Development and optimization of a protocol for automated RNA purification using the PAXgene™ Blood RNA System

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Introduction

Gene expression analysis in peripheral blood is an important tool in molecular diagnostics and clinical research of cancer, monitoring diseases at the molecular level, and clinical trials of new drugs. One of the major challenges for reliable analysis is the alteration of gene expression starting immediately at the time of aspiration, even after short-term storage of native specimens, due to ex vivo gene induction, repression, and RNA degradation.

PreAnalytiX developed and launched a product system* that enables the collection, stabilization, storage, and transportation of human whole blood specimens, together with a rapid and efficient protocol for isolation and purification of cellular RNA. The system comprises blood collection tubes and an RNA isolation and purification kit. Since the introduction of the PAXgene Blood RNA System, researchers have expressed a need for a low-throughput, automated (LTA) solution for RNA preparation from blood collected in PAXgene Blood RNA Tubes, with purification based on the proven silica-membrane spin-column technology in the PAXgene Blood RNA Kit.

The aim of this research study was to develop and optimize a protocol for LTA RNA purification on the recently launched QIAcube® robotic platform using PAXgene Blood RNA Tubes and the PAXgene Blood RNA Kit.

Note: The use of the PAXgene Blood RNA Kit on the QIAcube is for research use only and not for use in diagnostic procedures. It has not received clearance or approval for clinical use in the USA, Canada, or Europe.

* PAXgene Blood RNA Tube (100), PreAnalytiX (Hombrechtikon, Switzerland), cat. no. 762165; PAXgene Blood RNA Kit (50), PreAnalytiX (Hombrechtikon, Switzerland), cat. no. 762164, 762174.

Materials and methods

Replicate blood samples were collected in PAXgene Blood RNA Tubes, and RNA was isolated and purified using either the standard manual procedure according to the PAXgene Blood RNA Kit Handbook (reference protocol) or the newly developed automated procedure (QIAcube protocol).

The automated solution for RNA preparation from PAXgene Blood RNA Tubes starts with resuspended nucleic acid pellets from 2–12 samples (tubes are centrifuged first in a laboratory centrifuge). Sample lysis with heating and shaking is fully automated on the QIAcube, followed by the bind–wash–elute purification protocol and the final heat denaturation of RNA, also on the QIAcube.

To enable comparison of the automated and manual protocol performance, purified RNA from both protocols was analyzed by UV spectrophotometry, by denaturing standard agarose and capillary gel electrophoresis with calculation of RNA integrity numbers (RIN, Agilent® 2100 bioanalyzer with Nanochip® arrays), in singleplex real-time quantitative RT-PCR (RQ-RT-PCR), and singleplex RQ-PCR assays to measure RNA yield, purity, quality, integrity, RT-PCR inhibition, cross contamination, and traces of genomic DNA (gDNA).

Results — Overview

Use of the automated RNA isolation and purification protocol on the QIAcube was easy and convenient with no failures in sample processing (no membrane clogging or sample loss). This demonstrates the high degree of reliability of the newly developed protocol.

Independent of the 2 protocols used, all RNA purities were high, with no salt contamination, as shown by typical UV scan profiles of pure RNA (Figure 1A) and A_{260}/A_{280} ratios within the range of 1.8 to 2.2. Sample preparation always yielded >3 µg RNA/2.5 ml blood. The overall RNA yield was statistically not different in both protocols, with mean ± SD of 8.5 ± 2.0 µg (automated) and 8.2 ± 1.5 µg (manual) (see Figure 2). Overall RNA quality was evaluated by denaturing standard agarose gel electrophoresis and revealed no gDNA. High RNA integrity was shown by clearly visible 18S and 28S rRNA bands in the gel (Figure 1B). Detailed RNA quality analysis was carried out by Agilent 2100 bioanalyzer RIN calculation from fluorescence profiles after capillary gel electrophoretic separation of RNA. The analysis revealed high and comparable RNA integrity using the PAXgene Blood RNA System with both protocols (Figure 3). As shown in Figure 4, no cross contamination was detectable in the automated protocol using beta-globin as a target sequence, which is known to be the most abundant transcript in whole blood samples and was used to detect traces of positive samples possibly carried over into negative samples. Quantification of gDNA in RNA samples verified that contamination was very low, <1% (w/w) gDNA per total nucleic acids, and comparable between the 2 protocols (Figure 5). No RT-PCR inhibition was detected with eluate volumes of up to 32% (v/v) sample input into reaction using RNA samples purified with the automated protocol (Figure 6).

Results — RNA purity and quality analysis

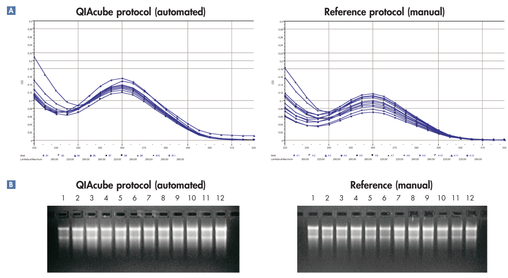

Figure 1. RNA purity and quality analysis from duplicate blood samples of 12 donors in PAXgene Blood RNA Tubes prepared automatically (QIAcube protocol) and manually (reference protocol) with the PAXgene Blood RNA Kit. A RNA was analyzed by UV spectroscopic scan of RNA from 220 to 320 nm for all samples. B RNA sample aliquots were analyzed by denaturing agarose gel electrophoresis (1.2 M formaldehyde; run time: 75 min, 120 V).

