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TECHNICAL NOTE

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Optimization of DNA Extraction from Low-Yield and Degraded Samples Using the BioRobot® EZ1 and BioRobot® M48

ABSTRACT: Robotic extraction of DNA from dilutions of blood and semen using either the BioRobots® EZ1 or BioRobots® M48 consistently produced lower recoveries than standard organic extractions of the same samples. In an effort to increase the efficiency of robotically extracted DNA, glycogen and carrier RNA were added following cell lysis. The addition of glycogen, postlysis, resulted in no improvement in DNA recovery with the BioRobot® EZ1. However, when carrier RNA was added to the cell lysate of limited and degraded samples extracted on the EZ1 or the M48, DNA recoveries dramatically increased four- to 20-fold. DNA yields obtained by robotic extraction in the presence of carrier RNA were as high, or higher, as those obtained by organic extraction lacking carrier RNA, while experiments that utilized carrier RNA in both types of extractions showed increased sensitivity for both methods. Furthermore, carrier RNA substantially increased the recovery of fragmented DNA with the EZ1.

KEYWORDS: forensic science, robotic DNA extraction, automated DNA purification, silica beads, BioRobot® EZ1, BioRobot® M48

Manual DNA extraction using some form of a standard organic extraction procedure is the norm for most laboratories performing DNA analysis for forensic casework. Increased case backlogs and the need for ever greater throughput is causing many laboratories to consider robotics, and recent attempts to adapt DNA extraction methods to robotic platforms have been reported (1–4). For forensic casework samples, any robotic procedure that is adopted should perform as well as, if not better, than the manual method it is replacing, especially with regard to the sensitivity and efficiency of DNA recovery, as well as the suitability of the purified DNA for amplification by PCR. Because forensic samples may be limited and/or compromised, it is crucial that DNA yield not be sacrificed for increased throughput. We report here on the optimization of two robotic platforms recently introduced and marketed for forensic applications: Qiagen's BioRobot® EZ1 and BioRobot® M48.

These robots are attractive from a casework standpoint because of their automated protocols, their ease of use, and their relatively small footprints. The BioRobot® EZ1 system is marketed as a "bench-top" instrument, and is capable of processing up to six samples at a time, while the M48 is larger, and can process from 6 to 48 samples in six-sample increments. Both instruments use the same silica-based extraction method, and are used in conjunction with disposable extraction tubes provided by the manufacturer in kits containing all necessary reagents. The extraction time, postlysis, for six samples using the BioRobot® EZ1 system is *c.* 20 min, and requires little manual input other than specifying the expected quantity of DNA extracted (i.e., trace or reference protocols) and the elution volume (i.e., 50, 100, and 200 μL). Like the EZ1, the BioRobot® M48 requires minimal user input, has a larger range of user-selectable elution volumes (50–400 μL), and completes the extraction of 48 samples in *c.* $2\frac{1}{2}$ h.

The goal of this study was to compare the quantity and quality of DNA extracted using the BioRobot® EZ1 and BioRobot® M48 systems with that of casework-validated, manual organic extraction procedures that are currently in use at the California Department of Justice, Richmond DNA Laboratory (Cal DOJ), and the Scientific Investigation Division of the Los Angeles Police Department (LAPD). DNA recovery was initially determined to be sub-optimal, especially with low-yield samples, and optimization of recovery was attempted by the addition of two carrier molecules to the postcell lysate. In this report, we describe the significant improvement in DNA recovery with these extraction robots through the simple addition of a carrier molecule. The extraction efficiency of degraded DNA was also evaluated, as was the effect of the carrier molecule on subsequent downstream sample analysis.

Materials and Methods

Sample Preparation

Liquid blood and semen were obtained by donation from laboratory staff. Dilutions of samples were prepared either in sterile phosphate-buffered saline (PBS; BioRobot® EZ1) or in sterile water (BioRobot® M48). In certain samples, either carrier poly-A RNA (Qiagen, Valencia, CA) or glycogen (Sigma Chemical,

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St. Louis, MO) was included following lysis and just before extraction.

