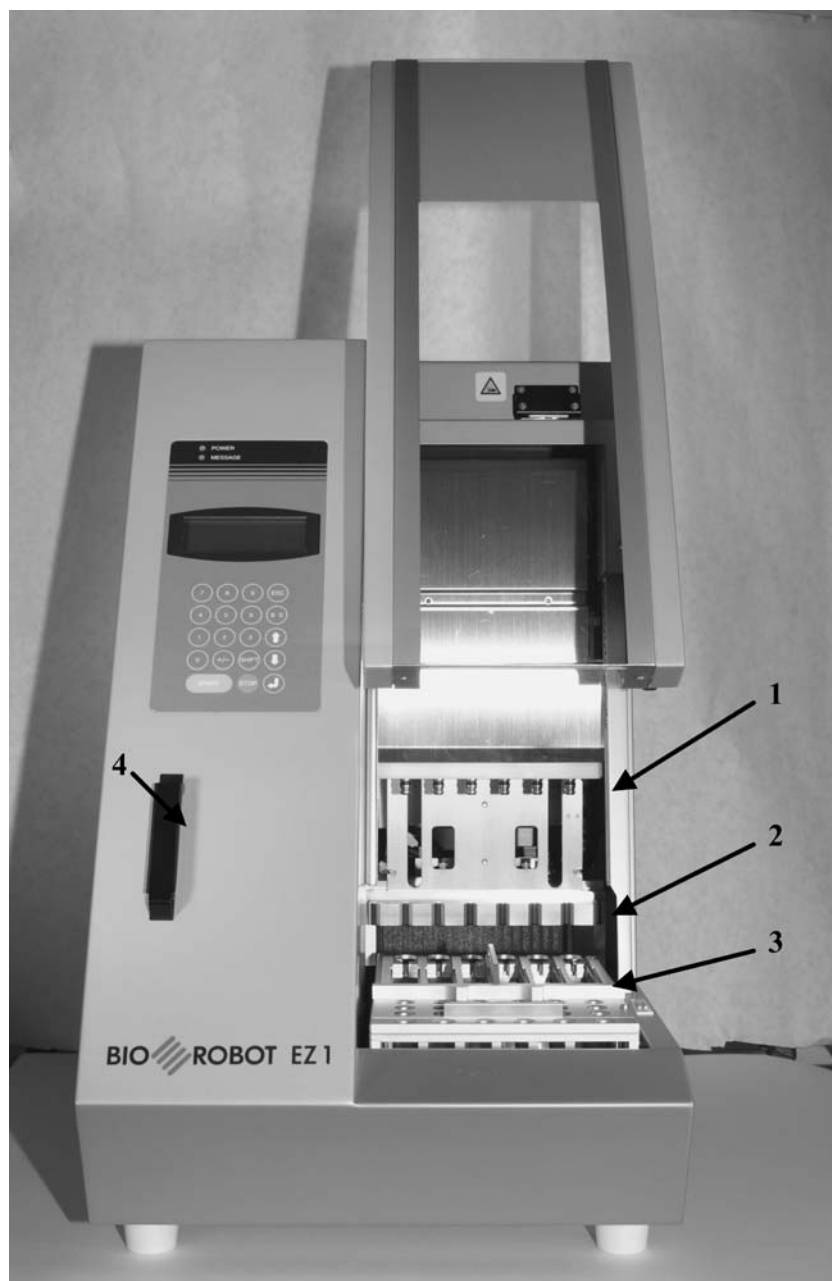
FIG. 1—*The BioRobot EZ1 workstation: 1) Six pipet heads 2) Magnet 3) Sample and Cartridge racks 4) Protocol card slot.*

range of evidence and reference samples typically encountered in forensic casework. The BioRobot EZ1 workstation was evaluated for the ability to obtain reproducible results from evidence and reference samples on a variety of substrates. Experiments were conducted to determine the efficiency of DNA recovery using the BioRobot EZ1. Comparisons were made between yields obtained with BioRobot EZ1 extractions and the traditional organic extractions employed at the San Diego Police Department Crime Laboratory. Possible sample-to-sample contamination both within and between extraction runs was investigated. The effect on DNA yield of eluting purified DNA in the different volumes offered by the protocol card was evaluated. Finally, the BioRobot EZ1 workstation was found to be an invaluable tool in removing PCR inhibitors that joften co-purify with DNA in the standard organic extraction method.

