High-throughput automation for RNA isolation from blood stabilized in PAXgene™ Blood RNA Tubes

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Introduction

Ex vivo changes in RNA content and profile in blood samples post-phlebotomy caused by degradation and gene induction are well documented (1, 2). These changes can lead to doubtful or inconsistent results, especially for sensitive quantitative or semi-quantitative analytical methods, such as real-time RT-PCR and microarrays. There is a need, therefore, to stabilize cellular RNA species and to stabilize the gene expression profile at the time of blood collection.

Stabilization of blood in PAXgene Blood RNA Tubes provides a convenient solution for collection of whole blood and immediate stabilization of the cellular RNA profile. PAXgene Blood RNA Tubes contain a proprietary reagent composition based on a patented RNA stabilization technology. This reagent composition protects RNA molecules from degradation by RNases and minimizes ex vivo changes in gene expression. PAXgene Blood RNA Tubes are intended for the collection of human whole blood and stabilization of cellular RNA for up to 3 days at 18–25°C or up to 5 days at 2–8°C. Currently available data show stabilization of cellular RNA for at least 6 months at –20°C or 12 months at –70°C. The manual version of the PAXgene Blood RNA system was cleared by the FDA and CE-marked in Europe for in vitro diagnostic purposes.

To allow a higher sample throughput combined with a controlled and completely documented process (e.g., for clinical trials), we have designed protocols and a kit for two state-of-the-art robotic platforms — the BioRobot® MDx and BioRobot Universal System. This study was conducted to show the performance of these robotic solutions. The purified RNA was tested in quantitative RT-PCR assays and on Affymetrix® GeneChip® probe arrays.

1. Muller et al. (2002) Leukemia **16**, 2395-99.

2. Rainen L et al. (2002) Clin. Chem. **48**, 1883-90.

Materials and methods

Blood was collected in PAXgene Blood RNA Tubes (PreAnalytiX), stored for 20–24 h at room temperature (18–25°C) and then frozen at –20°C. The frozen samples were thawed for 2 h at room temperature before processing. For automated cellular RNA isolation, the PAXgene Blood RNA MDx Kit (PreAnalytiX) was used on both the BioRobot MDx and the BioRobot Universal System (QIAGEN). As reference, the manual PAXgene 96 Blood RNA Kit or (for Affymetrix GeneChip experiments) the PAXgene Blood RNA Kit were used. The quantity and quality of the RNA samples were analyzed by spectrophotometric analysis (SpectraMax® spectrophotometer, Molecular Devices) and on an Agilent® 2100 bioanalyzer. Downstream gene expression analyses were performed using quantitative, real-time RT-PCR assays and Affymetrix GeneChip arrays. For the array analysis, a new PNA-based GeneChip Globin Reduction Protocol combined with the GeneChip Blood RNA Concentration Kit (Affymetrix) was performed prior to labeling.

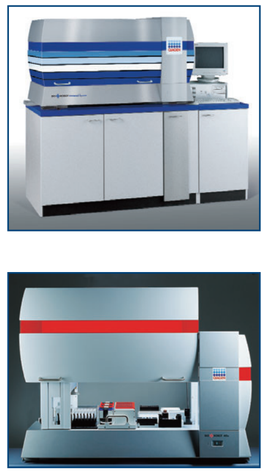


Figure 1 BioRobot workstations for the PAXgene Blood RNA MDx Kit. **A** BioRobot Universal System. **B** BioRobot MDx.

Key parameters and workflow

- RNA yield — ≥3 µg for 95 % of all samples with white blood cell counts of 4.8 x 10^6 – 1.1 x 10^7 cells/ml
- RNA purity — A_{260}/A_{280} ratio between 1.8 and 2.2 for 95% of all samples
- Genomic DNA contamination — <0.2% (w/w) for 95% of all samples
- Total failure rate — no RNA visible in denaturing agarose gel in <0.1% of all samples
- Reproducibility — CV ≤25% between units and different donors for 95% of all samples
- RT-PCR inhibition — none detected, with eluates comprising up to 30% of the reaction volume
- Total processing time for 96 samples — ≤4 hours, including pre-run handling and setup, robot run, downstream processing, and cleanup; <0.5 hour hands-on time
- Samples per run — protocols for batches of 48 and 96 samples
- Sample volume — 2.5 ml human whole blood stabilized in a PAXgene Blood RNA Tube
- Elution volume — 120 µl

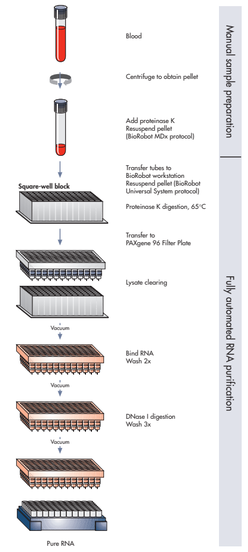


Figure 2 Workflow using the PAXgene Blood RNA MDx Kit.