BioRobot® EZ1 System

The BioRobot® EZ1 workstation and EZ1 DNA Tissue kit (48 extractions) were obtained from Qiagen. All extractions used the Magtration System 6GC version 1.1N11 software and the Forensic Trace Sample protocol. All extractions were performed by adding 10 μL of each diluted blood sample to 190 μL of Buffer G2 and 10 μL of Proteinase K (provided at a concentration of 600 mAU/mL in the EZ1 DNA Tissue kit), followed by brief vortexing and incubation for 1 h. For semen samples, 10 μL of each diluted sample was added to 190 μL of Buffer G2 and 10 μL of proteinase K and 3 mg of dithiothreitol (DTT; USB, Cleveland, OH), followed by brief vortexing and incubation at 56°C overnight. All samples were briefly vortexed twice during the incubation and briefly centrifuged at the end of the incubation. One microgram of carrier RNA (QIAmp Micro Kit, catalog #56304) was added before automated DNA extraction on the BioRobot® EZ1 workstation. Sealed reagent cartridges, elution tubes, tipholders containing filter-tips, and sample tubes are added to the workstation. The Trace Sample Protocol includes the following steps: addition to and mixing of magnetic silica particles with samples, magnetic separation, and washing, followed by elution. The DNA was eluted in a volume of 50 μL of sterile water (5).

BioRobot® M48 System

The BioRobot® M48 workstation and the MagAttract DNA Mini M48 kit (192 extractions) were obtained from Qiagen. Extractions were performed by adding 25 μL of each blood dilution to 165 μL of Buffer G2 and 10 μL of Proteinase K (provided at a concentration of 600 mAU/mL in the Mini M48 kit), followed by incubation at 56°C for 30 min. For semen samples, 25 μL of each semen dilution was combined with 155 μL of Buffer G2, 10 μL of Proteinase K, and 10 μL of 1 M DTT (Sigma Chemical), 10 mM sodium acetate (pH 7.5) before an overnight incubation at 56°C. Digested samples were briefly vortexed at least twice during the incubation. Automated extractions on the BioRobot® M48 were conducted using the Qiasoft M Operating System software (vers. 2.0E001) and the Trace protocol and eluted in a volume of 50 μL of sterile water.

Organic DNA Extraction Procedure

Samples processed at Cal DOJ were incubated at 56°C in a stain extraction buffer (1 h for blood, overnight for semen) of the following composition: 10 mM Tris, pH 8.0, 10 mM EDTA, 100 mM NaCl, and 2% SDS with 10 μL of a 20 mg/mL Proteinase K (Invitrogen, Carlsbad, CA) was added. These cell lysates were extracted twice with phenol/chloroform/isoamyl alcohol, and finally concentrated into Tris-EDTA buffer (TE^{-4}, 10 mM Tris-HCl pH 8.0 and 0.1 mM EDTA) using centrifugation dialysis with Centricon YM-100 (Millipore, Bedford, MA) concentration columns. This protocol has been validated in our laboratory according to SWGDAM guidelines for use with casework samples, and is the protocol used currently. For the extraction of semen, 3 mg of DTT (USB) was added to the lysis buffer before incubation. After Centricon YM-100 concentration, the eluted DNA was brought to a final volume of 50 μL TE^{-4}, the elution volume of the BioRobot® EZ1.

A similar validated protocol for organic extractions was performed at the LAPD. Briefly, 25 μL of diluted blood was added to 0.5 mL of digest buffer (10 mM Tris, pH 7.5, 10 mM EDTA, 50 mM NaCl, 2% SDS) containing 15 μL of 10 mg/mL Proteinase K (Promega, Madison, WI), and incubated for 60 min at 56°C. Semen dilutions were extracted, similarly, with the exception that 20 μL of 1 M DTT (Sigma Chemical), 10 mM sodium acetate (pH 7.5) was added before overnight incubation at 56°C. These lysates were extracted three times with phenol/chloroform/isoamyl alcohol, and then washed three times with Tris-EDTA buffer (TE, 10 mM Tris-HCl pH 8.0 and 1 mM EDTA) and concentrated using Centricon YM-100 centrifugal filter devices (Millipore). The concentrated DNA solutions recovered from the Centricon filters were brought to a final volume of 50 μL in TE.

DNA Quantification

DNA quantification for samples processed at Cal DOJ was performed using one of two real-time, TaqMan, PCR assays designed at the Cal DOJ DNA laboratory. A singleplex assay, which amplifies an *c*. 180 bp target at the TH01 locus (the length is sample specific depending on the allele(s) present), was used for the majority of quantitations performed on DNA extracted with the BioRobot® EZ1. This assay, described by Timken et al. (6), was performed on an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA) in a reaction volume of 20 μL containing 10 μL of TaqMan® 2 × Universal Master Mix, No AmpErase® UNG (Applied Biosystems), 1 μL of 3.2 μg/μL nonacetylated BSA (Sigma Chemical), with the nuTH01-probe concentration at 200 nM, and the nuTH01-F primer and nuTH01-R primer concentrations at 300 nM each, and 4 μL of sample. The thermal cycling parameters were as follows: enzyme activation at 95°C for 10 min, followed by 45 cycles of 95°C for 15 sec and 60°C for 60 sec.