Materials and Methods

Evidence bloodstains, fetal tissue, urine and reference samples (typically cuttings of dried bloodstains on S&S filter paper, liquid blood, or reference mouth swabs) were pre-treated with 190 μL digest buffer (10 mM Tris-HCL, pH = 7.5, 10 mM EDTA, 50 mM NaCl, 2% SDS (w/v) in distilled water) and 10 μL proteinase K (10 mg/mL) for at least 1 h at 56°C. If applicable, the substrates were removed and the absorbed liquid was collected by means of spin baskets (Promega Corporation, Madison, WI) prior to BioRobot EZ1 purification. BioRobot EZ1 purification of pre-treated samples was performed with the EZ1 DNA Tissue kit (Qiagen Corporation, Valencia, CA) and the "Trace" protocol on the EZ1 Forensic Protocol Card (Qiagen, Valencia, CA). A final elution volume of 50 μL was selected for DNA purified from evidence

samples. The final elution volume selected for reference samples was 200 μL.

Microscopic examinations were typically performed on saliva-based evidence such as stains, chewing gum, and cigarette filters. These samples were first washed in distilled water and the cells pelleted by centrifugation at 13 K rpm for 2–5 min, then re-suspended in approximately 50 μL of distilled water. Microscopic examinations were performed using 2–5 μL of sample. 145 μL of digest buffer and 10 μL proteinase K were then added and the samples incubated at 56°C for at least 1 h. Substrates were removed and liquid was collected via spin baskets prior to BioRobot EZ1 DNA extraction (described above) with a final elution volume of 50 μL.

Sexual assault samples were processed with a differential extraction procedure (5–8) with minor modifications made to accommodate the BioRobot EZ1 volume requirements. Distilled water washes were employed for all samples for initial microscopic examinations. The initial cell digest was performed by adding 145 μL of digest buffer and 10 μL of proteinase K to the approximate 50 μL of resuspended cells, and then incubating at 56°C for 1–2 h. After centrifugation and removal of the non-sperm fractions, a second treatment of 500 μL digest buffer and 20 μL proteinase K for 1 h at 56°C was used to ensure complete lysis of any remaining non-sperm cells in the sample. Following the standard wash steps and a second microscopic examination, the sperm fractions, consisting of approximately 50 μL, were treated with 135 μL of digest buffer, 10 μL of proteinase K, and 10 μL of DTT (1 M DTT, 10 mM Sodium Acetate, pH = 5.2) and incubated at 56°C for 1–2 h. Non-sperm fractions extracted with the BioRobot EZ1 were eluted in a final volume of 200 μL. Sperm fractions were eluted in a final volume of 50 μL.

The root ends (0.5–1 cm) of plucked hair samples were treated with 180 μL of digest buffer, 10 μL proteinase K, and 10 μL of DTT. The samples were incubated at 56°C for at least 6 h. Additional aliquots of 10 μL proteinase K and 10 μL DTT were added and the samples were incubated at 56°C for at least 2 h or until the hair samples were completely dissolved. For all hair extractions a portion of the hair adjacent to the root was extracted as a control as per SDPD protocol.

Organic extractions were carried out according to the standard method employed at the San Diego Police Department Crime Laboratory using Centricon-100 molecular filters (Millipore, Bedford, MA) for the washing and concentration of extracted DNA.

All extracted DNA samples were quantified using a slot-blot methodology with the QuantiBlot Human DNA Quantitation kit (Applied Biosystems, Foster City, CA) with chemiluminescent detection using SuperSignal West Femto Maximum Sensitivity Substrate (Pierce Biotechnologies, Rockville, IL) with the CCDBio image detection system (Syngene Technology, Frederick, MD) and supplied software.

PCR amplifications were performed using the Applied Biosystems 9700 thermal cycler (Applied Biosystems) using the AmpF*l*STR Profiler Plus amplification kit (Applied Biosystems). All amplifications targeted approximately 1.5 ng of template DNA using the manufacturer's recommended protocol. PCR products were electrophoresed using the ABI 310 Genetic Analyzer (Applied Biosystems), sized using the GeneScan™ software and genotyped using the Genotyper™ software.

DNA Recovery From BioRobot EZ1

Previously purified samples of known DNA concentration were used to create duplicate sample sets containing 7.5 ng to 200 ng total DNA in 200 μL. One sample set was then extracted using the BioRobot EZ1 without digest buffer or proteinase K pre-treatment. The duplicate sets were then quantified and the yields were compared.

Variable Elution

Replicate sets of bloodstains containing 5 μL of blood were extracted using the BioRobot EZ1 with elution volumes of 200 μL, 100 μL, and 50 μL. The DNA yields of resulting sets of purified samples were quantified and compared.

Cross Contamination

Concentrated blood samples consisting of 100 μL of liquid blood in 100 μL of distilled water were extracted on the BioRobot EZ1 interspersed with blank samples containing 200 μL of distilled water. No pre-treatment of the samples or blanks was performed for this experiment as no substrate was present. The order of the samples and blanks was reversed for a second extraction run. All samples were eluted in a final volume of 200 μL. For quantification the volume of the blank samples tested was ten times that of the blood samples. For the subsequent STR amplification, between 0.25 and 0.75% of the concentrated blood samples were amplified so that an adequate DNA profile was obtained. Ten percent of each of the blank samples, which is as much as could be accommodated in the reaction, was amplified in order to detect any possible cross-contamination.

