Automated, low-throughput RNA purification from whole blood using the PAXgene® Blood RNA system

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Introduction

Gene expression analysis of peripheral blood is an important tool for monitoring diseases at the molecular level and is used in molecular diagnostics, clinical research, and clinical trials of new drugs. We have developed and optimized a low-throughput, automated protocol for RNA preparation from blood collected in PAXgene Blood RNA Tubes*. The automation technology is based on a manual procedure that uses the proven silica-membrane spin-column technology of the PAXgene Blood RNA Kit*.

The aim of this study was to evaluate a new automated RNA purification protocol on the QIAcube® robotic platform and to compare its performance to the manual protocol.

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

The QIAcube

The QIAcube. The QIAcube instrument is a robotic system for biological sample preparation that includes a liquid handling unit, a robotic arm with grabber, a centrifuge, and a thermoshaker unit.

Materials and Methods

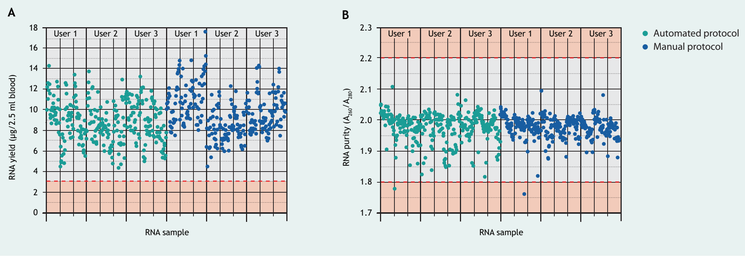
Replicate blood samples from 48 adult consented donors were collected in a total of 576 PAXgene Blood RNA Tubes. RNA was purified using either the manual procedure according to the *PAXgene Blood RNA Kit Handbook* (reference protocol) or the new automated purification procedure (test protocol). To enable comparison of test and reference protocol performance, purified RNA from both protocols was analyzed spectrophotometrically, by capillary gel electrophoresis, in real-time quantitative RT-PCR, and in PCR assays to measure RNA yield, purity, integrity, relative transcript levels, and levels of genomic DNA, respectively.

Results

Automated purification of RNA from all specimens collected in PAXgene Blood RNA Tubes resulted in no failures in sample processing. Furthermore, the quantity and quality of purified RNA from both the test and reference protocols were comparable in that all samples processed on the instrument yielded >3 micrograms RNA per tube, contained <1% (w/w) residual genomic DNA, and exhibited comparable transcript levels. Purified RNA exhibited A_{260}/A_{280} ratios of 1.8–2.2.

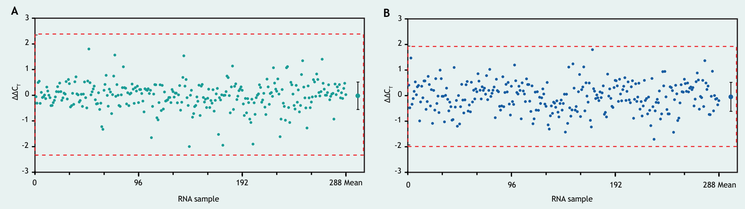
RNA Yield and Purity Analysis

RNA yield and purity analysis. Blood samples from 48 different donors were collected in PAXgene Blood RNA Tubes (12 tubes per donor, 576 tubes in total). The contents of the tubes from 6 donors were pooled and subsequently re-aliquoted into 72 samples, which were divided and processed automatically with the QIAcube (test protocol) and manually (reference protocol) using the PAXgene Blood RNA Kit. Tubes were processed by 3 different operators. Each operator used 3 different lots of the PAXgene Blood RNA Kit for extraction and processed quadruplicate samples from each of the 8 donor pools. RNA was quantified and purity was analyzed using UV spectroscopy. **(A)** RNA yields (µg/2.5 ml blood) and **(B)** purity (A_{260}/A_{280} ratio) of all individual samples are shown for every combination of protocol, operator, QIAcube instrument or laboratory equipment, and kit lot.

