Journal of Clinical Laboratory Analysis 21:244–248 (2007)

Comparison of Manual and Automated Nucleic Acid Extraction From Whole-Blood Samples

Kathrin Riemann,[1]* Michael Adamzik,[1,2] Stefan Frauenrath,[1] Rupert Egensperger,[3] Kurt W. Schmid,[3] Norbert H. Brockmeyer,[4] and Winfried Siffert[1]

[1]*Institute of Pharmacogenetics, University Hospital Essen, Essen, Germany*
[2]*Department of Anaesthesiology, University Hospital Essen, Essen, Germany*
[3]*Institute of Pathology and Neuropathology, University Hospital Essen, Essen, Germany*
[4]*Hospital for Dermatology and Allergy, Ruhr University Bochum, Bochum, Germany*

Nucleic acid extraction and purification from whole blood is a routine application in many laboratories. Automation of this procedure promises standardized sample treatment, a low error rate, and avoidance of contamination. The performance of the BioRobot M48 (Qiagen) and the manual QIAmp® DNA Blood Mini Kit (Qiagen) was compared for the extraction of DNA from whole blood. The concentration and purity of the extracted DNAs were determined by spectrophotometry. Analytical sensitivity was assessed by common PCR and genotyping techniques. The quantity and quality of the generated DNAs were slightly higher using the manual extraction method. The results of downstream applications were comparable to each other. Amplification of high-molecular-weight PCR fragments, genotyping by restriction digest, and pyrosequencing were successful for all samples. No cross-contamination could be detected. While automated DNA extraction requires significantly less hands-on time, it is slightly more expensive than the manual extraction method. J. Clin. Lab. Anal. 21:244–248, 2007.

Key words: DNA purification; quality; quantity; roboter; PCR

INTRODUCTION

Many different methods can be applied for extraction of DNA from several specimens. In genetic laboratories DNA is routinely extracted from whole-blood samples and applied, e.g., to standard polymerase chain reaction (PCR) techniques. Common extraction procedures include phenol chloroform purification or the use of commercially available kits. However, these manual preparation protocols are not appropriate for mid-sized to high-throughput DNA extraction, which is necessary for the setup of a DNA repository. Numerous samples have to be handled in a time-saving manner to generate DNA suitable for further processing.

To be suitable for long-time storage, DNA has to fulfill certain criteria. These criteria include a minimum of quantity, high-molecular-weight components, as well as adequate purity to guarantee high-quality results in subsequent analyses (1). Automation of DNA extraction provides the advantage of standardized large sample treatment and avoidance of errors during routine handling and contamination due to unsealed intermediate steps (2,3). While most manual DNA extraction kits are based on silica membranes, the BioRobot M48 (Qiagen, Hilden, Germany) uses magnetic-beads technology. After cell lysis the DNA binds to membranes or beads, respectively, followed by two washing steps and elution in water or buffer.

In this study a commercial kit for manual preparation (Qiagen) and an automated processing technique (Qiagen) for DNA extraction and purification from blood were compared in terms of performance. Furthermore, various routine PCR downstream applications were

*Correspondence to: Dr. rer. nat. Kathrin Riemann, Institute of Pharmacogenetics, University Hospital Essen, Hufelandstr. 55, 45147 Essen, Germany. E-mail: kathrin.riemann@uk-essen.de

Grant sponsors: IFORES, University Hospital Essen; Competence Network HIV/AIDS.

Received 25 October 2006; Accepted 22 February 2007
DOI 10.1002/jcla.20174
Published online in Wiley InterScience (www.interscience.wiley.com).

tested and compared using DNA extracted with both methods. The time, effort, and costs required for both techniques were also analyzed.

