Automation of RNA isolation from human blood samples stabilized in RNAgard® Blood Tubes - Integration with Roche MagNA Pure Compact System

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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 (Cat# 62201-131) are designed for the immediate stabilization of cellular RNA in human blood samples, which provides a safe and efficient method for standardized collection and transport of whole blood specimens. Purification of RNA from blood stabilized in RNAgard Blood Tubes has been optimized with the BioMaxi® Blood RNA Purification Kit (Cat# 64201-601). High yields of excellent quality RNA with unaltered gene expression are obtained and performed well in a wide range of downstream research applications, including but not limited to, bioanalyzer, RT-qPCR and gene expression arrays. In order to accommodate the need for automated processing of samples, here we present an easy workflow for RNA isolation from blood samples collected in RNAgard Blood Tubes using the magnetic glass particle-based MagNA Pure Compact System (Roche Applied Science). The system includes both MagNA Pure Compact Instrument (Cat# 03731146001) and kits such as MagNA Pure Compact RNA Isolation Kit (Cat# 04802993001). We show that, when using this RNA isolation platform, high yields of excellent quality RNA can be isolated from blood stabilized in RNAgard Blood Tubes, even after two weeks of ambient temperature storage. Low to high throughput sample processing can therefore be easily achieved using the MagNA Pure Compact System. This provides a comprehensive solution for automated RNA isolation from blood samples stabilized in RNAgard Blood Tubes.

Materials and Methods

Blood sample processing: Human blood from two healthy donors was collected in RNAgard Blood Tubes by an outside contractor, shipped to Biomatrica and kept at room temperature for 14 days. Triplicate blood samples were processed for RNA isolation following the protocol below.

1. Invert the RNAgard Blood Tube 3-5 times to ensure proper mixing, and empty 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 conical tube to bring the total volume to 12 mL, cap the tube and incubate 15 minutes at room temperature while shaking (500-750 rpm).
3. Vortex the conical tube at 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 room temperature for 10 minutes at 9000 x g in fixed rotor, or 30 minutes at 4500 x g in swinging bucket rotor.
5. Carefully decant the supernatant, and invert the tube onto an absorbent paper towel 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 wipe.
7. Resuspend the pellet with 200 µL of lysis buffer from MagNA Pure Compact RNA Isolation Kit until homogeneous, and then transfer the supernatant into a sample tube provided.
8. Place the sample tube in the MagNA Pure Compact tube rack, and start the “RNA Cell” protocol or “RNA Blood” protocol as described in the MagNA Pure Compact RNA Isolation Kit manual.

RNA analysis: RNA was purified from human blood samples stabilized in RNAgard Blood Tubes using the MagNA Pure Compact System, as described above. Integrity of the isolated RNA was assessed by gel, and RNA Integrity Numbers (RIN) were determined using Agilent 2100 Bioanalyzer. Total RNA yield per blood tube and RNA purity (A_{260}/A_{280}) were determined by UV spectrophotometry. *c-fos* and *IL-1ß* expression, normalized to 18s rRNA, were examined by RT-qPCR relative to day 0, using the ΔΔCt method. Presence of genomic DNA contamination was determined in the RNA samples purified at two different time points by qPCR amplification of an RNaseP amplicon, against a genomic DNA standard curve.

