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Comparisons of Three Automated Systems for Genomic DNA Extraction in a Clinical Diagnostic Laboratory

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Purpose: The extraction of nucleic acid is initially a limiting step for successful molecular-based diagnostic workup. This study aims to compare the effectiveness of three automated DNA extraction systems for clinical laboratory use. **Materials and Methods:** Venous blood samples from 22 healthy volunteers were analyzed using QIAamp® Blood Mini Kit (Qiagen), MagNA Pure LC Nucleic Acid Isolation Kit I (Roche), and Magtration-Magnazorb DNA common kit-200N (PSS). The concentration of extracted DNAs was measured by NanoDrop ND-1000 (PeqLab). Also, extracted DNAs were confirmed by applying in direct agarose gel electrophoresis and were amplified by polymerase chain reaction (PCR) for human beta-globin gene. **Results:** The corrected concentrations of extracted DNAs were 25.42 ± 8.82 ng/μL (13.49-52.85 ng/μL) by QIAamp® Blood Mini Kit (Qiagen), and 22.65 ± 14.49 ng/μL (19.18-93.39 ng/μL) by MagNA Pure LC Nucleic Acid Isolation Kit I, and 22.35 ± 6.47 ng/μL (12.57-35.08 ng/μL) by Magtration-Magnazorb DNA common kit-200N (PSS). No statistically significant difference was noticed among the three commercial kits ($p > 0.05$). Only the mean value of DNA purity through PSS was slightly lower than others. All the extracted DNAs were successfully identified in direct agarose gel electrophoresis. And all the product of beta-globin gene PCR showed a reproducible pattern of bands. **Conclusion:** The effectiveness of the three automated extraction systems is of an equivalent level and good enough to produce reasonable results. Each laboratory could select the automated system according to its clinical and laboratory conditions.

Key Words: Automated system, nucleic acid extraction, DNA, polymerase chain reaction

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INTRODUCTION

Nucleic acid extraction is an important initial step of modern molecular diagnostics. A variety of methods are available to extract nucleic acids for analysis. The choice of methods in any laboratory depends on their major sample source and the nature of the assay.[1] Blood, urine, tissue, and body fluid are used in a clinical laboratory, but fresh or frozen blood is the most widely used specimen for nucleic acid extraction. Extracted DNAs are applied to clinical analysis such as genotype analysis, diagnosis of infectious disease, and transplant engraftment assessment. The first limiting factor of a successful molecular-based diagnosis is nucleic acid extraction because the process is labor-intensive and is affected by many interfering sources and inherently scarce amount of target quantity.[2] It is very important to obtain nucleic acid with sufficient amount and good purity. Recently, many commercial kits for nucleic acid extraction from clinical specimens have been introduced.[3]

We aim to compare the efficiency of commercial automated nucleic acid extraction systems from venous blood for clinical laboratory application.

MATERIALS AND METHODS

Twenty-two samples for genomic DNA extraction from healthy volunteers

Venous whole blood samples from 22 healthy volunteers were obtained with K_2-EDTA tubes (BD Vacutainer® BD, Franklin Lakes, NJ, USA). This study was performed with informed consent from all volunteers and conformed to the principles of the Helsinki Declaration.

Genomic DNA extraction

Nucleic acids from each 200 μL of EDTA-whole blood sample were extracted. After cell lysis and protein denaturation, extractions with automated systems were performed. The final extracted elution volumes were 100 μL by MagNA Pure LC, 200 μL by Magtration System 12GC, and 200 μL by QIAcube (Qiagen, Hiden, Germany).

Nucleic acid extraction with automated systems

QIAamp® Blood Mini Kit with QIAcube

QIAamp® Blood Mini Kit (Qiagen, Hilden, Germany) with QIAcube (Qiagen) uses spin column technology. From the 200 μL input volume of EDTA-whole blood sample, a final 200 μL extracted volume was obtained. The steps were as follows: Samples were lysed and heated in the orbital shaker. Each lysate was transferred to a spin column in a rotor adapter and if the lysate needed to be homogenized or cleared, it was transferred to the middle position of the rotor adapter. Nucleic acids were bound to the silica membranes or purification resins of the spin column and washed to remove contaminants. The spin column was transferred to a collection tube for elution of purified nucleic acids.