A second multiplex qPCR TaqMan assay was used for quantification of the DNAseI-digested samples. This assay amplifies two nuclear genome targets of different length, the TH01 target described above as well as a shorter amplicon of 69 bp at the CSF1PO locus (7), and therefore, provides information about the quantity as well as the quality of the DNA. In addition, this assay incorporates an internal positive control (IPC), including primers and a TaqManMGB probe to amplify and detect a synthetic oligonucleotide (77 nt) spiked into the qPCR master mix. The IPC oligonucleotide was designed to be nonhomologous to naturally occurring sequences, and experiments have demonstrated that the IPC primers and probe do not detect human DNA or other genomes commonly encountered in forensic samples (7). The reaction conditions were as above, with the exception that an additional 0.5 μL of 5 U/μL AmpliTaq Gold® was added. The primers and probes were at the following concentrations: 600 nM of each nuTH01-primer, 200 nM of nuTH01-probe, 400 nM each of nuCSF-primer, 100 nM of nuCSF-probe, 50 nM each of IPC primer, 200 nM IPC-probe, with 90,000 copies of an IPC oligonucleotide (7). The assay was performed on an ABI 7500 Sequence Detection System (Applied Biosystems), and the data were analyzed using Applied Biosystems 7500 Real Time PCR System SDS software v1.3.

For both of the above assays, Promega Human Genomic Female Standard DNA (Promega, Cat # G1521) was used as a DNA standard. The concentration of extracted DNA was determined for each sample in triplicate.

Samples extracted at the LAPD were quantified using the TaqMan, human, nuclear DNA, qPCR (TaqMan HUMTHO1) assay as described by Richard et al. (8), which amplifies a 62 bp amplicon that is 31 bp downstream of the polymorphic repeat region of the

HUMTH01 locus (Accession D00269). This assay was slightly modified to include 0.16 μg/mL BSA (fraction V, Sigma Chemical) in each reaction, and contained 5 μL of DNA extract in a total reaction volume of 25 μL. DNA extracts were quantified, in duplicate, on an ABI 7000 Sequence Detection System by comparison with an eight-point DNA standard curve (50–0.023 ng/μL). DNA standards were prepared at the LAPD using human placental DNA purchased from Sigma Chemical.

Serological Screening Tests and Extractions Performed on Liquid Blood and Semen Dilutions

Serially diluted liquid blood and semen samples were extracted using either the BioRobot® EZ1 or the BioRobot® M48, and compared with a standard organic extraction procedure. Samples extracted with the EZ1 were prepared in sterile PBS in dilutions up to 1:2500 for liquid blood and 1:20,000 for semen. Carrier RNA (Qiagen) and glycogen (Sigma Chemical) were tested in an attempt to maximize DNA yield (9–12). One microgram of carrier RNA or 100 μg of glycogen was added to the sample, postlysis, followed by DNA extraction with the BioRobot® EZ1 or a standard organic procedure.

At the LAPD, 25 μL aliquots of each dilution of liquid blood or semen were extracted, using either the M48 BioRobot® or the organic method, and compared. For blood extractions, 2–16,384-fold dilutions of liquid blood in sterile water were prepared, and 1 μg of carrier RNA was added postlysis. Based upon the superior yields obtained from samples that included carrier RNA, the semen dilutions, which ranged from 3- to 1.1×10^6-fold, were all extracted in the presence of 1 μg of carrier RNA.

Liquid blood and semen dilutions prepared at the LAPD were also screened with presumptive tests for blood and semen using phenolphthalein (13) and acid phosphatase (AP) (14) reagents, respectively. Phenolphthalein (Fisher Chemical, Fair Lawn, NJ), and sodium-α-naphthylphosphate and Fast Blue B dye (both from Sigma Chemical) were used to prepare the phenolphthalein and AP reagents, respectively.

Degradation Study

HL60 DNA (ATCC™, Manassas, VA) was digested with DNaseI (Invitrogen), for progressive lengths of time. Approximately 10.3 μg DNA in 36 μL and 9 μL of 10 × DNaseI Reaction Buffer were added to 45 μL sterile water. A 10 μL aliquot was transferred to a tube containing 2 μL of 25 mM EDTA (0 time point), and to the remaining reaction mix, 2.0 μL of DNaseI (1 U/μL) was added and incubated at room temperature (24°C). Aliquots of 10 μL were removed after 5, 10, 15, 30, 45, and 60 min and transferred to tubes containing 2 μL of 25 mM EDTA. All samples were then incubated at 65°C for 15 min. The extent of DNA fragmentation was assessed by agarose gel electrophoresis alongside the DNA molecular weight size marker XIV 100-bp ladder (Roche Diagnostics, Indianapolis, IN).