MATERIALS AND METHODS

DNA Extraction

Genomic DNA from 420 whole-blood samples collected within the Competence Network HIV/AIDS for the setup of a DNA repository were extracted using two different methods. Manual DNA extraction of the blood samples was performed with the QIAmp® DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's manual. A maximum of 30 samples (maximum centrifuge load) can be manually processed in each extraction round with the supplied fast-spin columns based on silica-gel membranes. The BioRobot M48 (Qiagen) was applied for DNA extraction from the same blood samples using the MagAttract DNA Blood Mini M48 Kit (Qiagen) following the manufacturer's instructions. The robotic workstation allows automation of the magnetic-particle purification technology. A maximum of 48 samples can be processed during each run. The input volume of blood and output volume after elution were 200 µL for both methods.

DNA Quantity and Purity

After DNA extraction the DNA concentration of each sample was determined by means of a NanoDrop® ND-1000 spectrophotometer (PeqLab, Erlangen, Germany). When assessing nucleic acids, the ND-1000 automatically measures a spectrum ranging from 220 nm to 350 nm. The ratio of the absorbance at 260 nm and 280 nm was used to define DNA purity, and a ratio of approximately 1.8 was used as a standard for pure DNA. As a secondary measure of DNA purity, the ratio of the absorbance at 260 nm and 230 nm was calculated and considered to represent pure DNA within the range of about 1.8–2.2. Additionally, the maximum absorbance of the magnetic beads used in the automated DNA extraction at 320 nm was noted to check for potential contamination due to remaining beads in the eluate.

Downstream Applications

The quality of manually and robotically extracted DNA was tested in several PCR-based applications. For amplification of high-molecular-weight PCR products, the *CYP2D6* gene was analyzed for its presence (*CYP2D6*5*) with primers described elsewhere (4). Using a modified slowdown PCR technique (5), the products of the *CYP2D6*5* PCR are as follows: the wild-type allele yields a 5.1-kb fragment and the deletion yields a 3.2-kb fragment. Manually and robotically extracted DNA was also used for single nucleotide polymorphism (SNP) genotyping. The *GNB3* C825T polymorphism was analyzed with *Bse*DI restriction digest after PCR. The restriction digest generated a 209-bp fragment for the TT genotype, and 152- and 57-bp fragments for the CC genotype. Heterozygous samples displayed three fragments (6). Furthermore, DNA was regenotyped for the same polymorphism by applying the more sensitive pyrosequencing technology (7).

Time and Cost Analysis

The cost per sample was calculated for manual and automated extraction, including the test kit, reagents, and consumables, based on list prices. The total time requirements for processing a maximum of samples and the actual hands-on time were taken during each run for both methods.

Statistical Analysis

The mean values and standard deviation (SD) were calculated for all measurements and compared by means of a two-tailed paired *t*-test. $P < 0.05$ was considered to be statistically significant. Statistical analysis was performed using Graphpad Prism 4.0 (Graphpad Software, San Diego, CA).

RESULTS

Whole-blood samples were used for the comparative analysis of DNA extraction and purification of the QIAmp® DNA Blood Mini Kit (Qiagen) and the BioRobot M48 (Qiagen). After the extraction procedures were completed, the DNA was immediately quantified by spectrophotometry. No dropout was detected in the manually prepared samples, but one sample processed by the robot yielded no DNA. Therefore, only 419 of 420 samples were included in subsequent analyses. Samples that were manually extracted displayed a mean concentration of 23.95 ± 0.65 ng/µL (range = 4.00–127.00 ng/µL). Extracts prepared by the robot showed a mean concentration of 15.23 ± 0.35 ng/µL (range = 1.20–56.30 ng/µL; Fig. 1a). The difference in quantity was statistically significant ($P < 0.0001$). Next, the quality of eluates was assessed by comparing the 260/280 ratios of DNAs. The mean value of the manual extracts was 1.90 ± 0.02 (range = 1.32–4.33), and the robot extracts displayed a mean value of 1.98 ± 0.02 (range = 1.15–4.98; Fig. 1b). This difference was also statistically significant ($P = 0.009$). As a secondary measure of purity, the 260/230 ratio was determined. DNA extracted with both