Bloodstain Samples

Different fabrics, including a maroon bandana, dark blue denim, black denim, black cotton, black leather and a multicolored cotton, were selected based on their relevance to forensic casework and the potential to cause inhibition of the polymerase chain reaction. Three replicate sets of bloodstains were created on the different fabrics using 250 μL stains of a 1/8 dilution of blood. Three sets of cuttings (4 × 4–6 × 6 mm) were made. Two of the sets were extracted with the BioRobot EZ1 with an elution volume of 50 μL. The third set was extracted using organic extraction.

Saliva Samples

A series of diluted saliva stains [50 μL neat, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and 1/128] were created on three fabrics (maroon bandana, blue denim and a multicolor cotton) using 50 μL of each dilution. One set of cuttings was taken from each fabric with sample sizes ranging from 5 × 5 mm to 7 × 7 mm. All samples were then extracted using the BioRobot EZ1 with an elution volume of 50 μL. Additionally, a second set of samples was taken from the blue denim and processed by the standard organic method.

Cigarette butts were analyzed by sampling one-third of the paper from the filter region thought to have been in contact with the smokers' lips, and then extracting using the BioRobot EZ1 with an elution volume of 50 μL.

Three cigar butts were analyzed as evidence in a robbery case. Each cigar butt had a portion cut away for DNA analysis. The sampled portions were comprised largely of plant material, and consequently a large volume was recovered when organically extracted. One half of each of the organically extracted samples was re-purified in the BioRobot EZ1. The original and BioRobot EZ1

re-purified samples from each of the cigars were quantified then amplified and the results for each compared.

Mock Sexual Assault Samples

Replicate sets of swabs were created containing a dilution series of semen (2 μL, 1.3 μL, 0.43 μL, 0.143 μL, 0.047 μL and 0.015 μL) and a constant volume of saliva (50 μL). One set of the swabs was then differentially extracted and purified using the BioRobot EZ1. The other set of swabs was differentially extracted according to the standard SDPD protocol.

Results and Discussion

DNA Recovery From BioRobot EZ1

The DNA recovery from the BioRobot EZ1 workstation is an important consideration if the automated extraction is to be used for evidence. The previously purified DNA samples re-extracted with the BioRobot EZ1 contained on average 60–70% of the DNA in the original samples (data not shown). Previously published values for the guanidine thiocyanate/silica bead extraction indicate a 70% expected recovery (9,11). It should be noted that other factors such as the amount of cellular material removed from the substrate, the efficiency of cell lysis, or the presence of other competing biomolecules (e.g., proteins) may ultimately influence the recovery of DNA from the extraction process. In general, a loss of DNA from that theoretically expected is likely from any extraction technique since none are completely efficient (14). Further comparisons could be made between the efficiency of BioRobot EZ1 extractions and the efficiencies obtained with other DNA extraction methods.

Variable Elution

In comparing the yields of samples eluted in 200, 100 or 50 μL volumes, a lower total yield of DNA was observed when employing a 100 μL elution volume compared to the 200 or 50 μL elution volumes which were similar (Fig. 2). As expected, the overall concentration of the eluants went up with decreased elution volume. It is unclear why the recovery of DNA is compromised with the 100 μL elution volume, but this phenomenon has been documented by a second laboratory (Tine Thorbjoernsen, Qiagen Corporation; Oslo, Norway. Personal communication). The nature of the samples being extracted should be evaluated when deciding on an elution
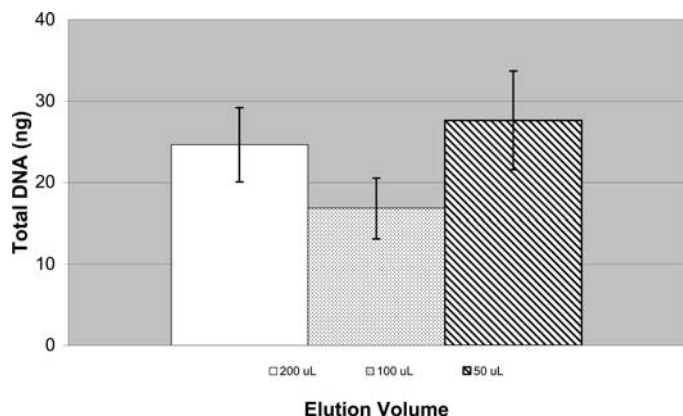

FIG. 2—*Histogram depicting the average recovery of total DNA from 5 μL of liquid blood from three elution volumes.*

volume for evidence samples, and should depend on an analyst's experience in dealing with similar evidence. The goal should be obtaining purified DNA extracts with not only sufficient DNA for downstream assays but with optimal concentrations for STR amplification. Careful consideration of the type of evidence sample should result in less sample manipulation and a decreased analysis time.

