Automation of RNA isolation from blood samples stabilized in RNAgard® Blood tubes, using the QIAcube sample prep platform

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Introduction

Clinical Research studies often require blood sample collection at multiple geographic sites under a wide range of conditions. RNAgard® Blood tubes are designed for the immediate stabilization of cellular RNA in human blood samples, providing an efficient method for standardized collection, transport and storage of whole blood specimens and isolation of their RNA material. Purification of RNA from blood stabilized in RNAgard Blood Tubes has been optimized with the BioMaxi® Blood RNA Purification Kit (Biomatrica). High yields of high quality RNA with unaltered gene expression are obtained, and perform well in a wide range of downstream research applications, including but not limited to, bioanalyzer, gene quantification by qPCR and gene expression arrays. In order to accommodate the need for high throughput processing of samples, here we present an easy workflow for automated RNA isolation from blood samples collected and stored in RNAgard Blood tubes, using column-based QIAcube® sample prep platform. We show that using this RNA isolation platform, high yields of excellent quality RNA can be isolated from blood stabilized in RNAgard Blood tubes, even after 2 weeks of ambient temperature storage.

Materials and Methods

Blood sample processing: Human blood from 2 healthy donors was collected in RNAgard Blood tubes (Cat # 62201-131) by an outside contractor, shipped to Biomatrica and stored at ambient temperature for 14 days, plus 2 additional days at 4°C. Triplicate samples per donor were equilibrated to room temperature and processed for RNA isolation, following the protocol below:

1- Invert the RNAgard Blood Tube 3-5 times to ensure proper mixing, remove the cap and pour the contents of the tube into a clean 50 ml conical tube.

2- Pipette 3 ml of **BioMaxi Precipitation Buffer** (Cat # RP1-001-FG) into the 50 ml conical tube to bring the total volume to ~12 ml and close the cap on the tube and incubate 15 minutes at room temperature while shaking (500-750 rpm).

3- Vortex the 50 ml conical tube vigorously (Maximum Speed) for at least 30 seconds, ensuring that the solution travels to the top of the tube to achieve proper mixing of the contents.

4- Centrifuge the tube at 4,500g for 30 minutes at room temperature in a swing-bucket rotor.

5- Carefully pour off the supernatant and leave the tube inverted on absorbent paper for 1-2 minutes. **Note:** A translucent reddish pellet should be visible at the bottom of the tube.

6- Blot the remaining drops of liquid off the rim of the tube with clean absorbent paper.

7- Follow QIAGEN® recommended protocol for automated RNA isolation from stabilized blood using QIAcube (Cat # 762164; Version 2; Pg 42, skipping steps 3-5; (Start sample processing on step 6: Add 350 µl resuspension buffer (Buffer BR1), and vortex until the pellet is visibly dissolved), according to the manufacturer's instructions.

RNA analysis: RNA was isolated from human blood samples stabilized in RNAgard Blood Tubes using the QIAcube automated platform, as described above. Integrity of the isolated RNA was assessed by agarose gel electrophoresis and RNA Integrity Numbers (RIN) was determined using the Agilent 2100 Bioanalyzer. Total RNA yield per blood tube and RNA purity (A_{260}/A_{280}; A_{260}/A_{280}) were determined by UV spectrophotometry.