MagNA Pure LC Nucleic Acid Isolation Kit I with MagNA Pure LC

MagNA Pure LC Nucleic Acid Isolation Kit I with MagNA Pure LC (Roche Diagnostics, GmbH, Mannheim, Germany) is based on magnetic-bead technology with a special buffer containing chaotropic salts and proteinase K. Nucleic acids are bound to the surface of the magnetic glass particles. Cellular debris were removed by several washing steps and the purified nucleic acids were eluted. From the 200 μL input volume of EDTA-whole blood, 100 μL output volume of extracted genomic DNA product was obtained, after the magnetic beads were separated from the solution. We used corrected concentrations of extracted DNAs because the final extracted volume of MagNA Pure LC Nucleic Acid Isolation Kit I was smaller than those of other kits.

Magtration-Magnazorb DNA common kit-200N with Magtration System 12GC

Magtration-Magnazorb DNA common kit-200N with Magtration System 12GC [Precision System Science (PSS) Co. Ltd., Chiba, Japan] adopts the principle of magnetic particle-based technology. The basic steps were as follows: Target cells were lysed by chaotropic reagents, causing the release of nucleic acids which then was absorbed on magnetic silica beads under high-ionic-strength conditions. Each nucleic acid could be selectively captured by changing the ionic strength. Unbound free proteins, nucleic acids, and other small cellular debris were removed by washing with 70% ethanol. Bound nucleic acid could be eluted from the beads with H_2O or a low ionic strength buffer. This system used magnetic beads which were aggregated by applying a magnet to the narrow portion of a tip. The separation and resuspension of magnetic particles were performed within the disposable tip. On this study, from 200 μL input volume of whole blood, we got 200 μL volume of extracted DNA.

Measurement of nucleic acid concentration and purity

The concentrations of extracted DNAs were measured by NanoDrop ND-1000 spectrophotometer (PeqLab, Erlangen, Germany). The NanoDrop ND-1000 is a full-spectrum spectrophotometer for measuring the absorbance of DNA, RNA, protein, and dye. It can measure a spectrum ranging from 220 to 750 nm automatically. The spectrum measurement is then performed with 2 optical fibers installed in the pedestal (emitting light of a Xenon lamp) and the sample arm (spectrometer with linear CCD array). DNA, RNA, protein, or dye in 1.5 μL volume can be measured without cuvettes or capillaries. DNA concentration and optical density (OD) ratio at 260/280 nm to evaluate purity of DNA[1] were calculated at the same time.

Electrophoresis of extracted DNAs for confirmation

The quality of the extracted nucleic acids by three automated systems was confirmed by direct gel loading and was amplified by PCR for human beta-globin gene. After quantifying the extracted nucleic acids by NanoDrop, the products were directly loaded into the 0.8% agarose gel with molecular markers. Reproducibility of each system was evaluated by loading the extracted DNAs from the same sample. For internal control human beta-globin gene identification was performed by PCR (Primers and reagents from Bioneer, Daejeon, Korea). We also used premix AccuPower HF PCR PreMix (Bioneer), containing forward primer 1.0 μL, backward primer 1.0 μL, and extracted DNA 2.0 μL (total 20 μL volume). The human beta-globin gene was amplified with forward (5'-ACACAACTGTGTTCACTAAC-3') and reverse (5'-CAACTTCATCCACGTTCA

Table 1. Characteristics of Three DNA Extraction Systems for Genomic DNA Extraction from Venous Blood Samples

Company	Unit	Qiagen	Roche	PSS
Automated machine	Name	QIAcube	MagNA Oure LC	Magtration System 12GC
Reagent kit	Name	Qiagen blood mini kit	MagNA pure LC DNA Isolation kit I	Magtration-MagaZorb DNA Common Kit-200N
Automation		Full	Full	Full
Principle		Spin column	Magnetic particle	Magnetic particle
Runtime by manufacturer / sample	Minute	5.08	2.81	2.5
Runtime by this study / sample	Minute	5.25	4.55	3.3
Maximum sample number / load	Number	12	32	12

Table 2. Results of Genomic DNA Extraction from Whole Blood Samples by Three Automated Systems