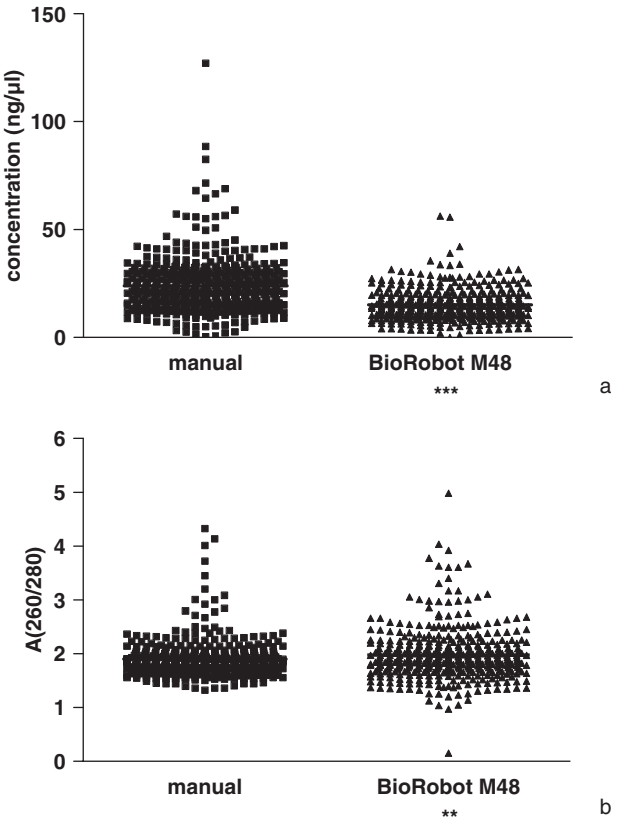

Fig. 1. Comparison of manual and automated DNA extraction. **a:** DNA concentration of eluate measured by spectrophotometer. **b:** DNA purity measured by spectrophotometer. $^{***}P<0.0001$; $^{**}P<0.01$.

methods showed ratios below the optimum (manual: 1.42 ± 0.08; roboter: 0.85 ± 0.06; $P<0.0001$). DNA processed by the robot was also tested for remaining magnetic beads in the eluate. The beads had a maximal absorbance at 320 nm. None of the samples exhibited contamination.

Further analyses of DNA quality included different PCR applications that are regularly applied in genetic laboratories. Detection of the *CYP2D6* null allele can be accomplished by a multiplex long PCR. Figure 2a shows the results of this approach. All manually extracted samples were successfully tested, and 1% of the robotically extracted DNAs failed to be amplified. None of the samples possessed the *CYP2D6*5* allele. Additionally, SNP genotyping for the *GNB3* C825T polymorphism was performed. Independently of the extraction procedure used, all samples were well amplified and digested, yielding all possible genotypes (Fig. 2b). Regenotyping of the PCR products for the same polymorphism by pyrosequencing confirmed the results of all samples with comparable peak intensities (Fig. 2c).

The sample capacity, costs, and time spent were compared for both systems (Table 1). The BioRobot M48 was able to process significantly more samples per run than the QIAmp method. The total time for handling a maximum of specimens was greater for the BioRobot M48, since a run at full capacity takes about 2½ hr. However, the actual hands-on time was less for the robot compared to the manual extraction procedures. The required chemicals and materials were more expensive for the robotic workstation.

DISCUSSION

The comparison of manual and automated nucleic acid extraction processes revealed a slight advantage for manually extracted DNA regarding technical issues. The yield of manually prepared DNAs was 57% higher than the yield of robot extracts when the procedures were performed according to the supplier's manual. Cell lysis in both methods is achieved by the use of different buffers that may have variable efficiencies. Likewise, the purity of manually extracted DNAs was closer to the optimum value compared to DNAs produced by the BioRobot, which argues for more protein takeover in the workstation. Only QIAmp applies proteinase K pretreatment, which degrades proteins that can be tightly bound to DNA and hence uncovers nucleic acids. Besides an optimized and validated protocol for difficult forensic specimens, there is no recommendation available for an improved performance for whole blood on the BioRobot M48 to date (8). Interestingly, the 260/230 ratios determined for both methods are far below the optimum. Remaining salts in the eluate usually account for these low values, even though the last washing step in both extraction systems requires an 80% ethanol wash. However, ionic strength is known to influence the absorbance of nucleic acids, especially the absorbance at 260 nm, which may have affected the 260/230 ratio (9).

