Automation of DNA isolation from blood samples stabilized in RNAgard® Blood Tubes using MagNA Pure Compact System - a complete solution for DNA and RNA isolation from the same sample

Angela Stassinopoulos and Vasco Liberal, Ph.D.

Introduction

RNAgard Blood Tubes (Cat No 62201-131) are designed for the immediate stabilization of cellular RNA in human blood samples, which provides an efficient method for blood RNA collection, transport and storage at room temperature. Purification of RNA from blood stabilized in RNAgard Blood Tubes has been optimized with BioMaxi Blood RNA Purification Kit (Cat No 64201-601) and MagNA Pure Compact System (Roche Applied Science). Clinical studies often require complete DNA and RNA profiles from blood samples. However, the comprehensive profile often needs high amount of blood samples, which complicates logistics if a large sample set is needed. In addition, DNA and RNA isolation from separated samples could cause operation errors as well as variation of the results. In order to accommodate the need for sequential RNA and DNA isolations from the same sample, we have developed an automated workflow for DNA and RNA isolation from the same blood samples stabilized in RNAgard Blood Tubes, using the magnetic glass particle-based MagNA Pure Compact System.

Materials and Methods

Blood sample processing: Human blood from 2 healthy donors was collected in RNAgard Blood Tubes by an outside contractor, shipped to Biomatrica and stored at room temperature for 2 weeks. Triplicate blood samples were processed for DNA and RNA isolation at both day 0 and day 14 as described below.

1. Invert the RNAgard Blood Tube 3-5 times to ensure proper mixing, empty the contents of the tube into a clean 50 mL conical tube.

2. Pipette 3 mL of BioMaxi Precipitation Buffer (Cat No RP1-001-FG) into the conical tube to bring the total volume to 12 mL, cap the tube and incubate at room temperature for 15 minutes 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 9,000 x g in a fixed rotor, or 30 minutes at 4,500 x g in a swinging bucket rotor.

5. Carefully decant the ~12 mL DNA containing supernatant into a labeled tube, but do not dispose of the pellet containing the RNA.

Note: *The pellet from step 5 is used for RNA isolation as described in Automation of RNA isolation from human blood samples stabilized in RNAgard® Blood Tubes - Integration with Roche MagNA Pure Compact System.*

6. For DNA isolation, transfer 1mL of the 12 mL of supernatant containing the genomic DNA to a sample tube provided in the MagNA Pure Compact Nucleic Acid Isolation Kit I-Large Volume (Cat No 03 730 972 001, Roche Applied Science).

7. Place the sample tube in the MagNA Pure Compact tube rack and start the “DNA_Blood_1000” protocol as described in the manual of MagNA Pure Compact Nucleic Acid Isolation Kit I-Large Volume.

DNA analysis: DNA was purified from human blood samples stabilized in RNAgard Blood Tubes using the MagNA Pure Compact System as described above. The quality of the isolated genomic DNA was assessed by agarose gel electrophoresis. Total DNA yield per 1mL of blood and DNA purity (A_{260}/A_{280}) were determined by UV spectrophotometry. The integrity of the DNA was further assessed with PCR amplification of a large fragment (7.5Kb) of the human *β-globin* gene.