Cross-Contamination

Although the sample and elution tubes remain open during the BioRobot EZ1 extraction process no DNA was detected in the blank samples in or between extraction runs by either the quantitation of the samples or the ensuing STR amplification. Amplification of up to 400 times more of the blank samples than blood samples resulted in no detectable DNA types in the blanks (Fig. 3).

The fact that reagents are compartmentalized in single-use cartridges, the extraction process occurs within the filter tip, and tip movement is linear and does not cross open samples, all combine to greatly reduce the risk of sample-to-sample contamination. The presence of the filter barrier in the tip provides adequate protection against concentrated samples contaminating samples in subsequent extraction runs. If a sample were to pass through the filter barrier in one extraction run, the presence of a new clean filter tip in the subsequent extraction run would prevent DNA from entering any subsequent samples.

Fabrics and Potential Inhibitors

Comparisons between the DNA yields obtained from the BioRobot EZ1 and organic extractions show that phenol-chloroform is able to extract more DNA from most fabrics (Fig. 4). The difference in yields could be attributable to the greater pre-treatment volume (500 μL) used in the standard organic extraction procedure. Reduced DNA yields with magnetic bead extractions compared with organic extractions have previously been observed (17,18). However, these studies suggested that the samples purified with magnetic beads were amplified more efficiently than those with phenol-chloroform. It has been demonstrated that silica-based extractions may also provide purer samples than organic extractions (16).

Regardless, the amount of DNA recovered from the BioRobot EZ1 extractions was sufficient for STR analysis. Increasing the pre-treatment incubation time of BioRobot EZ1 extracted samples increased the DNA yield (data not shown), most likely by allowing more of the sample to be drawn off the substrate. It was noted that samples with dye color present in the sample after pre-treatment with digest buffer and proteinase K were completely clear after purification on the BioRobot EZ1. In contrast, samples extracted off black leather and the multicolor cotton fabrics had dye co-purify with organic extraction. It was not possible to test all problematic substrates in this study; however, the results suggest that the BioRobot EZ1 may be an effective means of eliminating dyes and PCR inhibitors present in many of the substrates encountered in forensic casework.