The evaluation of downstream applications revealed no inhibiting factors in either type of product for PCR. It was possible to amplify both low- and high-molecular-weight PCR products. Only 1% of samples extracted with the BioRobot failed to generate fragments greater than 3 kb in length. SNP genotyping was successful with all DNAs extracted either way. The most common genotyping methods–restriction digest and pyrosequencing–were applied without any problems. Thus, no further purification steps after preparation are necessary even if the absorbance ratios are not optimal for some purity criteria. Cross-contamination as well as contamination of remaining magnetic beads could be excluded for the robotic workstation. The BioRobot provides an integrated tip guard to prevent dripping by

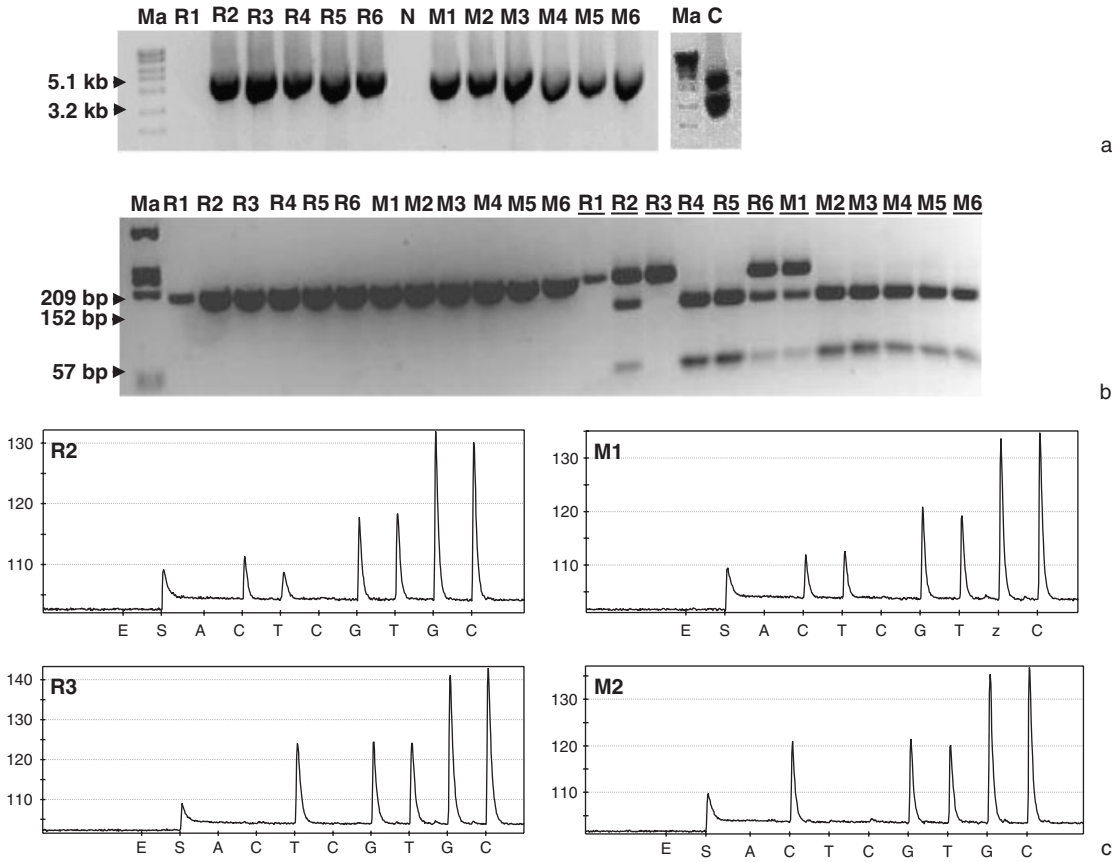

Fig. 2. PCR downstream applications with manually and robotically extracted DNA. **a:** *Cyp2D6*5* PCR generating a 5.1-kb fragment representing the wild-type, and a 3.2-kb fragment for the deletion. **b:** *GNB3* PCR generating a 268-bp fragment followed by restriction digest for C825T polymorphism. **c:** C825T genotyping by pyrosequencing. Ma, marker; R, robotically extracted DNA; N, negative control; M, manually extracted DNA; C, Control; R, M, digested PCR products.

TABLE 1. Time and cost comparison for manual and automated DNA extraction

Method	Maximum number of samples/run	Total time (min)/run[a]	Hands-on time (min)/run	Cost (€)/sample
QIAmp	30	100	90	2.57
BioRobot M48	48	170	20[b]	4.11[c]

[a]Total time includes hands-on time.
[b]Hands-on time for the BioRobot M48 does not include time necessary to homogenize stored blood samples.
[c]Cost includes price of Starter Pack for consumables.

the tips and UV sterilization between runs. Furthermore, no contamination during manual extraction was obvious. All genotype results obtained by restriction digest were confirmed by the more sensitive pyrosequencing technique. Therefore, the DNA quality achieved by both systems is sufficient for general genetic methods, as has already been shown to be viable for RNA extraction (10).