Kit name (input / output volume) sample number	QIAamp blood mini kit (200 / 200 μL)		Magna LC Nucleic Acid Isolation Kit I (200 / 100 μL)		Magtration-Magnazorb DNA common kit-200N (200 / 200 μL)	
	Corrected DNA concentration*	Purity	Corrected DNA concentration*	Purity	Corrected DNA concentration*	Purity
#1	21.64 ng/μL	1.93	18.88 ng/μL	1.93	14.96 ng/μL	1.60
#2	32.86 ng/μL	1.82	23.78 ng/μL	1.83	26.65 ng/μL	1.78
#3	32.20 ng/μL	1.91	25.51 ng/μL	1.91	26.53 ng/μL	1.77
#4	21.34 ng/μL	1.82	19.92 ng/μL	1.97	13.91 ng/μL	1.70
#5	18.87 ng/μL	1.83	20.68 ng/μL	1.90	15.27 ng/μL	1.71
#6	26.37 ng/μL	1.86	24.18 ng/μL	1.88	25.71 ng/μL	1.72
#7	24.21 ng/μL	1.99	22.98 ng/μL	1.90	22.09 ng/μL	1.70
#8	25.64 ng/μL	1.75	18.27 ng/μL	1.92	18.64 ng/μL	1.69
#9	22.54 ng/μL	1.82	21.47 ng/μL	1.93	28.62 ng/μL	1.73
#10	15.32 ng/μL	1.92	16.99 ng/μL	1.91	16.50 ng/μL	1.65
#11	19.74 ng/μL	1.86	17.50 ng/μL	1.88	21.24 ng/μL	1.63
#12	35.61 ng/μL	1.85	30.51 ng/μL	1.89	34.39 ng/μL	1.70
#13	27.93 ng/μL	1.71	29.00 ng/μL	1.95	23.17 ng/μL	1.66
#14	23.23 ng/μL	2.04	21.38 ng/μL	1.87	26.41 ng/μL	1.62
#15	52.85 ng/μL	1.59	25.78 ng/μL	1.91	22.00 ng/μL	1.67
#16	20.26 ng/μL	1.87	19.97 ng/μL	1.89	19.19 ng/μL	1.56
#17	21.94 ng/μL	1.81	28.54 ng/μL	1.90	21.77 ng/μL	1.65
#18	33.03 ng/μL	1.83	9.59 ng/μL	1.71	35.08 ng/μL	1.76
#19	13.49 ng/μL	1.86	16.20 ng/μL	1.60	12.57 ng/μL	1.79
#20	14.62 ng/μL	1.79	16.52 ng/μL	1.90	15.72 ng/μL	1.65
#21	34.01 ng/μL	1.90	46.70 ng/μL	1.95	31.57 ng/μL	1.85
#22	21.51 ng/μL	1.82	24.06 ng/μL	1.90	19.68 ng/μL	1.90
Mean ± SD	25.42 ± 8.82 ng/μL	1.84 ± 0.09	22.66 ± 7.24 ng/μL	1.88 ± 0.08	22.35 ± 6.47 ng/μL	1.70 ± 0.08
Range (min-max)	13.49 - 52.85 ng/μL	1.59 - 2.04	9.59 - 46.70 ng/μL	1.60	12.57 - 35.08 ng/μL	1.56 - 1.90

*Corrected DNA concentration = [elution volume (100 μL) × DNA quantity by NanoDrop ND-1000)] / sample volume (200 μL).

CC-3') primers with the reaction parameters; 94˚C, 5 min and 94˚C, 30 sec, and 53˚C, 30 sec for 30 cycles, and 72˚C, 5 min for extension. PCR products were separated on 2.0% agarose gel and visualized by ethidium bromide staining.

Statistical analysis

The mean and standard deviation (SD) were calculated for all measurements and one-way analysis of variance (ANOVA) with Tukey's Honestly Significant Differences (HSD) post hoc tests were performed with the results. Pearson's correlation coefficient was used for understanding the correlations between the extracted DNAs of each commercial extraction kit. We estimated the correlation between each extraction method. A p value less than 0.05 was considered as significantly statistically different. Statistical analysis was performed by SPSS 12.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Concentrations and purity of extracted DNAs

The characteristics of three DNA extraction systems for genomic DNA from ethylenediaminetetraacetic acid (EDTA)-blood samples were summarized in Table 1. All of the concentrations (ng/μL) and purity (A260/A280) of extracted DNA from 22 whole blood samples were quantified by NanoDrop ND-1000 (Table 2). The average concentration of the total extracted DNAs by three commercial kits was 23.47 ± 7.58 (mean ± SD) ng/μL (min-max, 9.59-52.85 ng/μL). There's no statistical difference in the corrected concentration of extracted DNAs ($p > 0.05$) among the three commercial kits, i.e., QIAamp® Blood Mini Kit (25.42 ± 8.82, 13.49-52.85 ng/μL), MagNA Pure LC Nucleic Acid Isolation Kit I (22.65 ± 14.49, 19.18-93.39 ng/μL), and Magtration-Magnazorb DNA common kit-200N Magtration System 12GC [Precision System Science (PSS) Co. Ltd.] (22.35 ± 6.47, 12.57-35.08 ng/μL).