Saliva Stains

Comparison of DNA yields from saliva stains demonstrated that for all samples the BioRobot EZ1 performs at a comparable efficiency to standard organic extraction. It should be noted that for BioRobot EZ1 extracted samples a slightly reduced yield was observed from the more concentrated samples (neat saliva through
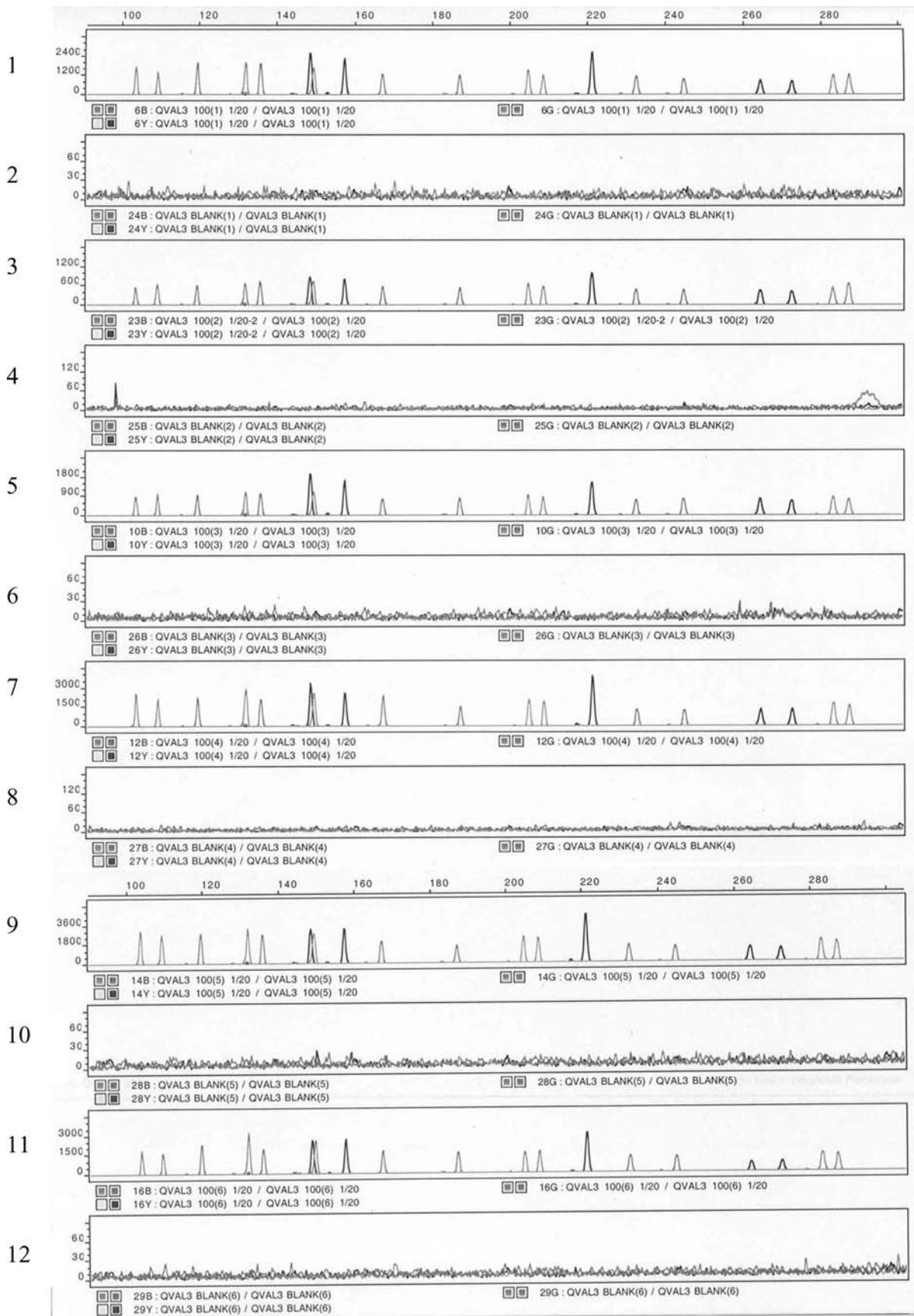
FIG. 3—*GeneScan*TM *electropherograms from the six blood samples and the six blank samples. For amplification the blood samples were diluted 1/20 in TE and the expected DNA profile was obtained. In contrast 20 μL of each undiluted blank sample was amplified with no DNA detected. Numbers 1, 3, 5, 7, 9, and 11 are the blood samples electropherograms. Numbers 2, 4, 6, 8, 10, and 12 are the blank samples electropherograms.*
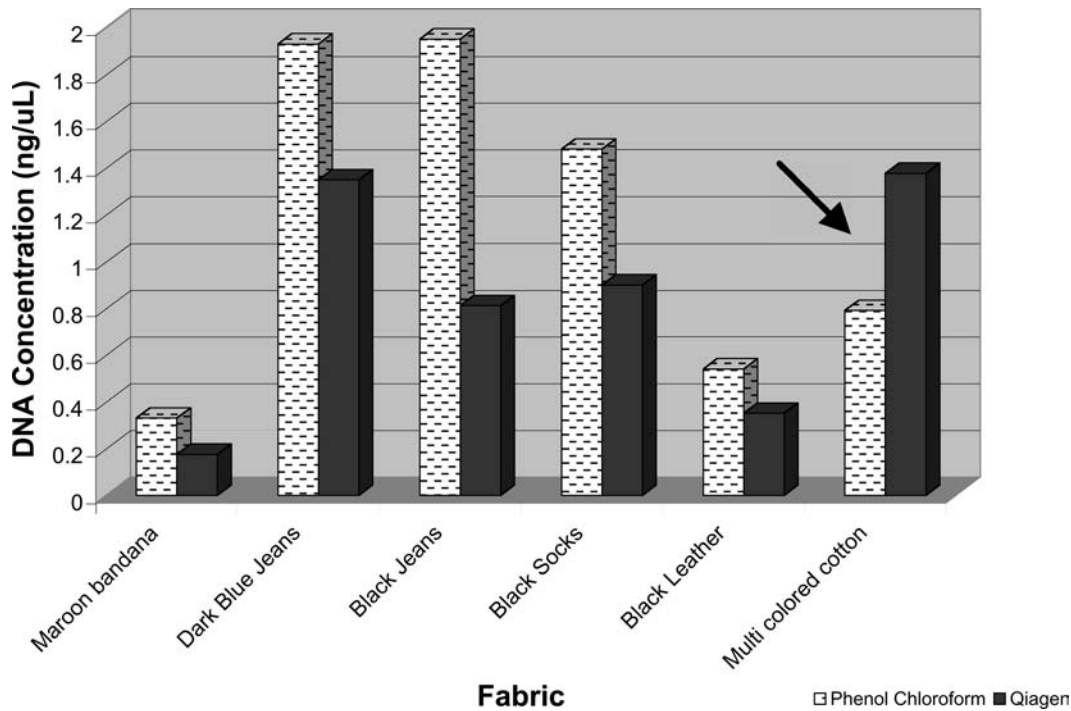
FIG. 4—*Results obtained from DNA extraction of bloodstains from a variety of fabric types routinely encountered in forensic casework using the BioRobot EZ1 and organic extraction. DNA concentrations are in ng/μL. The results for the BioRobot EZ1 are an average of the replicate extractions. Arrow denotes results for the extraction of blood from multicolored cotton where BioRobot EZ1 yields exceeded the yields for organic extraction.*
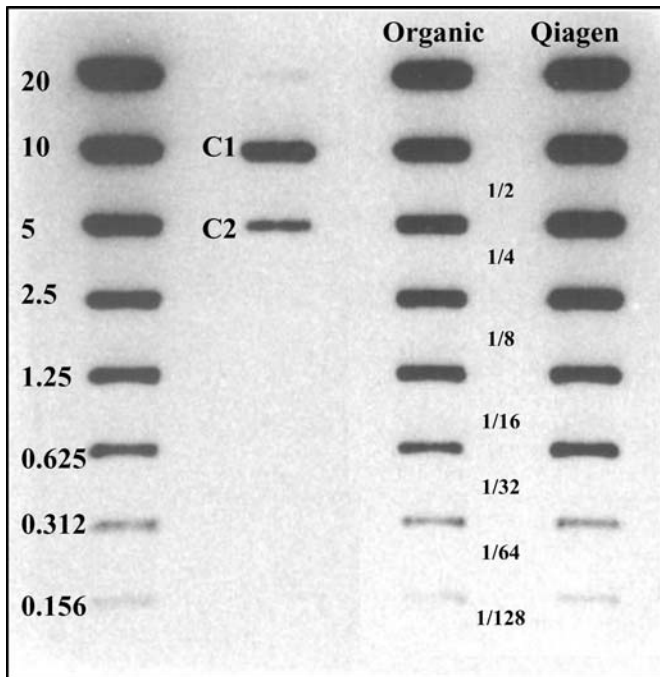