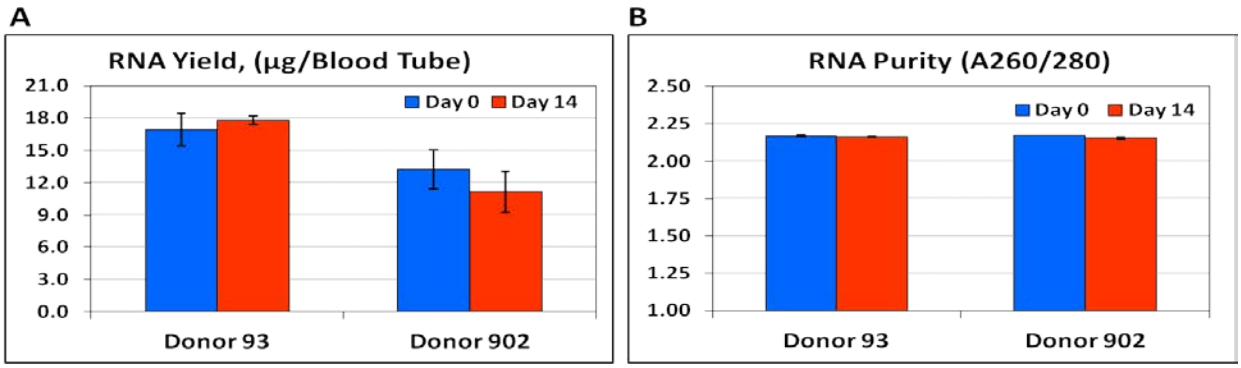

Figure 1: High yields of pure RNA can be isolated from blood samples collected in RNAgard Blood Tubes using MagNA Pure Compact System. Blood from two healthy donors was collected in RNAgard Blood Tubes and kept at ambient temperature for 14 days. RNA was isolated from triplicate samples per donor, as described above. RNA yield per blood tube **(A)** and RNA purity (A_{260}/A_{280}) **(B)** were determined by UV spectrophotometry.

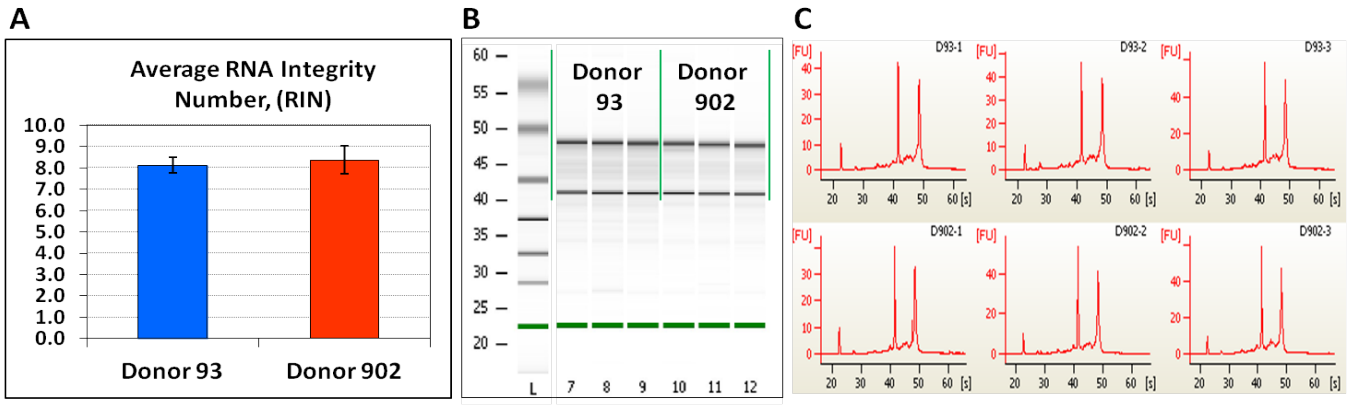

Figure 2: RNA isolated from blood samples collected in RNAgard Blood Tubes using the MagNA Pure Compact System is of high quality. Blood from two healthy human donors was collected in RNAgard Blood Tubes and kept at ambient temperature for 14 days. RNA was isolated from triplicate samples per donor using the MagNA Pure Compact System as described above. RNA integrity and quality was assessed by Agilent 2100 Bioanalyzer. **(A)** Average RIN for all samples; **(B)** A representative Bioanalyzer gel-like image; **(C)** Bioanalyzer graphs.