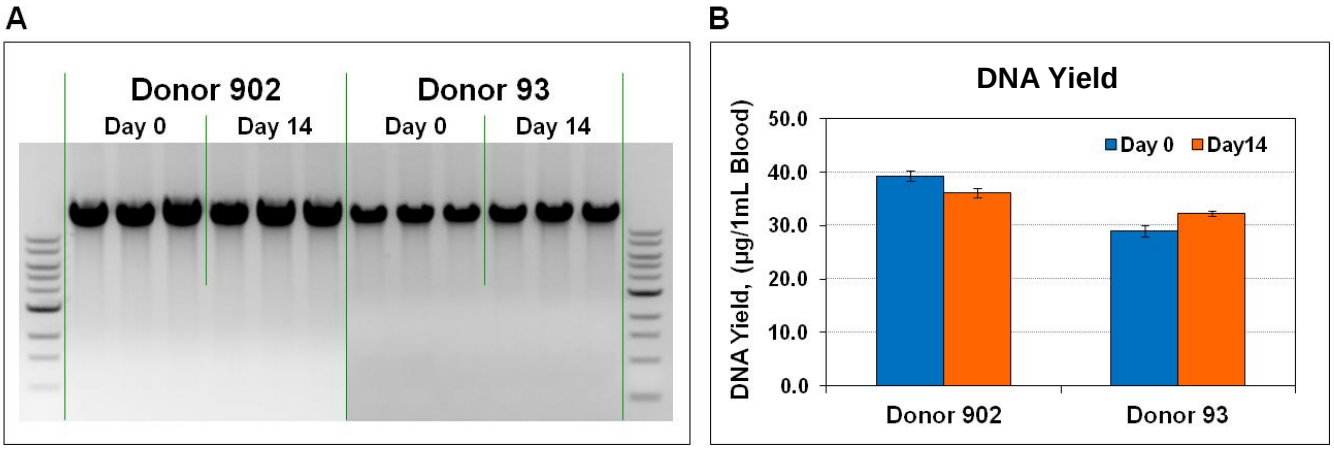

Figure 1: High yield of great quality genomic DNA is isolated from blood samples stored in RNAgard Blood Tubes using MagNA Pure Compact System. Blood from 2 healthy donors was collected in RNAgard Blood Tubes and triplicate samples were stored at room temperature for 0 or 14 days. DNA was isolated from the supernatant of the same blood samples processed for RNA isolation, as described above. DNA quality was assessed by agarose gel electrophoresis **(A)** and DNA recovery per 1mL of blood was determined by UV spectrophotometry **(B)**.

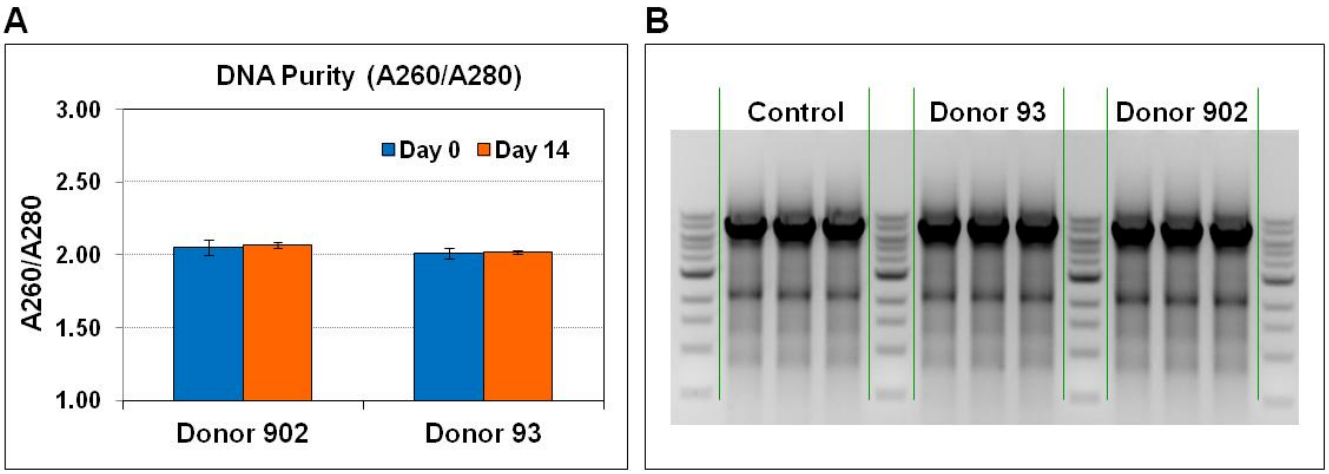

Figure 2: DNA isolated from blood samples stored in RNAgard Blood Tubes using the MagNA Pure Compact System is of great purity and high integrity. Blood from 2 healthy donors was collected in RNAgard Blood Tubes and triplicate samples were stored at room temperature for 0 or 14 days. DNA was isolated from the supernatant of the same blood samples for RNA isolation, as described above. The purity of the isolated DNA (A_{260}/A_{280}) was determined by UV spectrophotometry **(A)**; by compared with DNA isolated from fresh blood samples (Control), DNA integrity was assessed by PCR amplification of a 7.5kb human *β-globin* fragment **(B,** red arrow**)**.

Results and Discussion

We have previously shown that high yield of excellent quality RNA was isolated from human blood samples stabilized in RNAgard Blood Tubes after 2 weeks of room temperature storage by using the automated MagNA Pure Compact System. In this study we further examined whether DNA could be isolated from the same blood sample stabilized in RNAgard Blood tubes. We show that after initial blood collection and storage in the RNAgard Blood Tubes, high yield of genomic DNA was isolated using the MagNA Pure Compact System (Figure 1A and 1B). Additionally, the isolated genomic DNA is of very high purity, as shown by the high A_{260}/A_{280} ratios (Figure 2A). Furthermore, we show the high integrity of the isolated genomic DNA by long range PCR amplification of a 7.5kb human *β-globin* amplicon (Figure 2B). **We conclude that DNA isolation can easily be coupled with RNA isolation from the blood samples stabilized in RNAgard Blood Tubes with the MagNA Pure Compact System**. Thus we provide a complete solution for automated nucleic acid purification from blood samples with low to high throughput.

***Note**: Please read all instructions for the RNAgard Blood System and RNA isolation using MagNA Pure Compact System prior to using this protocol.*

RNAgard® is a registered trademark of Biomatrica.

Order RNAgard Blood Tubes