Results — RNA yields

- Average yield — 7 µg/tube
- 3300 tubes processed with final protocol version in 42 runs (1740 tubes on the BioRobot MDx; 1560 tubes on the BioRobot Universal System)
- Samples with yields ≤3 µg — only 0.9% of samples with yields ≤3 µg
- Reproducibility — average CV = 16%
- Total failure rate — no RNA visible in denaturing agarose gel electrophoresis for only 0.09% of all samples

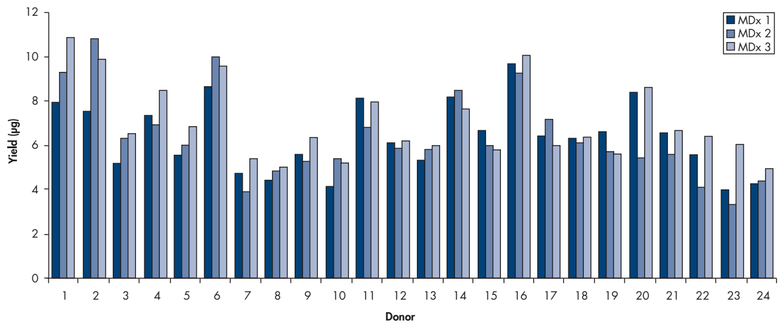


Figure 3 Blood samples from 24 donors (12 replicates per donor) were collected into PAXgene Blood RNA Tubes and processed on 3 different BioRobot MDx workstations (**MDx 1**, **MDx 2**, **MDx 3**) using the PAXgene Blood RNA MDx Kit. The yields were calculated by measuring absorbance at 260 nm.

Results — RNA purity

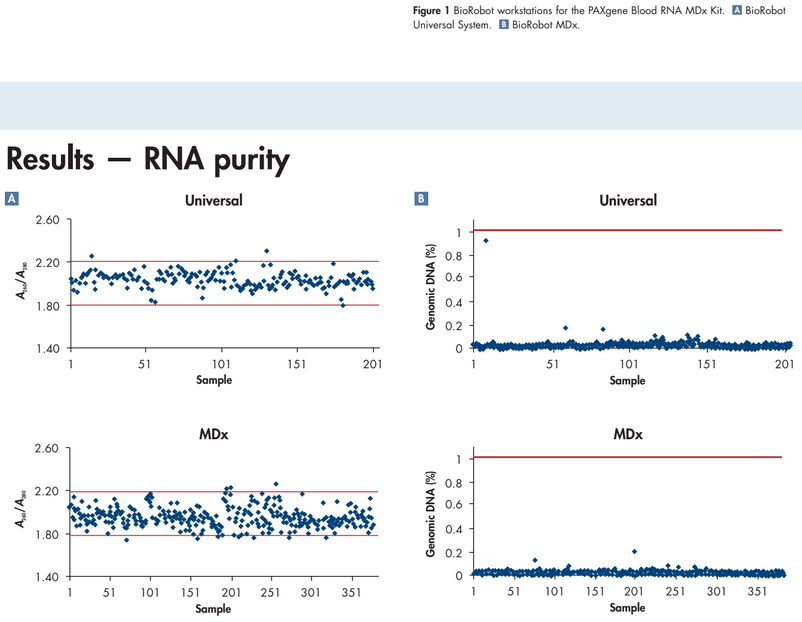
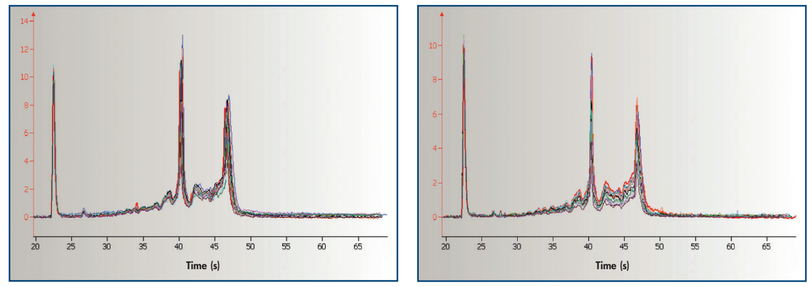


Figure 4 Blood was collected into PAXgene Blood RNA Tubes, and RNA was purified from the samples using the PAXgene Blood RNA MDx Kit on the BioRobot MDx (**MDx**) or BioRobot Universal System (**Universal**). **A** Ratio of the absorbance at 260 to 280 nm (A_{260}/A_{280}). **B** Genomic DNA was quantified using a TaqMan® real-time PCR assay with primers and probe for the β-actin gene. The amount of DNA present is given as a percentage of the total nucleic acid content.