FIG. 5—*Slot-blot results for 5 × 5 mm cuttings of diluted saliva stains. The left-most columns contain the DNA standards and calibrators (listed in total nanograms).*

TABLE 1—*Comparative extraction and STR data for cigar samples.**

Sample	DNA Concentration	STR Data
Item 1A	0.02 ng/μL	No STR data
Item 1A-Q	0.05 ng/μL	Full STR profile
Item 1B	0.11 ng/μL	Amel and D8S1179
Item 1B-Q	0.05 ng/μL	Full STR profile
Item 1C	undetected	No STR data
Item 1C-Q	undetected	Amelogenin

* Results obtained from three cigar samples purified with the BioRobot EZ1 and with the standard organic extraction. Samples 1A, 1B, and 1C were purified organically. Samples 1A-Q, 1B-Q, and 1C-Q were purified using the BioRobot EZ1.

EZ1 recovery of DNA was also seen from stains containing as low as 0.039 μL of saliva. The amount of DNA recovered from all samples was sufficient for further DNA analysis.

Five of the six-cigarette butt samples yielded full STR profiles while the sixth sample yielded a partial STR profile (data not shown). These results were consistent with the amount of cellular material observed microscopically and the single partial profile is likely due to a small amount of cellular material in the original sample rather than poor extraction efficiency. These results demonstrate that cigarette butt evidence samples are amenable to extraction with the BioRobot EZ1. An elution volume of 50 μL is recommended for such samples.

Two of the three cigar samples re-purified with the BioRobot EZ1 yielded full DNA profiles whereas no or little DNA typing information was obtained from the corresponding organically extracted samples (Table 1). Based on these results, the BioRobot EZ1 extraction clearly had a benefit on the outcome of the DNA typing. For example, for one of the cigar samples (Item 1B) amplification of 13 μL of the organically extracted sample exhibited inhibition whereas 20 μL of the less concentrated BioRobot EZ1 re-purified counterpart yielded a full DNA profile (Fig. 6). These results imply