Results — RNA yield analysis

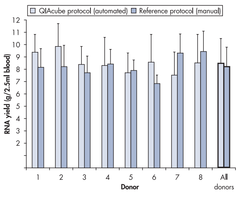

Figure 2. RNA yield analysis of blood samples from 8 donors. 24 replicate PAXgene Blood RNA Tubes per donor were divided, and RNA was prepared automatically (QIAcube protocol) and manually (reference protocol) using the PAXgene Blood RNA Kit, each in 3 consecutive runs of quadruplicate samples per run (run 1: donors 1–3 [n = 3 x 4 = 12]; run 2: donors 4–6 [n = 3 x 4 = 12]; run 3: donors 7–8 [n = 2 x 4 = 8]). RNA was quantified by UV spectroscopic measurement at 260 nm. Means (bars) and standard deviations (SD, vertical lines) of RNA yield from twelvefold replicates are given for all samples.

Results — RNA integrity analysis

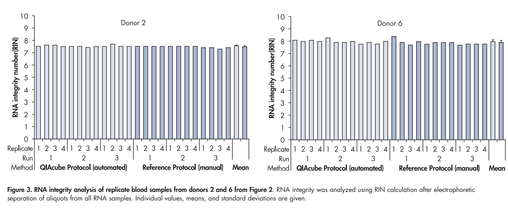

Figure 3. RNA integrity analysis of replicate blood samples from donors 2 and 6 from Figure 2. RNA integrity was analyzed using RIN calculation after electrophoretic separation of aliquots from all RNA samples. Individual values, means, and standard deviations are given.

Results — Cross-contamination analysis

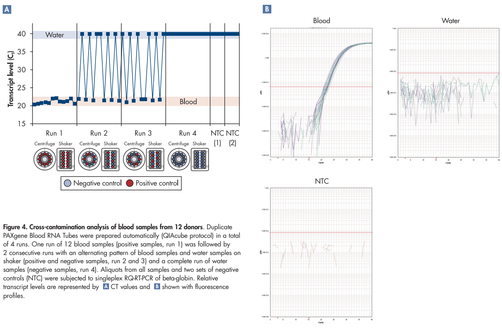

Figure 4. Cross-contamination analysis of blood samples from 12 donors. Duplicate PAXgene Blood RNA Tubes were prepared automatically (QIAcube protocol) in a total of 4 runs. One run of 12 blood samples (positive samples, run 1) was followed by 2 consecutive runs with an alternating pattern of blood samples and water samples on shaker (positive and negative samples, run 2 and 3) and a complete run of water samples (negative samples, run 4). Aliquots from all samples and two sets of negative controls (NTC) were subjected to singleplex RQ-RT-PCR of beta-globin. Relative transcript levels are represented by A CT values and B shown with fluorescence profiles.

Results — gDNA contamination analysis

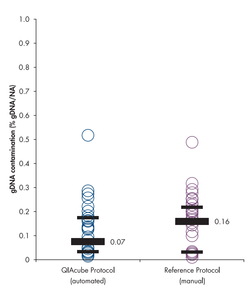

Figure 5. gDNA analysis from blood samples of 8 donors. Each sample was collected in 8 replicate PAXgene Blood RNA Tubes. Four were prepared automatically (QIAcube protocol) and 4 manually (reference protocol) with the PAXgene Blood RNA Kit in 4 consecutive runs (n = 32 [8 x 4] per method). Traces of genomic DNA were quantified by singleplex RQ-PCR of beta-actin from all samples (thick lines: median, small lines: upper and lower quartiles, circles: data points of individual samples).

Results — RT-PCR inhibition analysis

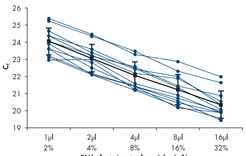

Figure 6. RT-PCR inhibition analysis of RNA from blood samples of 12 donors in PAXgene Blood RNA Tubes. RNA was prepared using the automated procedure (QIAcube protocol) with the PAXgene Blood RNA Kit. Increasing volumes of RNA eluates from all donors were subjected to singleplex RQ-RT-PCR of IL-1beta. Individual CT values (blue lines) and means (black thick line) with standard deviations (SD, vertical lines) are given for all samples.

Conclusions

- This research study demonstrates that the new automated protocol using proven silica-membrane spin-column technology and existing chemistry with the QIAcube robotic platform provides an efficient and reliable alternative low-throughput solution to the manual standard protocol.
- Using the automated protocol, performance of the PAXgene Blood RNA System was comparable with the manual procedure.
- The automated processing reduces protocol complexity, user interaction, and hands-on time, resulting in a more convenient and easy-to-follow workflow compared with the current, manual, standard PAXgene Blood RNA procedure.
- Manual errors and interventions are reduced, resulting in a higher degree of standardization for RNA preparation.
- The new automated technology is identical to the manual standard procedure (the same kit is used). No new technology is required (e.g., a change from spin-column to bead technology).

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