Short Tandem Repeat (STR) Typing

The quality of DNA extracted using the EZ1 was evaluated by STR analysis with the AmpFℓSTR® Identifiler™ PCR amplification Kit (Applied Biosystems). Amplifications were performed in a 25 μL final reaction volume containing 10 μL PCR Reaction Mix, 5.0 μL primer set, 0.5 μL Taq Gold® DNA Polymerase, and 10 μL of 1.0 ng input DNA. If <1 ng of DNA was available, 10 μL of DNA extract was used for the amplification. The amplification parameters were as follows: predenaturation and enzyme activation at 95°C for 11 min, 28 cycles of 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min, a final extension at 60°C for 60 min, and a 5°C hold indefinitely. Amplification products were electrophoresed on an ABI 3100 Genetic analyzer (Applied Biosystems) and evaluated using the GeneScan® version 3.7 and Genotyper® version 3.7 software (Applied Biosystems).

The suitability of DNA extracted from blood and semen using the BioRobot® M48 was assessed using the AmpFℓSTR® ProfilerPlus™ PCR amplification Kit (Applied Biosystems). Amplification reactions contained 20 μL PCR Reaction Mix, 10.0 μL primer set, 1.0 μL Taq Gold® DNA Polymerase, and 20 μL of DNA solution (~ 2.0 ng input DNA), resulting in a final reaction volume of 50 μL. Extracts containing <2 ng of DNA in 20 μL were used in amplifications without prior dilution. The amplification parameters were as described by Applied Biosystems. Capillary electrophoresis was performed on an ABI 310 Genetic analyzer (Applied Biosystems), and amplified products were evaluated using the GeneScan® version 3.7 and Genotyper® version 3.7 software (Applied Biosystems).

Results and Discussion

Extraction Efficiency Study

Initial experiments that compared DNA yields for whole blood, extracted with the organic method versus the BioRobot® EZ1, resulted in essentially no difference (Fig. 1); however, yields in these experiments with whole blood were relatively high (*c*. 30–100 ng). As yields started to approach 10 ng or less, the organic extraction method consistently outperformed the BioRobot® EZ1. Similar results were observed using the BioRobot® M48 (data not shown).

When experiments comparing extraction yields for serially diluted whole blood and semen were performed, greater yields were consistently obtained with the organic method (Tables 1A and B). Poorer recoveries from low-yield samples suggested that DNA binding to the silica-coated magnetic particles may be proportional to the concentration of nucleic acid molecules in the lysate up to a certain threshold, or that there is loss of a fixed amount of DNA due to nonspecific adhesion to sites on the silica beads or the walls of the container. Whatever the cause, these results led us to conclude that the BioRobot® EZ1 and BioRobot® M48, used as

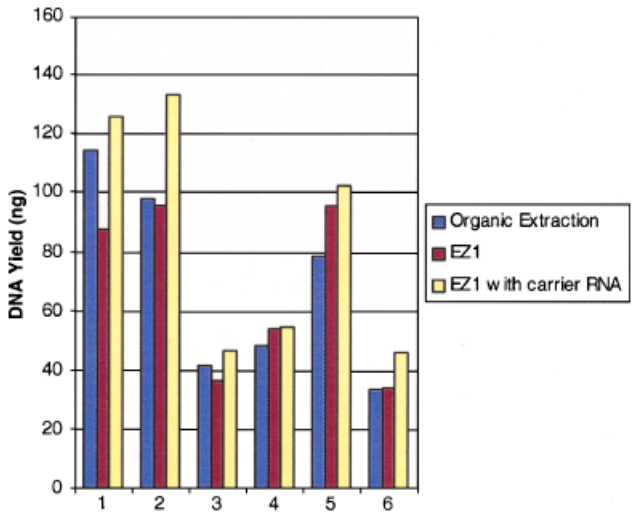

FIG. 1—*Comparison of the extraction efficiency of the BioRobot® EZ1 system with the standard organic extraction method. DNA was extracted from 10 μL volumes of liquid whole blood from 6 different individuals.*

TABLE 1A—*DNA yields from aliquots of serially diluted liquid blood samples extracted using the BioRobot® EZ1, the BioRobot® EZ1 with carrier RNA (cRNA), and the organic extraction methods (one representative run shown in table).*

Liquid Blood Dilutions	Volume of Liquid Blood Extracted (μL)	BioRobot® EZ1, DNA (ng)	BioRobot® EZ1 with cRNA, DNA (ng)	Organic Extraction, DNA (ng)
1:10	0.1	8.025	10.000	7.900
1:50	0.02	0.213	2.250	1.840
1:250	0.004	0.050	0.260	0.263
1:1250	0.0008	0.000	0.040	0.038
1:2500	0.0004	0.000	0.013	0.000

Quantification was performed using the real-time, TaqMan PCR—nuTH01 assay (average of triplicate quantifications shown in table).