−1/32 dilution of saliva) but for low concentration samples (1/64–1/128 dilutions of saliva) the recovery approaches that of organic extraction (Fig. 5). The results of this experiment demonstrated that the BioRobot EZ1 has the ability to extract DNA from small amounts of saliva found on a variety of substrates. For the three fabrics investigated, the BioRobot EZ1 consistently yielded DNA from stains containing 0.078 μL of saliva. Successful BioRobot
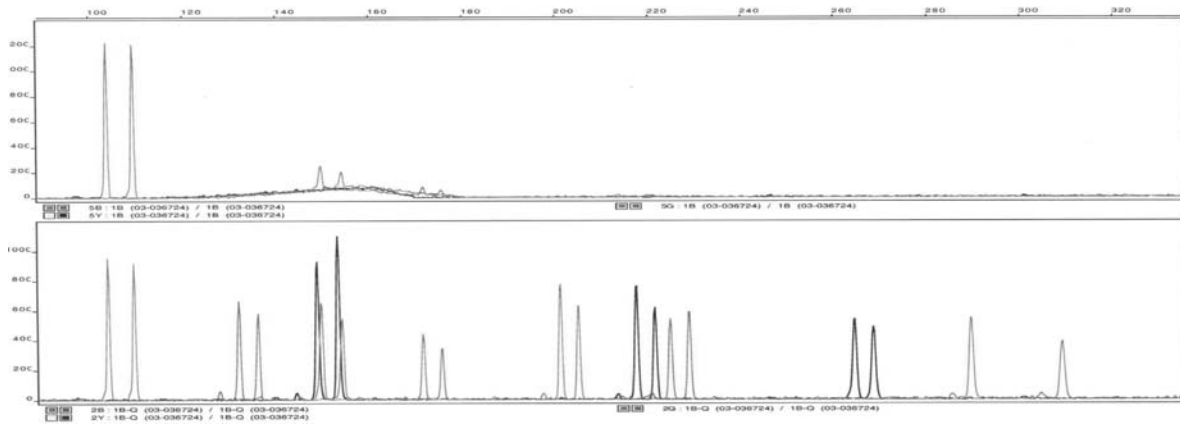
FIG. 6—*STR results using the AmpFlSTR Profiler Plus amplification kit. The top electropherogram depicts the results from Item 1B extracted organically. The bottom electropherogram is from Item 1B-Q extracted on the BioRobot EZ1.*
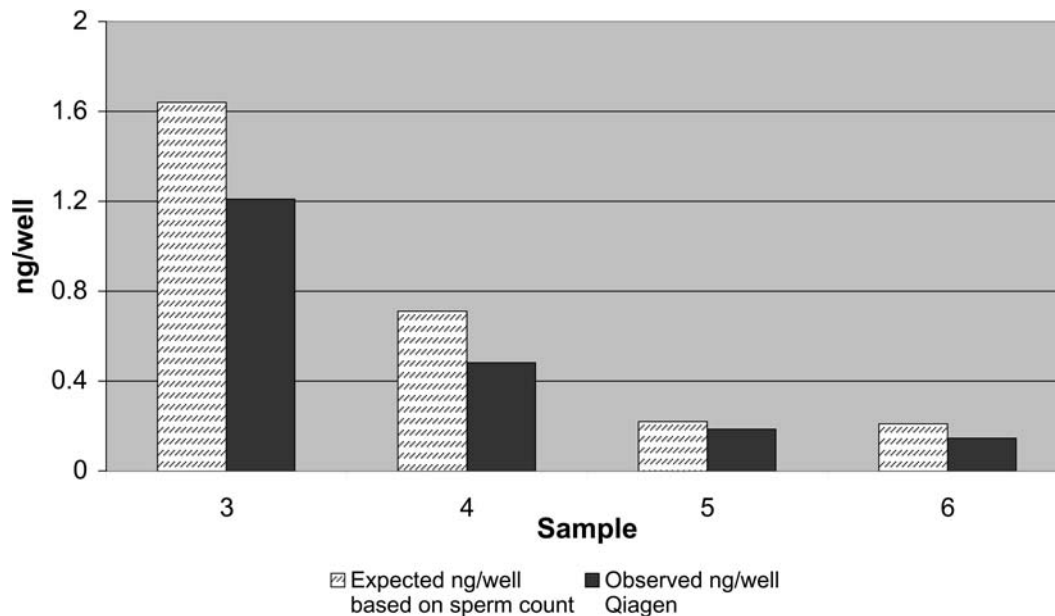

FIG. 7—*Histogram of theoretical DNA yields and observed DNA yields from BioRobot EZ1 extractions of differentially extracted semen stains. Expected DNA yields were calculated from microscopic evaluation of sperm cells counts and based on 3 pg of DNA per sperm cell. Samples with the largest number of sperm were excluded from the graph.*

that some form of inhibitor present in the organically extracted sample was removed when re-purified using the BioRobot EZ1.

The results from the analysis of the cigars as well as from previous studies with magnetic bead or silica-based extractions (14–18) further suggest that the BioRobot EZ1 purification can remove inhibitors from certain problematic samples. This is likely due to the fact that inhibitors attracted to the aqueous phase of organic extractions are not co-purified with the DNA in BioRobot EZ1 extractions because they do not bind to the silica beads. It is important to note that our experiments do not encompass all potential inhibitors to PCR and additional work could be done to determine which inhibitors may not be removed with the magnetic bead technology. Chui et al. have found that analysis of fecal material with both silica and magnetic particle-based extraction methods did not completely remove PCR inhibitors (19).

Mock Sexual Assault Swabs

Comparisons of the STR profiles generated from the mock sexual assault samples demonstrate that the BioRobot EZ1-based differ-ential extraction performs as well as the organic method. After the initial lysis of the non-sperm fraction and its collection, it is recommended that a second digest treatment with a larger volume be employed to ensure complete lysis of any remaining non-sperm cells in the sample. It is important to note that the use of the BioRobot EZ1 for the extractions of the sperm and non-sperm fractions allows for the elution of the purified DNA from the two fractions in different volumes. Thus an analyst can modify the final volume of the purified sample depending on the cell counts observed during microscopic examinations.

