

Automation of RNA isolation from blood samples stabilized in RNAgard® Blood tubes, using the magnetic bead-based Agencourt RNAdvance Blood 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 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 the magnetic bead-based Agencourt® RNAdvance™ Blood Kit (Cat# A35603 Beckman Coulter, Inc). We show that using this RNA isolation platform, high yields of great quality RNA can be isolated from blood stabilized in RNAgard Blood tubes, even after 2 weeks of ambient temperature storage. The combination of the great RNA stabilization provided by Biomatrica's RNAgard Blood Tubes and the high throughput capacity of Agencourt's RNAdvance Blood Kit provides an excellent solution for ambient temperature blood sample collection, storage and RNA isolation for very high sample number processing requirements.

Materials and Methods

Blood sample processing: Human blood from 3 healthy donors was collected in RNAgard Blood Tubes by an outside contractor, shipped to Biomatrica at 4°C and stored at room temperature for up to 14 days. At day 0, day 3, day 7 and day 14 of ambient sample storage, samples stabilized in RNAgard Blood Tubes were carefully mixed to obtain homogenized samples and triplicate 400µl aliquots of the stabilized blood samples per donor were processed for RNA isolation, following the Agencourt RNAdvance Blood Kit from RNA-stabilizing blood collection tubes.

RNA analysis: RNA was isolated from human blood samples stabilized in RNAgard Blood Tubes using the Agencourt's RNAdvance Blood Kit, as described above. Total RNA yield per ml of whole blood input and RNA purity (A_{260}/A_{280}) were determined by UV spectrophotometry. To Expression of C-Fos and IL1b transcripts, normalized to B-actin expression, was determined after 14 days of ambient temperature storage by rt-qPCR relative to expression at day 0, using the $\Delta\Delta C_t$ method. The presence of genomic DNA contamination in the purified RNA samples was determined in samples purified at 2 different time points by qPCR amplification of an RNase P amplicon, against a genomic DNA standard curve.

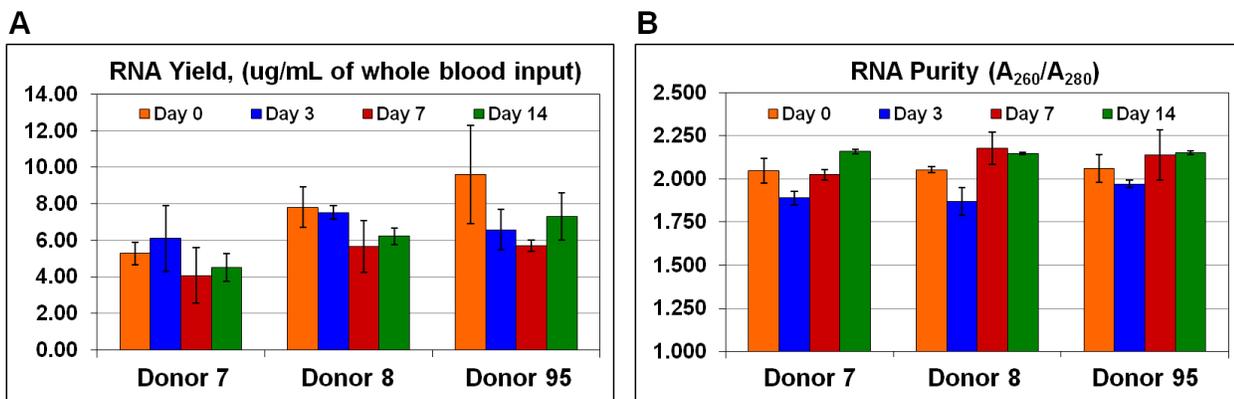


Figure 1: High yields of RNA with high purity can be isolated from blood samples stored in RNAgard Blood Tubes using the Agencourt RNAdvance Blood kit. Blood from 3 healthy human donors was collected in RNAgard Blood Tubes and stored at room temperature for up to 14 days. RNA isolations were performed at days 0, 3, 7 and 14 from triplicate samples per donor, using the Agencourt RNAdvance Blood system. Total RNA yield normalized for 1ml of whole blood input (A) and RNA purity (A_{260}/A_{280}) (B) were determined by UV spectrophotometry.

