Klin. Biochem. Metab., 13 (34), 2005, No. 1, p. 10–13.

MagNA Pure System for DNA Extraction from Whole Blood Clinical Verification of Pre-Analytical Phase of DNA Testing

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SUMMARY
The objective of our work was to verify the clinical usefulness of Roche MagNA Pure system for extraction of human genomic DNA from whole blood, and to compare it to Qiagen microcolumn-based extraction system. We examined the MagNA Pure LC DNA Isolation Kit I on the MagNA Pure LC Instrument. Intra-assay precision determined on pooled blood samples was 5.4% and day-to-day precision was 7.8%. DNA purity was 1.9 ± 0.1. The mean recovery within the DNA range 2.2–35.0 μg was 43%. A comparison of the MagNA Pure extraction process to Qiagen procedure performed on 32 human venous blood specimens gave statistically very similar DNA yields (6.2 ± 1.0 vs. 6.5 ± 1.0 μg). A regression equation was Y = 0.86X + 0.66 (r = 0.93, P < 0.001). Three various real-time PCRs evaluated via the crossing point analysis showed similar amplification profiles. Both compared extraction systems provide DNA of high quantity, purity and PCR amplificability. The MagNA Pure instrument contributes to reliable and comfortable preparation of DNA samples for consequent molecular analyses.
Key words: DNA, automated extraction, paramagnetic microparticles, verification, PCR.

Introduction

Directive 98/79/EC guides laboratory medicine in Europe to large and preferable using properly tested and documented in vitro diagnostic medical devices and their accessories.

From that point of view the role of bio-analysts working in clinical laboratories more closely associates with completely validated technologies, intra-laboratory verification of commercial methods and products, long-term total quality management, and making standard operation protocols including the pre-analytical phase of testing.

A sufficient amount of purified DNA molecules (without any signs of degradation) is a fundamental pre-analytical factor influencing molecular biology examination and correct clinical interpretation of analytical results.

Conventional time-consuming extraction procedures based mainly on ethanol precipitation of DNA after cell lysis and removal of contaminants by organic solvents have recently been replaced by faster and more automated separation methods.

The principle of these approaches is DNA affinity to the silica surface of chromatographic microcolumns (spin or vacuum systems), or paramagnetic DNA-binding microparticles (1–3).

The objective of our work was to verify the clinical usefulness of Roche MagNA Pure system for extraction of human genomic DNA from whole blood, and to compare this robotic technology to Qiagen microcolumn-based extraction system, which has routinely been used in our laboratory since 1999.

Materials and Methods

We examined and verified the MagNA Pure LC DNA Isolation Kit I on the MagNA Pure LC Instrument (Roche Diagnostics, Mannheim and Penzberg, Germany).

Intra-assay precision. Pooled blood from six individual venous EDTA blood specimens was extracted eighteen-times in one series via the High Performance (HP) Protocol. DNA was isolated from 200 μl of the pool and eluted with 150 μl of the Elution Buffer. DNA yield and purity were determined by spectrophotometric (OD260) measurement and calculation of OD260/280 ratio, respectively.

Inter-assay precision. Mixing ten other individual blood specimens made pooled blood for that experiment. A first part of the pool was used for preparation of twenty-five 250-μl-pool aliquots. The aliquots were frozen at –20 °C (= series A). Samples of B-series were formed by addition of one volume of the pool into two volumes of DEPC saline solution. Twenty-five 250-μl series B aliquots were frozen at –20 °C. Five extraction runs were performed in five days. In each run five 200 μl samples from the series A and B were processed. The elution volume was 150 μl. DNA was characterized spectrophotometrically.

Recovery. A third part of the above prepared pool, which contained 8.3×10^6 leukocytes per ml, was centrifuged at 1500 × g for 10 min at 4 °C. Using a pipette, buffy coat was removed. 100 μl of the buffy-coat-poor blood (5 ×104 leukocytes per ml; separation efficiency 99.4%) was added to 100 μl of purified DNA samples of known amounts (final DNA concentrations ranged from 11.2 to 174.2 μg/ml), and consequently re-extracted by the MagNA Pure HP Protocol. The elution volume was

100 µl. The above-mentioned samples of purified DNA had primarily been extracted by the QIAamp Blood Mini Kit (Qiagen, Hilden, Germany) prior to the recovery experiment. DNA concentration was determined spectrophotometrically.