The mean purity and range of extracted DNAs (A260/A280) of each kit was 1.84 ± 0.09, 1.59-2.04 by Qiagen, 1.88 ± 0.81, 1.60-1.97 by Roche, and 1.70 ± 0.08, 1.56-1.90 by PSS.

Comparisons of the concentration of extracted DNA by each automated system

Pearson's correlation coefficient (r) was 0.43 ($p = 0.045$) between Qiagen and Roche; between Qiagen and PSS was 0.6 ($p = 0.003$); and between Roche and PSS was 0.39 ($p = 0.069$) (Fig. 1).

Downstream applications

Regardless of the concentration of extracted DNAs, band patterns of them in direct agarose gel applications (0.8% agarose) (Fig. 2A) and post human beta-globin gene polymerase chain reaction (PCR) products (2.0% agarose) (Fig. 2B) were performed to evaluate the quality and quantity of extracted nucleic acids. Consistent band patterns of extracted DNAs (Fig. 3A) and post beta-globin gene PCR products were identified (Fig. 3B) from the same samples.

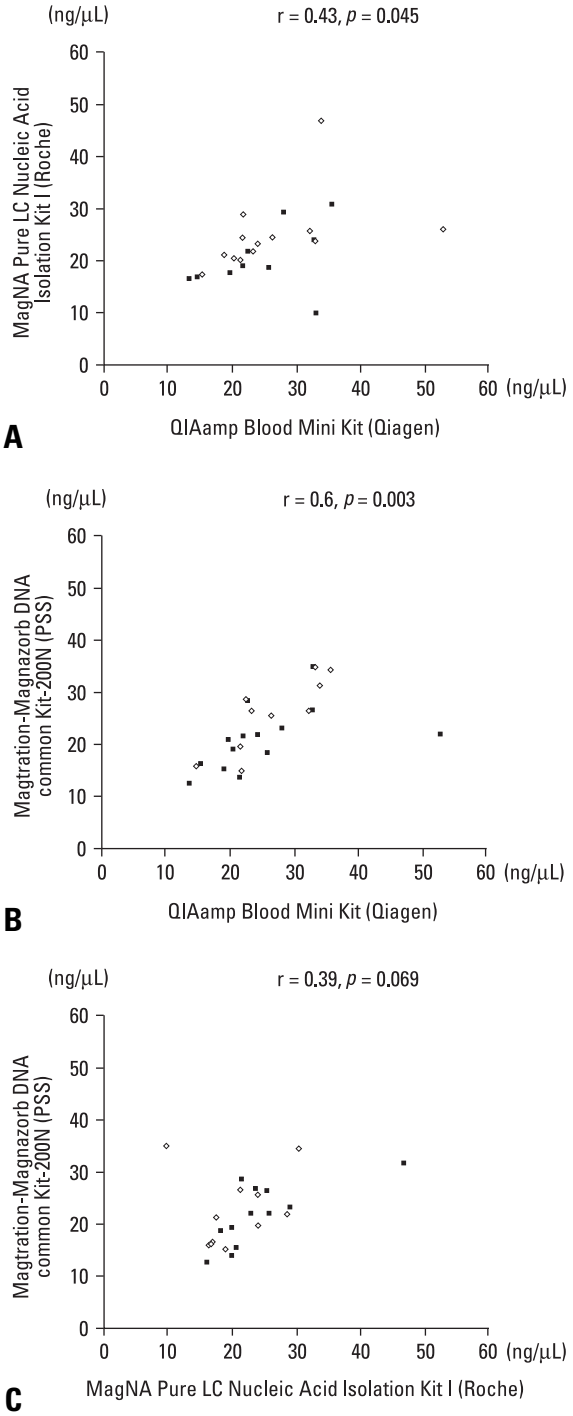

Fig. 1. Correlations between extracted DNAs of three automated DNA extraction systems.