TABLE 1B—*DNA yields from aliquots of serially diluted semen samples extracted using the BioRobot® EZ1, the BioRobot® EZ1 with carrier RNA (cRNA), and the organic extraction methods (one representative run shown in table).*

Semen Dilutions	Volume of Neat Semen Extracted (μL)	BioRobot® EZ1, DNA (ng)	BioRobot® EZ1 with cRNA, DNA (ng)	Organic Extraction, DNA (ng)
1:100	0.01	14.375	38.750	35.750
1:200	0.005	0.369	14.250	12.750
1:1000	0.001	0.066	2.900	1.813
1:2000	0.0005	0.000	1.263	0.870
1:10,000	0.0001	0.000	0.153	0.323
1:20,000	0.00005	0.000	0.181	0.114

Quantification was performed using the real-time, TaqMan PCR—nuTH01 assay (average of triplicate quantifications shown in table).

recommended by the manufacturer, might be suitable for the extraction of DNA from reference samples, but might not be suitable for the extraction of DNA from evidentiary samples.

Optimization of the BioRobot® EZ1 and BioRobot® M48 DNA Extraction Systems

The addition of two different "carrier" molecules, glycogen and RNA, has been used with some success with various extraction methods to increase DNA recovery. Glycogen, when added to the cell lysate, has been reported to improve the yield of DNA extracted from clinical samples (11,12). Similarly, the addition of yeast carrier tRNA has been utilized to increase DNA yields for some extraction methods, especially those incorporating ethanol precipitation (15).

In an attempt to improve the recovery of DNA from low-yield samples with the BioRobot® EZ1, glycogen and carrier poly-A RNA were added to cell lysates prepared from serially diluted blood and semen samples just before extraction. The DNA yields obtained from robotic extractions, in the presence and absence of each of these carrier molecules, were then compared with DNA recoveries attained with the organic method. The addition of glycogen resulted in no improvement in DNA recovery, and actually appeared to have a slight, deleterious effect (data not shown). However, when carrier RNA was added to the cell lysates of serially diluted liquid blood and semen samples, a significant increase in DNA yield was achieved, as much as 40-fold in some cases when compared with samples extracted without added RNA (Tables 1A and B).

Based upon the results of multiple experiments, the addition of carrier RNA appeared to be an effective way of enhancing DNA recovery for low-yield samples, working equally well with blood or semen, with yields as close to, if not better than, those obtained with standard organic extraction (Tables 1A and B). Furthermore, in the absence of carrier RNA, several of the highest dilutions yielded no DNA, as determined by qPCR, while with added carrier RNA, significant amounts of DNA were recovered (Tables 1A and B). Additionally, no adverse effects or interference were observed as the result of the addition of this carrier molecule: negative controls showed no evidence of the presence of DNA by qPCR.

Substantial increases in the sensitivity of extraction of liquid blood and semen dilutions in the presence of carrier RNA were also observed in independent experiments performed with the BioRobot® M48. Using the BioRobot® M48, DNA recoveries from serial blood dilutions were *c*. 4-fold greater in the presence of carrier RNA than in its absence, thus allowing DNA to be obtained from as little as $\sim 0.002\,\mu L$ of blood and $\sim 0.0001\,\mu L$ of semen (Tables 2A and B).

In order to establish that the measured DNA concentrations accurately represented the quantity of profilable DNA present, STR analysis using the AmpFℓSTR® ProfilerPlus™ PCR kit was performed. In these trials, full profiles were obtained from two out of four 1:4096 blood dilutions ($\sim 0.006\,\mu L$ of liquid blood) and four out of four 1:9375 semen dilutions ($\sim 0.003\,\mu L$ of semen) tested, in accordance with the measured DNA yields (data not shown). However, based upon the DNA yields measured for the M48 panel, it is probable that had the volume been reduced from 50 to 25 μL, full profiles could have been obtained for the next lowest dilution.

Interestingly, a reproducible enhancement in DNA recovery was also observed when carrier RNA was included during organic extraction. This one change caused DNA yields obtained by organic extraction to exceed those achieved using the BioRobot® M48. While the mechanism underlying this effect is unknown, previous studies have documented the adsorption of DNA to polypropylene and other plastic surfaces (16). Poly-A RNA/salmon sperm DNA has also been used effectively to enhance the extraction of DNA for low copy number methods (17), presumably by binding to sites on the surface of the container that would otherwise retain nucleic acids.