Comparison of MagNA Pure and Qiagen extraction procedures. Thirty-two individual venous blood samples of 200-µl volume were isolated with the MagNA Pure system and Qiagen microcolumns according to the manufacturers' instructions. The final elution volume was 150 µl. Evaluation was based on: (i) spectrophotometric analysis; (ii) real-time PCR amplification in the LightCycler (factor V Leiden, prothrombin G20210A, and MTHFR C677T mutation assays), and (iii) 0.5% agarose gel electrophoresis of DNA and PCR products.

Statistical analysis. Results were expressed as means, standard deviations (SD), and coefficients of variation (CV) in percentages. Data were compared using the median test, correlation analysis, or linear regression analysis.

P values lower than 0.001 were considered statistically significant.

Results

Intra-assay precision testing 200 µl pooled blood (N = 18) revealed an average DNA yield of 5.37 µg and CV 5.4%. The day-to-day precision experiment (five runs with five A- and B-series samples) showed CVs 7.8% (A-series, average DNA yield 7.5 µg) and 10.7% (B-series, 2.8 µg), respectively. DNA purity was 1.9 ± 0.1.

The mean recovery rate in the buffy-coat-poor samples containing DNA within the range 2.2–35.0 µg was 43%. However, the recovery was inversely related to DNA amounts in the samples (see Fig. 1).

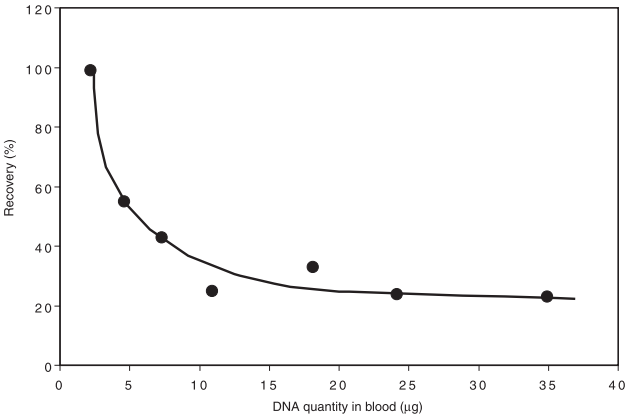

Fig. 1. Recovery of the MagNA Pure procedure used for DNA extraction from buffy-coat-poor blood samples with different DNA quantity.
The mean recovery was 43%.

A comparison of the MagNA Pure extraction process with Qiagen microcolumn procedure was performed on 32 blood specimens with a normal number of white cells. Both extraction approaches gave statistically very similar DNA yields (6.2 ± 1.0 vs. 6.5 ± 1.0 µg). More detailed data are summarized in Table 1. Results of linear regression analysis are illustrated in Figure 2. A regression equation was Y = 0.86X + 0.66 (Pearson's correlation coefficient = 0.93, P < 0.001).

Table 1. Comparison of DNA yields using the MagNA Pure LC DNA Isolation Kit and the QIAamp DNA Blood Mini Kit

Parameter	MagNA Pure	Qiagen
DNA concentration (µg/ml ± SD)	42 ± 8	43 ± 9
DNA yield mean (µg ± SD)	6.2 ± 1.0	6.5 ± 1.0
CV (%)	20	21
Median yield	5.7	6.0
Minimal yield	4.9	4.4
Maximal yield	9.5	9.7
Purity (mean ± CV)	2.0 ± 0.1	2.0 ± 0.2

The differences were not statistically significant.