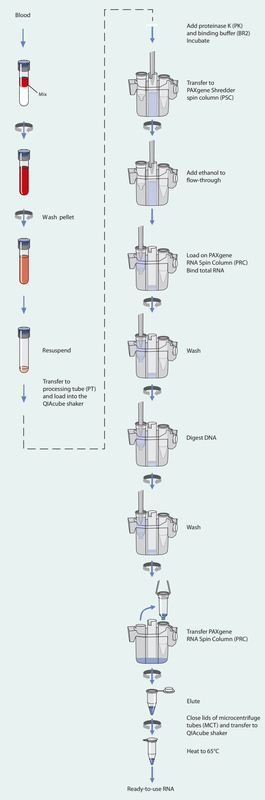


Transcript Level Analysis

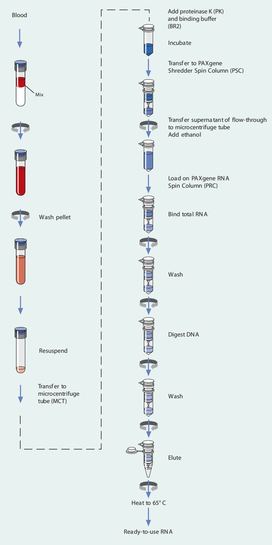
Transcript level analysis. Comparison of relative **(A)** c-fos and **(B)** IL-1ß transcript levels in 288 paired RNA preparations from replicate blood samples isolated with both the automated (test) and the manual RNA (reference) protocol and the PAXgene Blood RNA System as described above. Relative transcript levels of c-fos and IL-1ß were determined by real-time, duplex RT-PCR using 18S rRNA as an internal standard. Possible differences in transcript levels between RNA prepared from paired blood samples using both extraction protocols (test and reference) were calculated with the ΔΔCt method. Individual ΔΔCt values for all sample pairs (4 replicates x 8 donor pools x 3 QIAcube instrument or laboratory equipment with kit lot combinations x 3 operators = 288 pairs for each gene) are plotted as single dots with means (larger dots) and standard deviations (black bars) for all samples shown. The dashed lines indicate the ±3x total precision of the assays (c-fos: 2.34 CT; IL-1ß: 1.93 CT).



Test protocol: automated PAXgene Blood RNA procedure

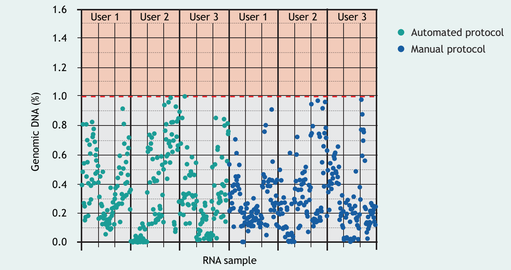


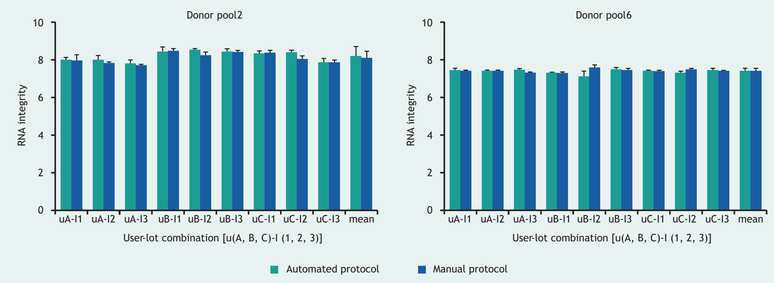
Reference protocol: manual PAXgene Blood RNA procedure



Analysis of RNA integrity and genomic DNA contamination

Analysis of RNA integrity and genomic DNA contamination. Samples shown in the figure below were subjected to genomic DNA analysis using real-time singleplex quantitative PCR of a ß-actin sequence. Genomic DNA was quantified using a standard curve method. Amounts of genomic DNA were related to the total nucleic acid content and the calculated percentage of genomic DNA contamination are shown for all individual samples. Two donor pools were randomly selected and all RNA samples were subjected to RNA integrity analysis using RNA integrity number (RIN) calculation after miniaturized-capillary gel electrophoresis with Agilent® Nanochips® run on the Bioanalyzer® 2100 system. The means and standard deviations of RIN values from quadruplicate replicates (processed with a single RNA preparation run) and from 36 sample replicates (processed with several RNA preparation runs with different combinations of protocol, operator, QIAcube instrument or laboratory equipment, and kit lot combinations) are presented as columns with bars. The combination of user (uA, uB, uC), kit lot (l1, l2, l3), mean for all combination (mean), and protocol is indicated on the X-axis and with the legend.





Conclusions

This performance evaluation demonstrates that the new automated protocol using proven spin-column technology and existing chemistry with the QIAcube robotic platform provides an efficient and reliable alternative low-throughput solution to the standard manual protocol.

- Using the automated protocol, performance of the PAXgene Blood RNA System was comparable to the manual procedure.
- Automated processing reduced protocol complexity, user interaction, and hands-on time, resulting in a more convenient and user-friendly workflow compared to the current standard manual 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 standard manual protocol and the same kit is used for both procedures.

The PAXgene Blood RNA Tube and **PAXgene Blood RNA Kit** are intended for the collection, storage, and transport of blood and stabilization of intracellular RNA in a closed tube and subsequent isolation and purification of intracellular RNA from whole blood for RT-PCR used in molecular diagnostic testing. Performance characteristics for the PAXgene Blood RNA System have only been established with FOS and IL1B gene transcripts. The user is responsible for establishing appropriate PAXgene Blood RNA System performance characteristics for other target transcripts.

For *in vitro* diagnostic use.