The sensitivity of two serological screening methods, the phenolphthalein (pheno) test for blood and the AP test for semen, was compared with the results obtained from the extraction of various blood and semen dilutions using the BioRobot® M48. Both presumptive screening tests are widely used in forensic labs and are very sensitive. Even at 1:16,384, the highest dilution of blood that was tested, the pheno test detected the presence of blood in accordance with previous studies (reviewed in (13)). The AP test results, however, were less effective at predicting the outcome of profiling a dilution of semen. For example, it is still possible to recover sufficient DNA to obtain a full profile from a semen dilution of 1:46,875; yet, more than 30 sec are required to produce a purplish product using the AP test at this dilution (Tables 2A and B). As 30 sec exceeds the AP test reaction time for which an item would be considered positive for the "presence" of semen (according to our validated protocol), it suggests that a more sensitive screening test for semen is needed.

Degraded DNA

Extractions were performed using degraded DNA to determine how these BioRobots® performed, relative to the organic method, for the extraction of compromised samples. Purified HL60 DNA was digested with DNaseI for varying lengths of time, and an aliquot of each time point was electrophoresed on an agarose gel for

TABLE 2A—*Average DNA yields from aliquots of serially diluted whole-blood samples extracted using the BioRobot® M48 and the organic extraction method, with and without carrier RNA (cRNA).*

Liquid Blood Dilutions	Volume of Liquid Blood Extracted (μL)	Phenolphthalein Reactivity (sec)	BioRobot® M48 DNA (ng)	BioRobot® M48 with cRNA, DNA (ng)	Organic Extraction DNA (ng)	Organic Extraction with cRNA, DNA (ng)
1:16	1.56	<3	72.7		58.6	
1:64	0.39	<3	18.3		20.9	
1:256	0.098	<3	4.08		6.33	
1:512	0.049	3	2.118	4.825	2.641	7.338
1:1024	0.024	3–5	0.833	3.302	1.368	7.401
1:2048	0.012	3–5	0.540	1.754	1.090	2.309
1:4096	0.006	5–7		1.080		1.177
1:8192	0.003	5–7		0.310		0.691
1:16384	~ 0.002	15–20		0.265		0.401

Quantification was performed using the real-time, TaqMan, HUMTH01 assay. The quantities listed below are based upon the extraction of duplicate samples in two independent experiments (i.e., a total of four samples). Phenolphthalein screening results were also based upon two trials.

TABLE 2B—*Average DNA yields from aliquots of serially diluted semen samples extracted using the BioRobot® M48 and the organic extraction method in the presence of carrier RNA.*

Semen Dilutions	Volume of Neat Semen Extracted (μL)	Acid Phosphatase Reactivity (sec)	BioRobot® M48 with carrier RNA, DNA (ng)	Organic extraction with carrier RNA, DNA (ng)
1:75	0.333	4–6	367	554
1:375	0.067	7–9	85	108
1:1,875	0.013	12–14	18.2	23.4
1:9,375	0.003	26–30	2.92	4.37
1:46,875	0.0005	>30	0.518	0.719
1:234,375	0.0001	>30	0.150	0.128

Quantification was performed using the real-time, TaqMan, HUMTH01 assay. The quantities listed below are based upon the extraction of duplicate samples in two independent experiments (i.e., a total of four samples). Acid phosphatase screening results were also based upon two trials.

visualization with ethidium bromide to confirm that a pattern of increasing fragmentation was obtained with increasing digestion time (data not shown). These samples were then extracted using the BioRobot® EZ1 with carrier RNA and the organic procedure for comparison. For this experiment, carrier RNA was also added to the samples that were organically extracted.

Notably, a significant loss of DNA occurred with both the DNaseI-treated samples and the untreated controls in both methods (Table 3). Nevertheless, extraction with the BioRobot® EZ1 with added carrier RNA resulted in recoveries comparable to those obtained with the organic method (Table 3).

In order to determine whether the quality of the DNA (extent of fragmentation) was a factor in the recovery of DNA with these robots, we used a real-time qPCR assay that can assess the degree of DNA fragmentation. This assay amplifies two targets of different lengths (nuTH01: 170–188 bp, nuCSF1PO: 67 bp). We have previously demonstrated (7) that the ratio of the shorter to the longer target quantity provides a good estimate of the extent of fragmentation. Both extraction methods resulted in some loss of shorter fragments versus longer fragments, as reflected by the reduction in the CSF/TH01 ratio for the highly degraded samples (Table 3). This result was predicted for the organic extraction, which incorporates a Centricon YM-100 wash/concentration step with a nucleotide cut-off of *c*. 125 bp for double-stranded DNA (Millipore Centricon User Guide). Nevertheless, both extraction methods resulted in roughly equivalent recoveries for all the DNaseI digestion time points. In addition, for the most degraded samples (20–60 min digestion time points), significantly better yields were obtained with the robot when carrier RNA was added than when it was absent (data not shown).