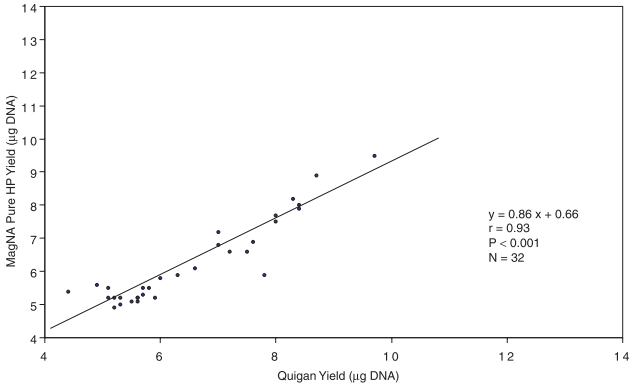

Fig. 2. Linear regression analysis comparing whole blood DNA yields using MagNA Pure and Qiagen extraction procedures

Table 2 demonstrates real-time amplification efficiency via the crossing point analysis of Leiden, G20210A, and C677T PCR assays. Using clinical samples all three reactions showed similar amplification profiles (upper part of Table 2). CP values of the analysed samples were normalized to the CP of the control sample used in each run under the same conditions. Differences between MagNA and Qiagen CP were not statistically significant in any tested assay.

A direct comparison of PCR efficiency was done with four DNA samples amplifying in the same run. Two of them had normal and the remaining had abnormally low Qiagen DNA yields. The data are manifested in the lower part of Table 2. In Leiden analysis the mean CP value of MagNA DNAs was higher than the Qiagen CP. The crossing points in the case of G20210A PCR were comparable to each other (Fig. 3). However, despite different DNA yields (see Table 2), concentrations, and integrity (Fig. 4A), Leiden and G20210A PCR products formed from those four MagNA Pure and Qiagen DNAs gave very comparable intensity of fluorescence on agarose gels (Fig. 4B and 4C).

Table 2. Crossing points (CP) comparison of MagNA and Qiagen DNAs via real–time PCR for factor V Leiden, prothrombin G20210A, and MTHFR C677T mutations

Extraction	Leiden (N = 51)		G20210A (N = 51)		C677T (N = 25)	
	CP (mean ± SD median)		CP (mean ± SD median)		CP (mean ± SD median)	
MagnaPure	27.43 ± 1.20	27.39	23.86 ± 0.78	23.87	20.96 ± 0.53	20.84
		NS		NS		NS
Qiagen	27.34 ± 0.97	27.26	23.58 ± 0.55	23.57	21.68 ± 0.58	21.15

Extraction	Leiden (N = 4)	G20210A (N = 4)	DNA Yield (µg)
	CP (mean ± SD)	CP (mean ± SD)	Mean ± SD
MagnaPure	28.78 ± 1.11	23.17 ± 0.42	6.21 ± 1.01
Qiagen	26.41 ± 0.67	22.27 ± 0.75	4.93 ± 0.85

Data are expressed as means, SD, and medians. NS: statistically non–significant changes. Upper part: CP comparison of samples from different PCR runs. Lower part: CP and DNA yield comparisons of the samples examined in one series.

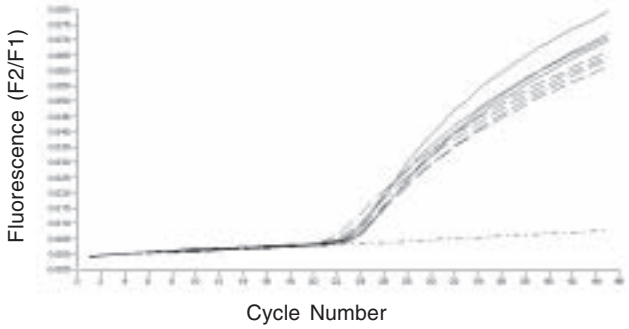

Fig. 3. PCR amplification of the prothrombin gene Solid line curves: MagNA Pure DNA templates; dashed line curves: Qiagen DNA templates; dot-and-dashed line curve: no DNA template in the reaction capillary.