Overall, the performance values of the QIAmp and BioRobot M48 were comparable to each other regarding further possible applications of extracted DNA, even though manual extraction achieved better technical results. Less hands-on time and the fact that a greater number of samples can be processed at once make the BioRobot M48 a real alternative for larger sample preparations, even though the cost per sample is higher than that for QIAmp extractions.

REFERENCES

1. National Genome Research Network (Nationales Genomforschungsnetz). Home page. http://www.ngfn.de.

2. Moss D, Harbison SA, Saul DJ. An easily automated, closed-tube forensic DNA extraction procedure using a thermostable proteinase. Int J Legal Med 2003;117:340–349.
3. Montpetit SA, Fitch IT, O'Donnell PT. A simple automated instrument for DNA extraction in forensic casework. J Forensic Sci 2005;50:555–563.
4. Hersberger M, Marti-Jaun J, Rentsch K, Hänseler E. Rapid detection of the CYP1D6*3, CYP2D6*4, and CYP2D6*6 alleles by tetra-primer PCR and of the CYP2D6*5 allele by multiplex long PCR. Clin Chem 2000;46:1072–1077.
5. Bachmann HS, Siffert W, Frey UH. Successful amplification of extremely GC-rich promoter regions using a novel 'slowdown PCR' technique. Pharmacogenetics 2003;13:759–766.
6. Siffert W, Rosskopf D, Siffert G, et al. Association of a human G-protein β3 subunit variant with hypertension. Nat Genet 1998;18:45–48.
7. Hauner H, Meier M, Jöckel KH, Frey UH, Siffert W. Prediction of successful weight reduction under sibutramine therapy through genotyping of the G-protein β3 subunit gene (GNB3) C825T polymorphism. Pharmacogenetics 2003;13:453–459.
8. Nagy M, Otremba P, Krüger C, et al. Optimization and validation of a fully automated silica-coated magnetic beads purification technology in forensics. Forensic Sci Int 2005;152:13–22.
9. Wilfinger WW, Mackey K, Chomczynski P. Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. Biotechniques 1997;22:474–481.
10. Knepp JH, Geahr MA, Forman MS, Valsamakis A. Comparison of automated and manual nucleic acid extraction methods for detection of enterovirus RNA. J Clin Microbiol 2003;41:3532–3536.