Automated nucleic acid purification for a variety of clinical applications

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The broad choice of protocols on the BioRobot® M48 workstation provides purification of DNA, RNA, or viral nucleic acids from many clinically relevant samples. Purified nucleic acids are well-suited for use in sensitive downstream applications, such as quantitative PCR and RT-PCR for genotyping and viral load monitoring.

Manual purification methods become labor-intensive and time-consuming as throughput increases. Automation of purification procedures enables streamlined workflows and less hands-on time. Standardized, automated purification of nucleic acids eliminates handling errors and increases reproducibility. In this paper, we evaluate automated DNA and viral RNA purification compared with manual methods for a variety of clinical samples and downstream applications.

Comparison of manual and automated DNA purification methods

Automated DNA purification on the BioRobot M48 workstation provided high-quality DNA from a variety of clinical samples, with yields and A_{260}/A_{280} ratios comparable to DNA purified manually using QIAamp® Kits (Table 1). The BioRobot M48 can process 6–48 samples per run providing the flexibility required for daily sample throughput and fast processing of priority samples

Table 1. Yield and Purity of DNA Purified Manually or on the BioRobot M48

Sample type	Amount (µl)	Samples tested	Automated (BioRobot M48)		Manual (QIAamp Kits)	
			A_{260}/A_{280}	Yield (µg)	A_{260}/A_{280}	Yield (µg)
Blood	200	31	1.85 ± 0.09	4.35 ± 1.14	1.85 ± 0.15	3.75 ± 1.61
Blood	350	9	1.87 ± 0.05	8.39 ± 1.39	n.d.	n.d.
Blood	700	36	1.87 ± 0.05	18.44 ± 6.12	n.d.	n.d.
Buccal cells	200	16	1.98 ± 0.17	1.30 ± 0.73	2.04 ± 0.44	2.28 ± 1.0
Paraffin-embedded tissues	200	4	1.95 ± 0.06	3.61 ± 1.42	1.80 ± 0.00	9.09 ± 4.45

n.d.: not determined.

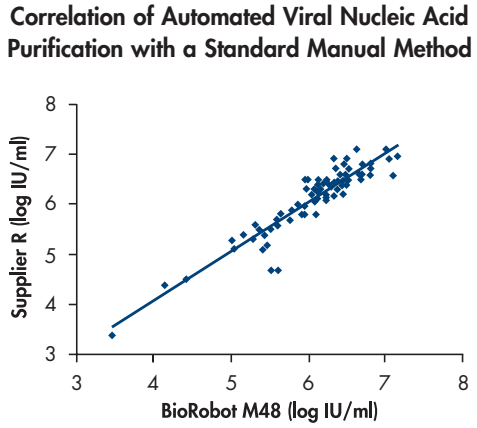

Figure 1 Viral nucleic acids were purified from 81 serum samples using a standard manual method (Supplier R) and the fully automated procedure on the BioRobot M48 workstation. Typical RNA virus C was detected using a quantitative RT-PCR-based assay (Supplier R). Comparison of viral load results for the two methods gave a high correlation (r^2 = 0.8629).

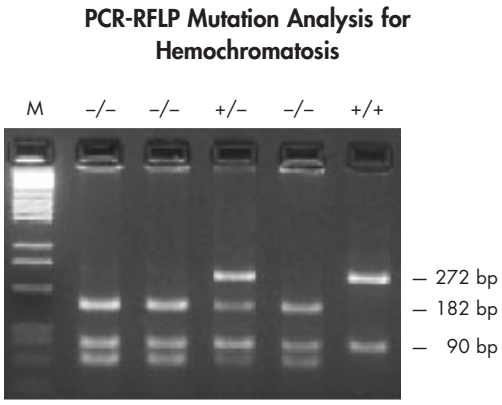

Figure 2 DNA was purified using the MagAttract® DNA Blood Mini M48 Kit on the BioRobot M48 workstation. Following PCR, the amplicons were restriction-digested and analyzed by agarose gel electrophoresis. Samples are shown from individuals homozygous for the H63D mutation (**–/–**), heterozygous (**+/–**), and homozygous for the wild-type allele (**+/+**). **M**: markers.

Viral load results with manual and automated viral RNA purification

Viral load monitoring is a valuable tool for determining the extent and course of a viral infection. Accurate quantification of viral loads requires reliable and efficient purification of viral nucleic acids prior to quantification. In order to assess the reliability of automated viral nucleic acid purification on the BioRobot M48 workstation, 81 serum samples were processed using a standard manual purification method for a typical RNA virus C and the fully automated procedure on the BioRobot M48. Comparison of the 2 methods gave a high correlation, indicating comparable reliability for viral load monitoring (Figure 1).