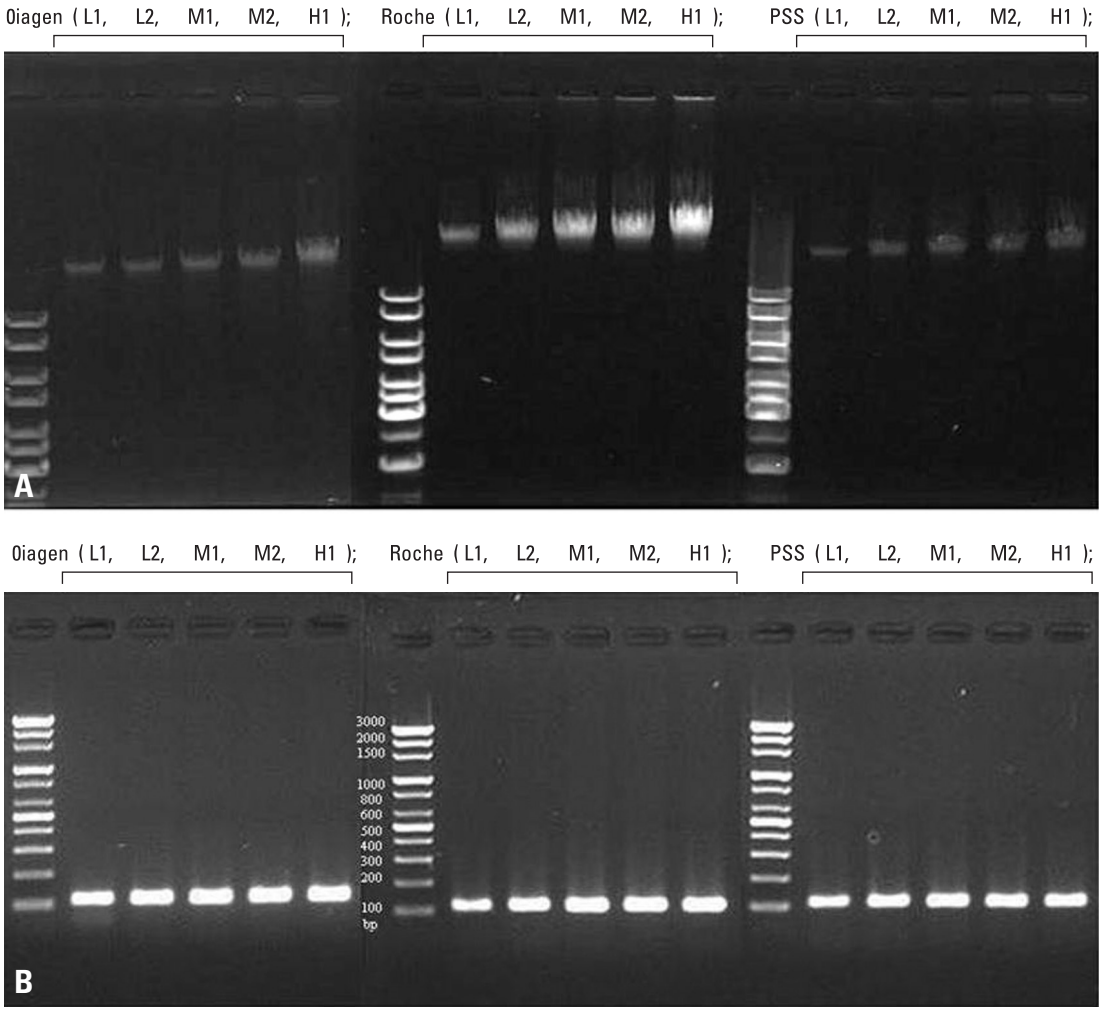

Fig. 2. Images of the DNA extracts and PCR products by three automated extraction systems. (A) Electrophoresis of DNA extracts by three automated DNA extraction systems in 0.8% agarose gel, 0.5% tris-borate-EDTA (L1, L2, M1, M2, H1; lowest, 2nd lowest, middle 1, middle 2, highest DNA quantity). (B) Electrophoresis of beta globin gene PCR products by three automated DNA extraction systems in 2% agarose gel, 0.5% tris-borate-EDTA (L1, L2, M1, M2, H1; lowest, 2nd lowest, middle 1, middle 2, highest DNA quantity). EDTA, ethylenediaminetetraacetic acid.