Typeable amounts of DNA were purified from all dilutions of semen evaluated using the BioRobot EZ1. The DNA yields from the extraction of the sperm fractions employing the BioRobot EZ1 approach the theoretically expected yields estimated from the observed number of sperm cells from microscopic examination (Fig. 7). Based on these experiments sexual assault samples containing as few as 300 sperm cells may be candidates for purification with the differential extraction method using the BioRobot EZ1. In cases with fewer than 300 sperm, the SDPD DNA laboratory has chosen to process these samples with a traditional organic

TABLE 2—*Sample types with DNA successfully isolated using the BioRobot EZ1.*

Liquid blood	Cigarettes
Reference bloodstains	Cigars
Reference mouth swabs	Stamps
BODE mouth swabs	Urine
Single hair roots	Fetal tissue
Saliva Stains	Semen
Evidence bloodstains	Chewing gum

extraction as improved DNA yields were obtained (data not shown).

STR Analysis

STR analysis of samples extracted with the BioRobot EZ1 produced the expected DNA profiles. The STR profiles generated all exhibited peak balance both within and across loci, as expected for high quality DNA. In general, in the absence of inhibition there was no difference in the quality of DNA profiles obtained from samples purified using the BioRobot EZ1 in comparison to organically extracted samples (data not shown).

Samples purified on the BioRobot EZ1 have sometimes been observed to contain a small amount of paramagnetic silica particles. While methods involving magnetic particles within an amplification reaction demonstrate their compatibility with the polymerase chain reaction (18), it has been stated that silica is a PCR inhibitor (14). To assess this concern, an experiment to determine the effect of the Qiagen paramagnetic silica particles on the PCR was completed. The results demonstrated that even in the presence of as much as one-fifth the amplification volume of paramagnetic particles the amplification reaction efficiency was not severely affected (data not shown).

Extraction Statistics

The SDPD Crime Laboratory has incorporated the use of the BioRobot EZ1 into forensic casework. DNA has been successfully purified from a number of different biological sources and from a number of different substrates that were deemed forensically significant (Table 2). As of the submission of this manuscript over 1000 samples have been processed with the BioRobot EZ1 including validation and casework samples.

In the total number of samples processed only two reference samples failed to produce a quantifiable amount of DNA upon slot-blot analysis. These reported instances of extraction failure occurred early in our experience with the EZ1 workstation. Further investigation into the cause of the failure revealed that the most probable cause was reagent cartridges that were not properly seated in the instrument.

Conclusions

The San Diego Police Department has developed procedures for DNA extraction from a wide variety of biological samples and substrates employing the BioRobot EZ1 workstation with the EZ1 DNA Tissue Kit and has validated it for forensic casework. In most cases, the DNA yields obtained with the BioRobot EZ1 workstation were comparable to those obtained from organic extractions followed by microconcentration. The single-use reagent cartridges, filter-tip reaction chamber, and the linear process employed by the BioRobot EZ1 limit the possibilities of sample-to-sample contamination or errors related to sample switching. In the extraction process with the BioRobot EZ1, tube-to-tube transfers normally performed by the analyst are drastically reduced lessening the likelihood of human error.

With the variety of DNA extraction methods available to the scientific community, forensic scientists have critical decisions to make concerning which methodologies might yield the best results for particular evidence samples. Timesaving DNA extraction methods that yield purified samples with high quality DNA are crucial for forensic laboratories to meet the rising demands for their services. Automated DNA extraction methods reduce analysis time and allow forensic DNA laboratories to process a high number of samples helping to eliminate backlogs. Large-scale robotic DNA extraction instruments are available to the forensic community, however these systems are often out of reach to smaller forensic laboratories due to their cost or where the number of samples analyzed is too few to justify the purchase of a large robotic platform. Small to medium-sized forensic laboratories can benefit from considerable timesaving and more effective case management without a loss in sample quality by incorporating the BioRobot EZ1 for DNA extractions in forensic casework.

The incorporation of the BioRobot EZ1 into forensic casework will not necessarily eliminate extractions using phenol-chloroform. While the majority of samples encountered in casework can be extracted effectively with the BioRobot EZ1 certain samples may still warrant organic extraction. The benefits of using the BioRobot EZ1 should be weighed in context with the case scenario and sample type. Factors such as visible stain size and age, and the abundance of sperm and epithelial cells should be assessed in deciding on a suitable extraction strategy. The BioRobot EZ1 is a tool, which if employed appropriately and in conjunction with organic extraction will reduce sample-processing times without sacrificing the quality of casework.

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