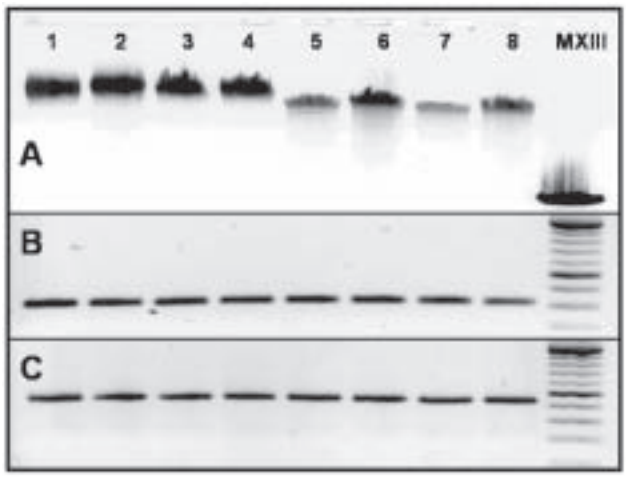

Fig. 4. Electrophoretic analysis of isolated DNA samples (4A), factor V PCR products (4B), and prothrombin PCR products (4C) Lines 1–4: DNA samples extracted with the MagNA Pure system; lines 5–8: DNAs extracted with Qiagen; MXIII DNA Marker (Roche).

Discussion

Clinical application of each new instrument is possible only after its full standardization and validation. The MagNA Pure LC Instrument, a robotic workstation for nucleic acid isolation, offers two optimized genomic DNA extraction protocols (Fast Protocol and High Performance Protocol), both allowing working with 20–200 µl of whole blood samples. These protocols differ at the time of DNA binding and elution. The volumes of reagents are identical. Our preliminary tests indicated that the Fast Protocol is not suitable for routine DNA extractions in our lab widely performing either qualitative or quantitative molecular analyses. DNA yields from the Fast Protocol were significantly lower than usual (1–3 µg, data not presented). That is why we preferred and fully tested only the MagNA Pure HP extraction protocol.

Intra- and inter-assay precisions below 8% established in the whole blood samples reflect very good stability of the extraction process. After blood dilution with saline (1 : 2) CV value increased by just 3%. Also DNA purity around 1.9 was excellent. Our previous examination of the QIAamp DNA Blood Mini Kit had shown the intra-assay variation 8.5% and purity 1.8 ± 0.1. A long-term DNA yield average has been 5.0 µg (range 3.1–9.4 µg) (4).

In the second part we examined DNA extraction recovery to estimate the accuracy of the MagNA Pure extraction procedure. We used purified DNAs added into the buffy-coat-poor blood pool. It was the only possible way how to make a model of the blood sample with known DNA amount. The received mean DNA recovery of 43% is analytically acceptable but the values closely depend on: (i) the content of DNA in individual samples reflecting various numbers of leukocytes in the blood; (ii) the blood volume used for DNA extraction. The DNA recovery rate for physiologic blood specimens was about 40–50%.

The analysis of real blood samples on the MagNA Pure system revealed DNA yields ranging from 5 to 10 µg. These data well correspond with the information provided by Roche (5) and other manufacturers using automated systems based on paramagnetic microparticles (Gentra, Promega, Qiagen, Tecan, etc.).

DNA samples isolated by both the procedures showed, in general, similar amplification efficiency. Using horizontal electrophoresis the MagNA Pure extraction seems to give more concentrated DNA with a higher degree of integrity. Paradoxically, these factors negatively influenced either Leiden or G20210A CP values in four tested samples with different DNA yields. We speculate that further changes in temperature profiles, amounts of DNA or other components added into PCR

mixtures could improve the results. On the other hand, good performance and robustness of used PCR reactions together with the melting temperature analysis carried out in the logarithmic phase of PCR reactions (after 45 cycles) diminish more or less the importance of those CP changes.

The MagNA Pure HP extraction of 32 blood samples lasts about 90 minutes including DNA dilution and transfer from well plates to DNA banking vials. The manual work (15–20 minutes) is connected only with placement of disposable plastics, reagents, and blood samples on the board; other parts of the process are fully automated. DNA extraction with Qiagen manual spin system takes approximately the same time but the number of samples per run is usually lower. If the number is ten or less, Qiagen extraction is ready in forty minutes. Therefore, standardized and fully automated extraction machines are useful pre-analytical devices for DNA laboratories examining more than twenty specimens a week.

In conclusion, both compared systems provide DNA samples of high quantity, purity and PCR amplificability. Thanks to the throughput of 40–70 samples per week, the MagNA Pure instrument contributes to reliable and comfortable preparation of DNA samples for consequent molecular analyses.

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Do redakce došlo 20. 10. 2004.

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