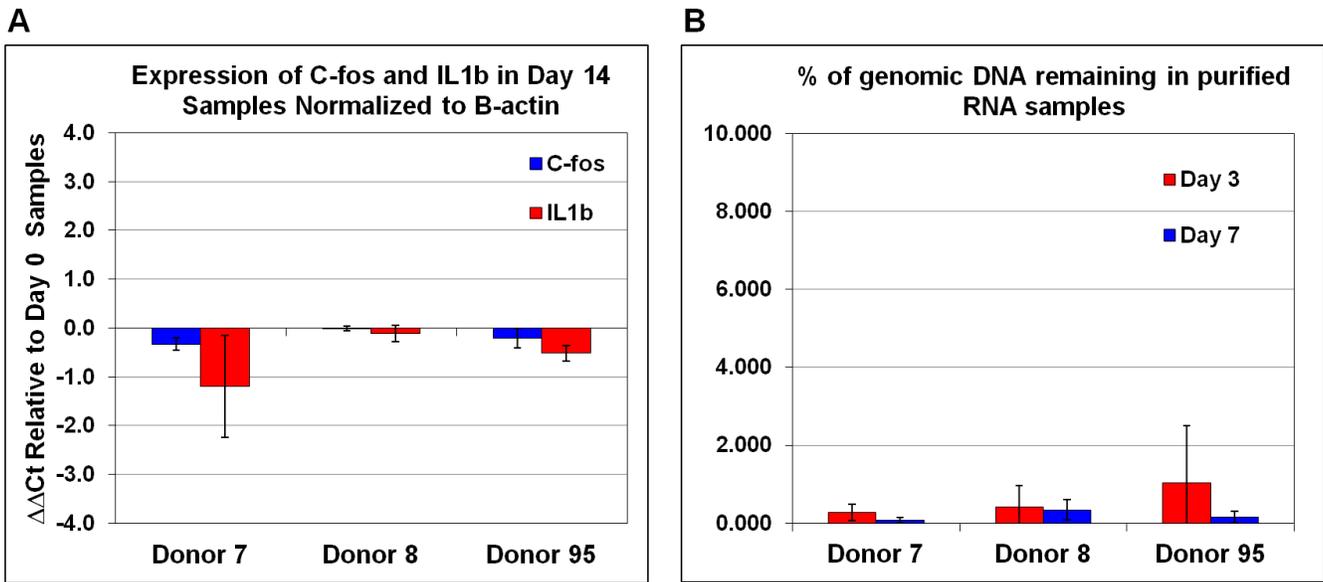


Figure 2: RNA isolated from blood samples stored in RNAgard Blood Tubes using the Agencourt RNAdvance Blood kit performs well in rt-qPCR and has an extremely low genomic DNA contamination. Blood from 3 healthy human donors was collected in RNAgard Blood Tubes and stored at ambient temperature for up to 14 days. RNA isolations were performed after the specified storage time from triplicate samples per donor, using the Agencourt RNAdvance Blood kit. Expression of C-Fos and IL1b transcripts, relative to B-actin expression, was determined after 14 days of ambient temperature storage by rt-qPCR, relative to expression at day 0 (A). The presence of genomic DNA contamination in the purified RNA samples was determined in samples purified at 2 different time points by qPCR amplification of an RNase P amplicon, against a genomic DNA standard curve (B).

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 Agencourt's RNAdvance Blood Kit, in order to increase sample processing throughput. We show that without any further modification to Agencourt's workflow, reproducible high yields of RNA are obtained over different times of ambient temperature stabilized blood sample storage using the magnetic bead-based Agencourt's RNAdvance Blood Kit following the manufacturer's recommended protocol, as assessed by UV spectrophotometry (Figure 1A). The isolated RNA samples are of high purity, as determined by the excellent A_{260}/A_{280} values (Figure 1B). Additionally, we show that even after 14 days of ambient temperature storage of the blood samples stabilized in RNAgard Blood Tubes, the RNA purified by the Agencourt's RNAdvance Blood Kit remains intact and representative of the transcript levels at the time of blood sample collection, as shown by the very little variation observed by quantitative rt-PCR (very low $\Delta\Delta Ct$ for both transcripts analyzed, for all donors), showing a constant gene expression profile even after 14 days of ambient temperature storage (Figure 2A). Finally, genomic DNA contamination of the RNA samples isolated using Agencourt's RNAdvance Blood Kit is extremely low (less than 1% for all samples tested), as shown by qPCR (Figure 2B). We conclude that the great RNA stabilization provided by RNAgard Blood Tubes can easily be coupled with the Agencourt's RNAdvance Blood Kit to achieve automated high throughput sample processing and deliver high yields of excellent quality RNA, compatible with downstream applications such as rt-qPCR.

Note: Please read all instructions for the [RNAgard Blood System](#) prior to using this protocol.

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