DISCUSSION

Recently, in the clinical laboratory the number of items and samples of molecular testing were rapidly increasing because many molecular methods have been adopted in modern medicine. The aim of this study is to evaluate the efficiency of automated DNA extraction systems for a clinical diagnostic laboratory. Three systems were selected because they could be used in clinical application and have appropriate capacity for our work load.

Venous blood sample is the most common clinical specimens for molecular diagnosis. It was reported that more than 70% of the original high-molecular weight DNA (> 25 kb) can be recovered from blood stored for 3 days with EDTA or acid citrate dextrose (ACD), even when stored at room temperature.[4] Generally, both EDTA and ACD specimen tubes provide good yields of nucleic acid for PCR and other assays.[1] Garg, et al. suggested that freezing the blood clot had no measurable effect on the quantity or purity of the DNA extracted.[5] Recently, commercial DNA extraction kits with automated devices were launched to overcome large demands of clinical laboratory. Our data could be helpful to adopt automated DNA extraction systems for a clinical laboratory.

We measured the concentration of extracted DNAs through NanoDrop ND-1000, direct gel applications, and beta-globin gene PCR product identification. All testing parameters showed generally good results. Magtration-Magnazorb RNA common kit-N200 (PSS) showed relatively lower purity, compared to the other kits, but it was not beyond an acceptable range. In general, the ratio of A260/A280 is acceptable purity between 1.6-2.0.[6,7]

This lower purity might be caused by contamination with organic solvents or proteins, they could lower optical density (OD), and also prevent accurate nucleic acid quan-