Mutation detection in hemochromatosis

Hemochromatosis is a disease resulting from significant iron overload. If untreated, organ and tissue damage can occur leading to death. Early detection is essential for proper diagnosis and treatment to prevent the disease's potentially life-threatening complications.

Hemochromatosis can have both genetic and environmental causes. In the United States, most cases are a result of a genetic predisposition. It is estimated that as many as 1 in 200 to 500 people are affected and as many as 1 in 10 carry the gene for hereditary hemochromatosis, making this one of the most common genetic disorders in the United States.

Two allelic variations of the *HFE* gene, H63D and C283Y, are significantly correlated with hereditary hemochromatosis. Using PCR-RFLP analysis, the allelic status can be unambiguously determined. The automated procedure on the BioRobot M48 workstation provided an efficient and reliable method for purification of high-quality DNA for successful PCR-RFLP analysis (Figure 2).

Factor V Leiden mutation detection

Hereditary defects in genes for blood clotting factors is a leading cause for the formation of potentially dangerous blood clots (thrombosis). Approximately 5–8% of the population of the United States has thrombophilia, a genetic predisposition for thrombosis, with the factor V Leiden mutation being one of the most frequent genetic mutations associated with thrombophilia. Among the various genetic components of thrombophilia identified to date, homozygosity for factor V Leiden correlates with the highest risk for developing thrombosis. Accurate and reliable genetic testing for factor V Leiden has the potential for early diagnosis and treatment as well as assessing potential susceptibility to thrombosis. Using melting-curve analysis on the LightCycler® system, normal and mutant alleles can be clearly identified. Automated DNA purification on the BioRobot M48 workstation provided high-quality DNA for mutation detection in research applications involving factor V Leiden (Figure 3).

Factor V Leiden Mutation Detection Using Melting-Curve Analysis

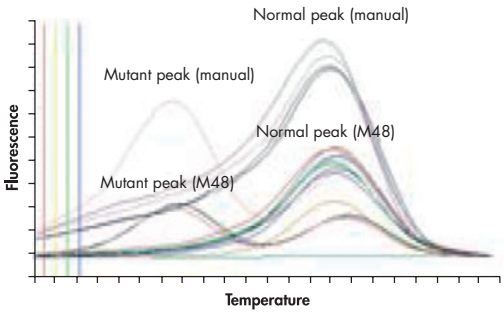

Figure 3 DNA was purified from human whole blood samples using the MagAttract DNA Blood Mini M48 Kit and a standard manual method. Purified DNA was amplified and melting-curve analysis was carried out using the LightCycler system. Melting-curve analysis clearly distinguishes normal and mutant alleles of the Factor V gene.

Conclusions

- Automated DNA purification on the BioRobot M48 provided consistent and reproducible yields from a variety of sample types, including blood, buccal cells, and paraffin-embedded tissues.
- Yields and A_{260}/A_{280} ratios were comparable for manual and automated purification. Automation on the BioRobot M48 reduces workload with less hands-on time and a streamlined workflow.
- Purified DNA and viral RNA performed well in a variety of downstream assays, including mutation analysis using PCR-RFLP and melting-curve analysis, and viral load monitoring by quantitative RT-PCR.

Ordering Information

Product	Contents	Cat. no.
BioRobot M48	Robotic workstation for automated purification of nucleic acids using MagAttract M48 Kits, computer, installation, 1-year warranty on parts and labor*	9000708
App. Package, M48, Genotyping	Software protocol package for genotyping applications on the BioRobot M48 workstation	9016146
App. Package, M48, Pathology	Software protocol package for pathology applications on the BioRobot M48 workstation	9016151
App. Package, M48, Infectious Disease	Software protocol package for infectious disease applications, v. 2.0, on the BioRobot M48 workstation	9016145
MagAttract DNA Blood Mini M48 Kit (192)	For up to 192 x 200 µl preps: MagAttract Suspension B, Buffers, and RNase-Free Water	951336
MagAttract DNA Blood Midi M48 Kit (192)	For up to 192 x 350 µl preps: MagAttract Suspension B, Buffers, and RNase-Free Water	951356
MagAttract DNA Mini M48 Kit (192)	For 192 DNA preps: MagAttract Suspension B, Buffers, Proteinase K	953336
MagAttract Virus Mini M48 Kit (192)	For 192 virus nucleic acid preps: MagAttract Suspension B and RNase-Free Reagents and Buffers	955336
MagAttract Viral RNA M48 Kit (96)	For 96 viral RNA preps: MagAttract Suspension F, Buffers	955235

*Warranty PLUS 2 (cat. no. 9237714) recommended: 3-year warranty, 1 preventive maintenance visit per year, 48-hour priority response, all labor, travel, and parts.

For more information about clinical applications on the BioRobot M48, visit www.qiagen.com/goto/M48clinical .

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