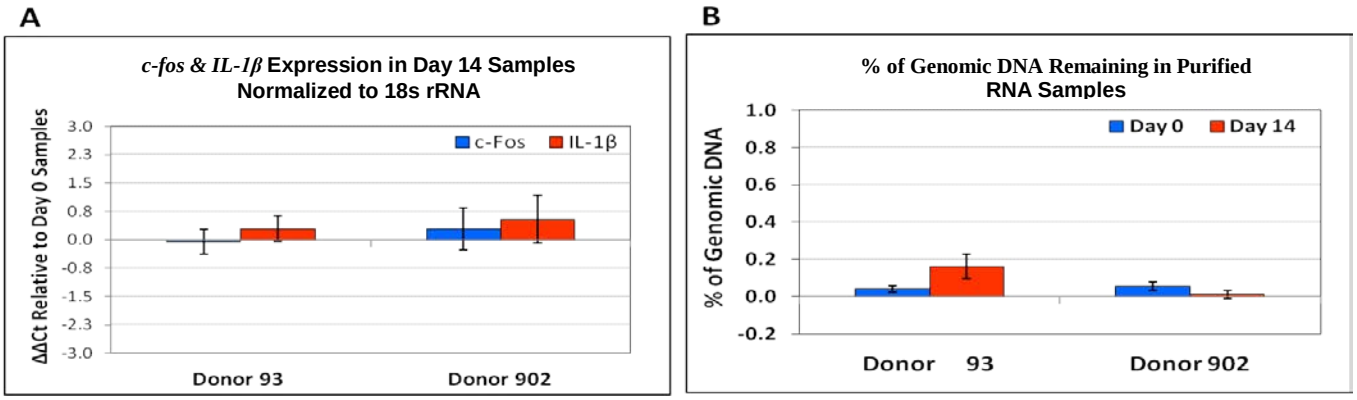

Figure 3: RNA isolated from blood samples collected in RNAgard Blood Tubes using the MagNA Pure Compact System performs well in RT-qPCR, and has virtually no genomic DNA contamination. Blood from two healthy human donors was collected in RNAgard Blood Tubes and kept at ambient temperature for 14 days. RNA was isolated from triplicate samples per donor using the MagNA Pure Compact System as described above. **(A)** Expression of *c-fos* and *IL-1β* transcripts, relative to 18s rRNA expression, was determined after 14 days of ambient temperature storage by RT-qPCR, relative to expression at day 0. **(B)** The presence of genomic DNA contamination was determined in RNA samples purified at two different time points, by qPCR amplification of an RNaseP amplicon against a genomic DNA standard.

Results and Discussion

We have previously shown that high yields of excellent quality RNA can be isolated by the BioMaxi Blood RNA Purification Kit from human blood samples stabilized in RNAgard Blood Tubes after two weeks of ambient temperature storage. In this study, we further tested whether RNA sample processing could be automated using Roche's MagNA Pure Compact System for increasing sample processing throughput. We show that after initial processing with the RNAgard Blood Tubes, reproducible high yields of pure RNA are obtained using the MagNA Pure Compact System (Figure 1A, B). Additionally, 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 after 14 days of ambient temperature storage of the blood samples stabilized in RNAgard Blood Tubes (Figure 2). Furthermore, we show that, in Figure 3A, even after 14 days of ambient temperature storage of the blood samples stabilized in RNAgard blood tubes, the RNA purified with the MagNA Pure Compact System remains intact. RT-qPCR shows low variation of transcript levels (very low ΔΔCt for both transcripts analyzed), suggesting a constant gene expression profile. Finally, genomic DNA contamination of the RNA isolated using the MagNA Pure Compact System is extremely low (less than 0.2% for all samples tested), as shown by qPCR (Figure 3B). We conclude that the RNA stabilization provided by RNAgard Blood Tubes can easily be coupled with the MagNA Pure Compact System to achieve automated processing upon delivering high yields of excellent quality RNA. Furthermore, by combining Roche's diverse MagNA Pure Sample prep platforms with Biomatrica's RNAgard Blood Tubes, low to high throughput sample processing could be easily achieved. This provides a comprehensive solution for automated RNA isolation from human blood samples stabilized in RNAgard Blood Tubes.

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

Order RNAgard Blood Tubes