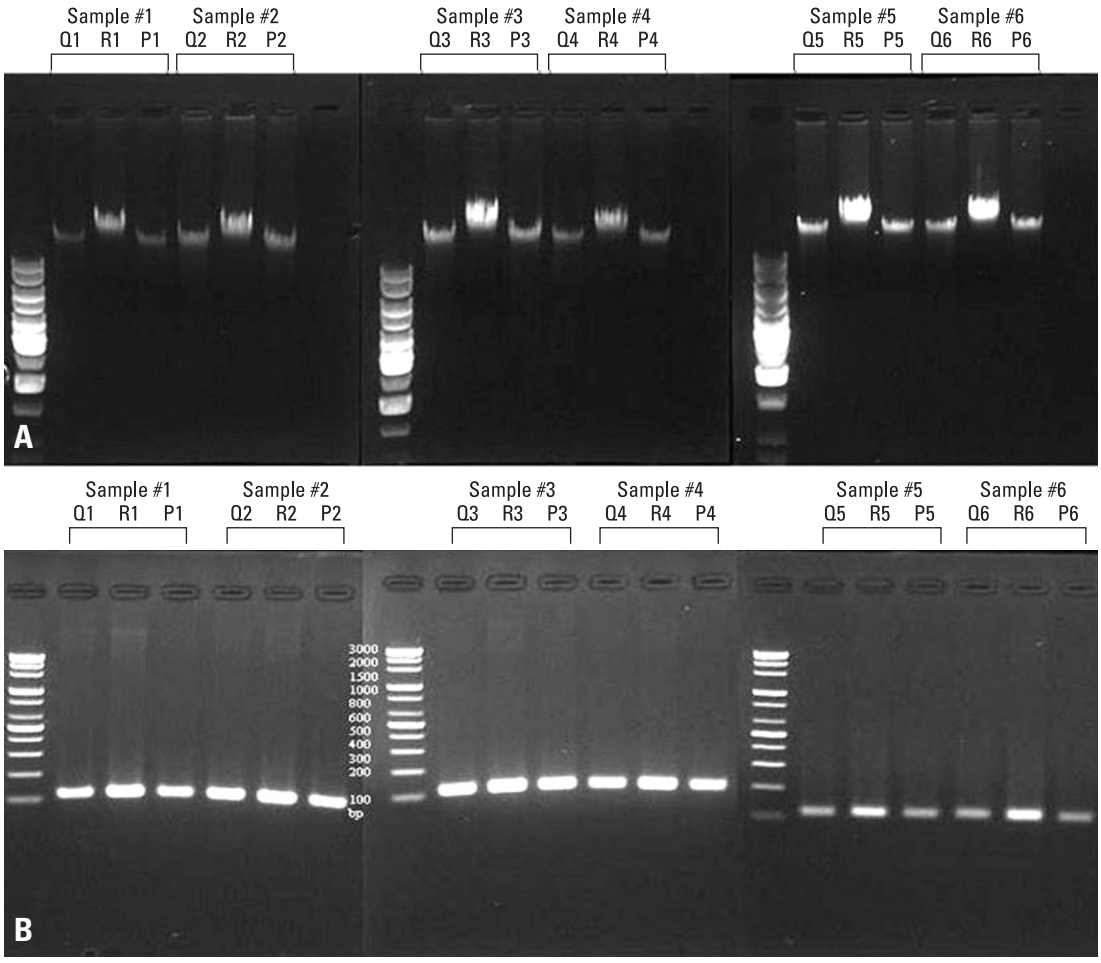

Fig. 3. Images of the DNA extracts and PCR products by three automated extraction systems. (A) Electrophoresis of DNA extracts from six sets of same samples by three automated DNA extraction systems in 0.8% agarose gel, 0.5% tris-borate-EDTA. (B) Electrophoresis of beta-globin gene PCR products from six sets of same samples by three automated DNA extraction systems in 2% agarose gel, 0.5% tris-borate-EDTA. Q, Qiagen; R, Roche; P, PSS; EDTA, ethylenediaminetetraacetic acid.

tification from the OD260 reading.[1] In addition, cross-contamination might be caused because of an aerosol.[8] There are no published reports of contamination in the different comparative studies undertaken until now.[9] Other causes of low DNA purity may be due to handling error of the operator, internal error of the extraction kit, and DNA calculator. Prospective large scale studies are necessary to clarify whether this lower purity result using Magtration-Magnazorb RNA common kit-N200 (PSS) is due to contamination.

For efficient nucleic acid extraction from various specimens, some commercial nucleic acid extraction kits with automated instruments have been developed for clinical application.[10-15] The choice of a nucleic acid extraction method depends on several factors, including assay targets (RNA/DNA-based), specimen type, sample throughput, laboratory workflow, cost, and the performance of the extraction system.[16]

The commercial nucleic acid extraction kits commonly adopt magnetic bead or silica matrix technology with a spin column or vacuum system. In our study, two commercial nucleic acid extraction systems, MagNA Pure LC nucleic Acid Isolation Kit I (Roche) and Magtration-Magnazorb RNA common kit-N200 (PSS), were magnetic bead technologies. In addition, the other Qiagen QIAamp® Blood Mini Kit (Qiagen) uses spin-column technology for genomic DNA extraction. In Pearson's correlation coefficient comparison, extracted DNAs by QIAamp® Blood Mini Kit (Qiagen) showed medium positive correlation ($r = 0.43$, $p = 0.045$) with those by MagNA Pure LC nucleic Acid Isolation Kit I (Roche). Extracted DNAs by QIAamp® Blood Mini Kit (Qiagen) showed positive correlation ($r = 0.6$, $p = 0.003$) with those by Magtration-Magnazorb RNA common kit-N200 (PSS). Extracted DNAs by MagNA Pure LC nucleic Acid Isolation Kit I (Roche) did not show statistically significant correlation ($r = 0.39$, $p = 0.069$) with those by Magtration-Magnazorb RNA common kit-N200 (PSS).

One of the disadvantages of spin column technology is that the columns cannot be placed tightly enough into the collection tubes during centrifugation. Consequently, cross-contamination of an aerosol may occur.[8] Compared to standard separation procedures, magnetic separation techniques have several advantages, including simplicity in handling and high automation potential.[17] However, there are still many disadvantages to all of these methods, such as labor-intensiveness, limited throughput, and technician dependent variability in the efficacy of extraction.[13] In our study, we used the corrected concentration of extracted DNAs for standardized comparison because the final eluted volumes of each commercial kit were not the same. The corrected DNA concentrations were calculated by dividing the output elution volume multiplied by DNA concentration by the input sample volume. The corrected average concentration of extracted DNAs of QIAgen kit was slightly higher than that of the other commercial kits but did not show statistically significant differences among the commercial kits in one-way ANOVA analysis.

The automation of DNA extraction has the advantage of a standardized sample treatment and avoidance of error during routine sample handling and contamination due to intermediate process[10,18] However, there are some debates on the efficiency of DNA extraction methods. Riemann, et al.[3] suggested that the quantity and quality of the generated DNAs were slightly higher with manual extraction method than automated extraction method.

In conclusion, the initial limiting step of DNA extraction for molecular-based diagnostic tests can be successfully automated with commercial systems with efficient and good performances. Each laboratory need to consider the automated system according to its clinical and laboratory conditions.

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