Results — RNA integrity



Sample	1	2	3	4	5	6	7	8	9	10	11	12
RIN	8	8.2	8.1	7.9	8	8.2	8	7.8	7.4	7.8	7.4	7.8

Sample	1	2	3	4	5	6	7	8	9	10	11	12
RIN	8.2	7.4	8	8.4	8.2	8	8.1	8.1	8	7.9	7.9	8.2

Figure 5 Blood was collected into PAXgene Blood RNA Tubes in 24 replicates from a single donor. RNA was purified using the PAXgene Blood RNA MDx Kit on the BioRobot Universal System. RNA was analyzed with an Agilent 2100 bioanalyzer. **A** Overlay of 12 samples on chip A. **B** Overlay of 12 samples on chip B. The RNA integrity numbers (RIN), indicated in the tables below the graphs, show the high quality of the purified RNA.

Results — performance in RT-PCR

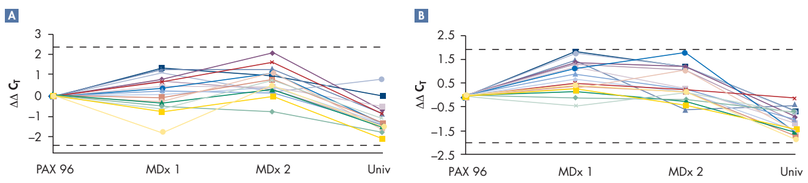
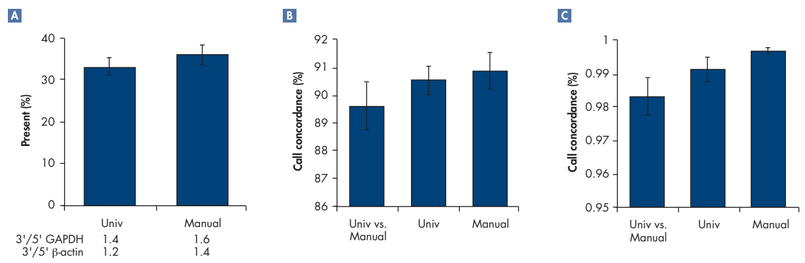


Figure 6 Blood was collected into PAXgene Blood RNA Tubes in twofold replicates from 20 donors. RNA was purified using the PAXgene Blood RNA MDx Kit on 2 different BioRobot MDx workstations (**MDx 1**, **MDx 2**), a BioRobot Universal System (**Univ**), and manually using the PAXgene 96 Blood RNA Kit (**PAX 96**). Purified RNA was analyzed using quantitative, real-time RT-PCR assays, with primers and probe for **A** the FOS and **B** the IL1B transcript. All values were within the ±3x total precision of the assay (2.34 C_T for FOS and 1.93 C_T for IL1B), indicated by the horizontal lines.

Results — GeneChip array analysis



	Univ	Manual
3'/5' GAPDH	1.4	1.6
3'/5' β-actin	1.2	1.4

Figure 7 Blood was collected into PAXgene Blood RNA Tubes in 12-fold replicates from a single donor. RNA was purified using the PAXgene Blood RNA MDx Kit on a BioRobot Universal System (**Univ**) and manually using the PAXgene Blood RNA Kit (**Manual**). The integrity of the RNA was verified with an Agilent bioanalyzer 2100 (average RIN for the BioRobot Universal System was 7.8; for manual purification, 7.7). Globin reduction was carried out, and six U133 2.0 plus GeneChips were hybridized with the purified RNA. **A** Present call rates and 3'/5' ratios of GAPDH and β-actin. **B** Call concordance. **C** Expression correlation between the robotic and the manual samples and in between the preparation methods. (These data were generated in cooperation with Affymetrix.)

Conclusions

- Walkaway purification of cellular RNA on the BioRobot MDx and BioRobot Universal System gave reproducible high yields of RNA from blood stabilized in PAXgene Blood RNA Tubes.
- Purification using the PAXgene Blood RNA MDx Kit provided RNA with high purity and very low genomic DNA contamination, ensuring reliable performance of these RNA samples in sensitive downstream assays.
- Both PAXgene automated systems provided fully automated and reliable RNA purification, enabling high-throughput RNA isolation and purification for gene expression analysis.

Acknowledgements

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