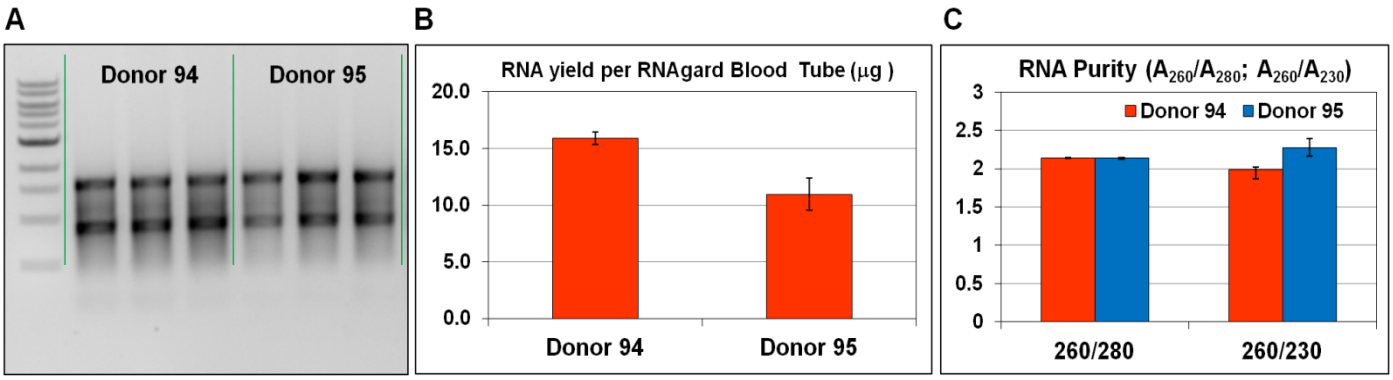

Figure 1: High yields of RNA with high purity can be isolated from blood samples stored in RNAgard Blood Tubes using the QIAcube platform. Blood from 2 healthy human donors was collected in RNAgard Blood Tubes and stored at room temperature for 14 days, plus 2 additional days at 4°C. After initial tube processing, RNA isolations were performed from triplicate samples per donor, using the automated (QIAcube) method. 5% of the purified RNA was analyzed by agarose gel electrophoresis **(A)**. Total RNA yield per blood tube **(B)** and RNA purity (A_{260}/A_{280}; A_{260}/A_{230};) **(C)** were determined by UV spectrophotometry.

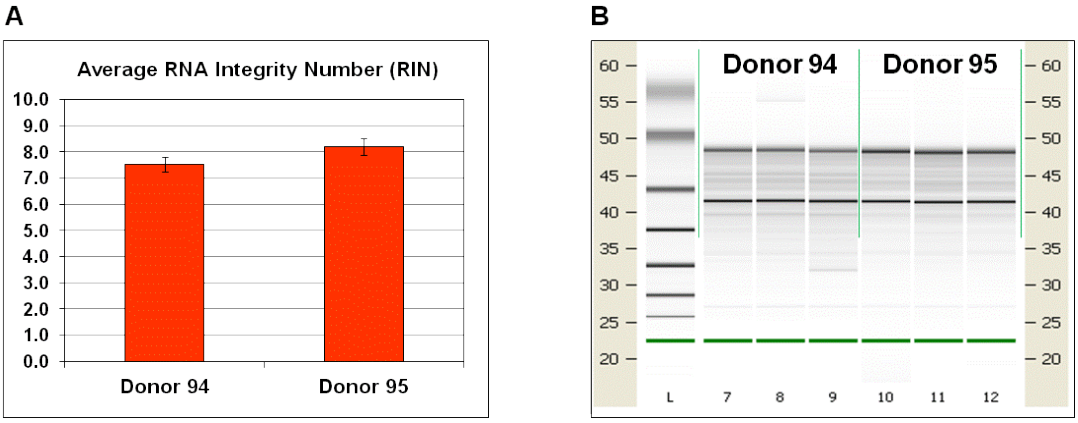

Figure 2: RNA isolated from blood samples stored in RNAgard Blood Tubes using the QIAcube platform is of great quality. Blood from 2 healthy human donors was collected in RGB Tubes and stored at room temperature for 14 days, plus 2 additional days at 4°C. After initial tube processing, RNA isolations were performed from triplicate samples per donor, using the automated (QIAcube) method. RNA integrity and quality was assessed by Agilent 2100 Bioanalyzer. Average RIN from duplicate runs of all samples **(A)** and a representative Bioanalyzer gel-like image **(B)** are shown.

Results and Discussion

We have previously shown that high yield of excellent quality RNA can be isolated from human blood samples stabilized in RNAgard Blood tubes even after 2 weeks of ambient temperature storage. In this study we tested whether RNA sample processing could be automated using QIAcube sample prep platform, in order to increase sample processing throughput. We show that after initial processing of the RNAgard Blood tubes, reproducible high yields of great quality RNA are obtained using the QIAcube, following the manufacturer's recommended kits and protocols, as shown by agarose gel electrophoresis analysis of the isolated RNA (Figure 1 A, B). The isolated RNA samples processed in the QIAcube are very pure, as determined by the excellent A_{260}/A_{280} and A_{260}/A_{230} values, assessed by UV spectrophotometry (Figure 1C). Finally, we show that even after 14 days of ambient temperature storage of the blood samples stabilized in RNAgard Blood tubes, the automated RNA isolation results in RNA of very high integrity, as determined by the RNA Integrity Numbers higher than 7.5 for both donors analyzed in this study, assessed using Agilent 2100 Bioanalyzer (Figure 2). We conclude that the great RNA stabilization provided by RNAgard Blood tubes can easily be coupled with the QIAcube sample prep platform to achieve automated high throughput sample processing and deliver high yields of excellent quality RNA.

***Note**: Please read all instructions for the RNAgard Blood System prior to using this protocol.*

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