STR Analysis

The quality of DNA extracted using the BioRobot® EZ1 system with carrier RNA was further assessed by STR amplification

TABLE 3—*Quantification of DNase I-digested HL 60 DNA before and after extraction using the BioRobot® EZ1 with carrier RNA (cRNA) and organic extraction methods (one representative run shown in table).*

DNaseI Digestion Time Points (min)	Before Extraction			Organic Extraction with cRNA			EZ1 with cRNA		
	nuTH01 DNA (ng)	nuCSF DNA (ng)	Degradation Ratio nuCSF/nuTH01	nuTH01 DNA (ng)	nuCSF DNA (ng)	Degradation Ratio nuCSF/nuTH01	nuTH01 DNA (ng)	nuCSF DNA (ng)	Degradation Ratio nuCSF/nuTH01
Undigested	9.390	8.420	0.9	4.190	4.160	1.0	3.730	3.200	0.9
5	4.240	6.350	1.5	0.900	1.620	1.8	1.710	2.520	1.5
15	1.340	4.560	3.4	0.589	1.800	3.1	0.575	1.460	2.5
20	1.080	4.670	4.3	0.571	2.270	4.0	0.577	1.720	3.0
30	0.637	3.950	6.2	0.203	0.894	4.4	0.267	1.260	4.7
45	0.332	2.680	8.1	0.219	1.180	5.4	0.149	0.831	5.6
60	0.320	2.980	9.3	0.233	1.250	5.4	0.168	0.913	5.4

The quantification was performed using the real-time, TaqMan PCR—nuTH01-nuCSF-IPC triplex assay (average of triplicate quantifications shown in table).

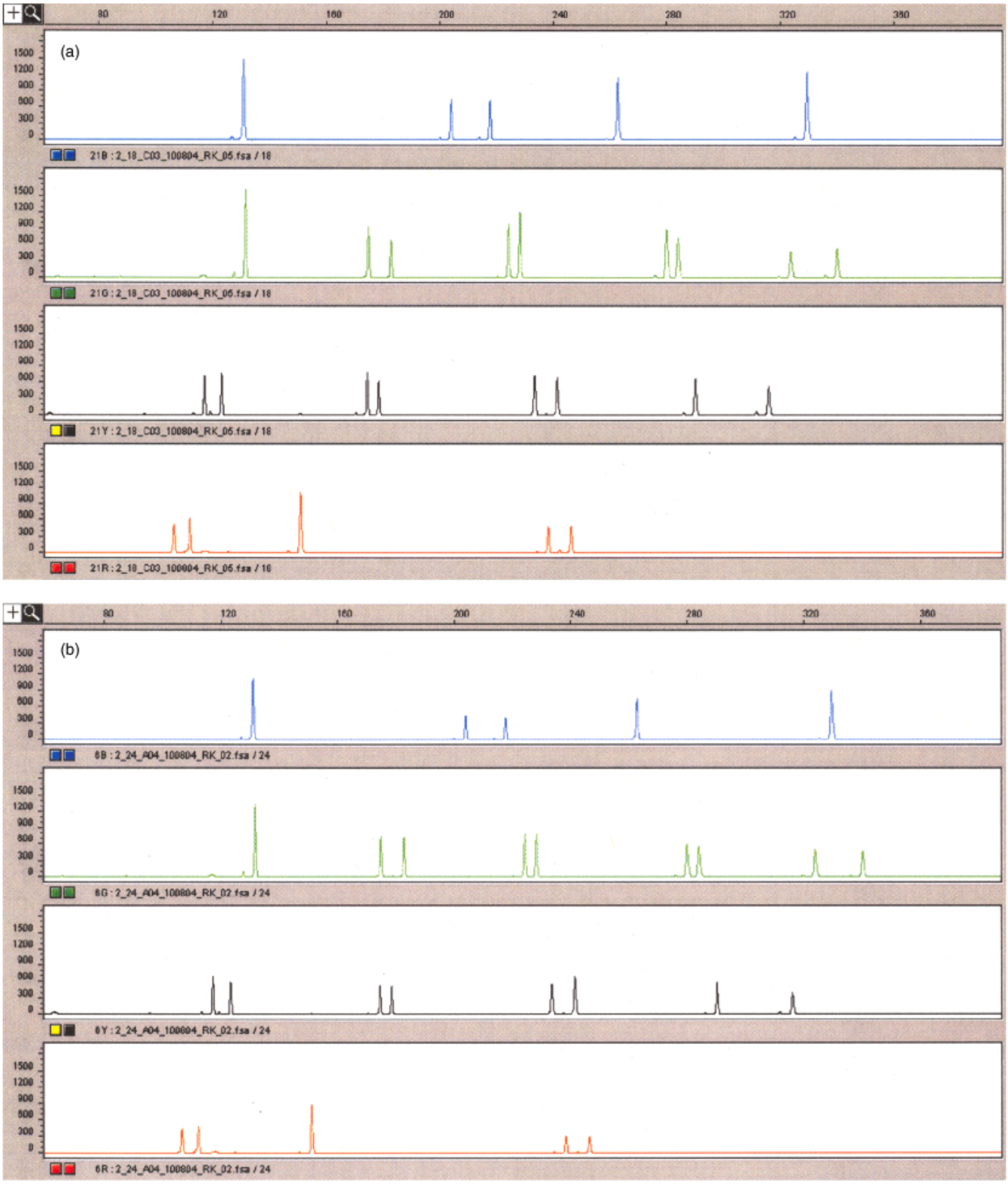

FIG. 2—*Comparison of AmpFℓSTR® Identifiler™ multiplex short tandem repeat profiles of DNA isolated from semen samples. DNA was extracted using the BioRobot® EZ1 system with carrier RNA (A) and standard organic extraction (B). One nanogram of input DNA was amplified in each case.*

using the AmpFℓSTR® Identifiler™ PCR amplification Kit (Applied Biosystems). The extraction of DNA on the BioRobot® EZ1 in the presence of carrier RNA produced no deleterious effects on STR typing. Complete STR profiles were obtained for input DNA amounts down to 250 pg of extracted DNA, which, in our hands, is typical for DNA extracted with the organic method. No artifacts were detected for the BioRobot® EZ1-extracted samples, and the same genotypes were obtained as with DNA extracted by the organic method, with peaks at roughly the same relative fluorescence unit (RFU) (Fig. 2).

Conclusion

Although Qiagen reports that "consistent high-performance in sensitive forensic applications illustrates the high-purity, integrity, and optimal concentration of DNA purified using the EZ1 DNA Tissue Kit and the BioRobot® EZ1 workstation" (18), all of the samples that they tested yielded relatively large amounts of DNA (0.04–1.2 μg). In practice, forensic casework samples frequently yield far <0.04 μg of DNA. We are aware of no reported observations that show good DNA recoveries for low-yield and degraded samples using the BioRobot® EZ1 workstation. In fact, Montpetti et al. (2) report that traditional organic extraction gives improved DNA yield for fewer than 300 sperm as compared with BioRobot® EZ1. Our results indicate that, for samples that contain less than ~ 10 ng of DNA, extraction with the EZ1 DNA Tissue Kit and the BioRobot® EZ1 workstation is not as sensitive as a standard organic extraction (i.e., one lacking carrier RNA), followed by centrifugation dialysis on Centricon YM-100 filtration devices. However, we have demonstrated that DNA recoveries for low-yield samples can be greatly improved, with either of the BioRobot® (EZ1 or M48) workstations and Qiagen's reagent kits, through the simple addition of carrier RNA following cell lysis. We should point out, however, that for some low-yield samples, robotically extracted DNA eluted in a 50 μL volume may require an additional concentration step.

Although the mechanism by which carrier RNA enhances the recovery of DNA in extractions is unknown, we offer two hypotheses. One possibility is that the RNA blocks sites on the sides of the container, on the centrifugation device filter, and on the surface of the silica beads, which would otherwise retain sample DNA. Secondly, if the thermodynamics of DNA adsorption to silica is governed primarily by entropy, as suggested by DNA-binding studies in chaotropic perchlorate solutions (19), then carrier RNA might enhance DNA adsorption to the silica by competing for the remaining solvent water molecules that are not bound to the chaotrope.

In this study, the addition of carrier RNA to the cell lysate increased the yield of DNA in both the robotic and organic extractions with no adverse effect upon downstream sample analysis (i.e., either for DNA quantification by qPCR or STR analysis). This one modification to the robotic protocols allows for recoveries equal to, or greater than, those obtained with a standard organic extraction procedure, making the BioRobot® EZ1 and BioRobot® M48 workstations, in conjunction with the EZ1 DNA Tissue kit and MagAttract DNA Mini M48 kit, respectively, an option for the extraction of DNA from forensic case samples. The robots' automated extraction protocol and nonreliance on toxic reagents make them attractive alternatives to manual organic extraction.

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