Automated biobanking workflow for room temperature collection, transport and storage of human blood samples for molecular RNA and DNA diagnostics.

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Abstract

Diagnosis and monitoring of disease based on gene expression profiles from blood requires reliable nucleic acid preservation during sample collection and shipment. Transcription profiles can change rapidly after sample collection, potentially affecting interpretation of gene expression and ultimately dictating inadequate treatment. Biomatrica's RNAgard® Blood Tube is a collection device for whole blood, which stabilizes the RNA in blood cells and allows for room temperature sample handling and storage. Coupled with a robust RNA purification method, it provides a complete solution for blood collection, storage and high yield RNA purification. RNA isolation can be performed with multiple automated platforms, allowing ease and flexibility in the workflow, and excellent results for low to high throughput sample processing.

Moreover, the RNAgard Blood Tube is a convenient solution for studies that require isolating and testing both DNA and RNA from the same patient. The logistics of such studies can be very complex, especially when large sample sets are required. Isolation of RNA and DNA from the same sample is ideal, since the use of separate samples could introduce operation errors and variation of results. To accommodate the need for RNA and DNA isolations from the same blood sample stabilized in RNAgard Blood Tubes, we have developed an automated workflow for sequential RNA and DNA purification, using the MagNA Pure System (Roche).

Biomatrica's room temperature nucleic acid preservation technology, coupled with automated purification, can provide accurate gene expression profiles, proving highly valuable for improved biomedical research and patient treatment.

Collection → Transport → Process → Archive → Distribute

Materials and Methods

Assembly of Instruments and Consumables

We assembled a complete list of instruments and consumables (supplies and reagents) required for the workflow. The consumables consisted of the following supplies from Biomatrica:

1. **VACUETTE® DNAgard® Blood Tubes**, used for ambient temperature collection, stabilization and shipping of whole blood, which protect genomic DNA in whole blood specimens for up to 14 months
2. **VACUETTE® RNAgard® Blood Tubes**, used for ambient temperature collection, stabilization and shipping of whole blood, which protect total cellular RNA in whole blood specimens for up to 14 days
3. **DNAstable® *Plus***, an ambient temperature liquid DNA stabilizer that protects purified genomic DNA in a liquid state for up to 1 year and in the dry state for more than 10 years, providing flexible workflow integration
4. **RNAstable®**, a unique RNA stabilizer that protects RNA from degradation at ambient temperatures for at least 12 years based on accelerated stability studies
5. **BioMaxi ™Blood SNA Purification Kit** for DNA extraction from whole blood.
6. **Mini-BioStore**, a large, ambient temperature, dry storage cabinet with active humidity control and a maximum storage capacity of >500,000 samples. (See Figure 1 below).

Additionally, the following consumables were obtained:

1. RiboPure™ Blood RNA purification kit (Qiagen) for RNA extraction from whole blood. Higher yield and quality can be also obtained through extraction using the BioMaxi Extraction Kit (Biomatrica).
2. DNA extraction through spin column, charge switch or magnetic bead based purification
3. 2D barcoded PicoTubes® (Tap Biosystems) for sample storage and high-throughput handling.
4. PicoTube® storage vehicle (Tap Biosystems)

Lastly, instrument solutions to implement the workflow were provided bv TECAN and Tap Biosystems.

Figure 1: Mini-BioStore
For high volume, ambient temperature sample storage.

Each Mini-BioStore unit holds 1440 x 384-well PicoTube plates or 2520 standard 96-well plates. Based on these numbers, we calculated two scenarios for large scale storage of blood samples:

Scenario 1: Using 384-well PicoTube plates, each Mini-BioStore would hold 552,960 donor blood samples.

Scenario 2: Using standard 96-well plates, each Mini-BioStore would hold 241,920 donor blood samples.

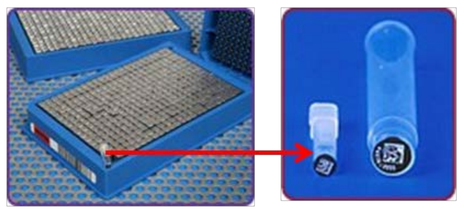


Figure 2: 2D barcoded PicoTubes and PicoTube Handling Device (Tap Biosystems) for nucleic acid storage

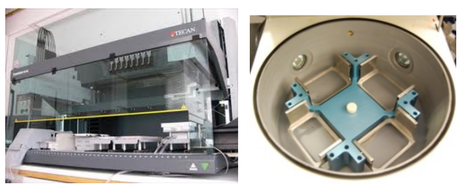


Figure 3: Liquid handling instrumentation (TECAN); High volume drying using speed-vac centrifugation.

Sample Collection, Processing and Storage of DNA and RNA

The sample collection phase of the study was conducted with 20 donors providing 6.5 ml of blood drawn into DNAgard Blood Tubes and 2.5 ml of blood drawn into RNAgard Blood Tubes. Blood drawn into these tubes is immediately stabilized in a liquid state allowing for ambient temperature transport.

Following overnight shipment of the samples, blood in the DNAgard Blood Tubes was subjected to a rigorous 5-day shipping simulation mimicking a worst-case shipping scenario, including exposing the samples to temperature fluctuations from freezing at -20°C to elevated temperature of 45°C for 18 hours.

Subsequently, DNA was extracted from blood using spin column based DNA purification kits. Following 8 days of storage in DNAstable *Plus* reagent, the extracted DNA was analyzed qualitatively and quantitatively. Results of these analyses are shown below.

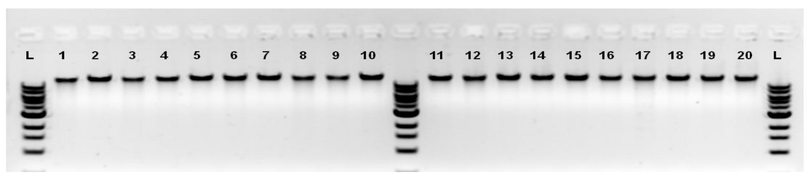


Figure 4: Analysis of genomic DNA integrity after recovery from dry-storage in DNAstable *Plus* by agarose gel electrophoresis. Genomic DNA isolated from DNAgard Blood Tubes after the shipping simulation was stored dry in DNAstable *Plus* for 8 days. DNA was rehydrated and 100 ng was analyzed by agarose gel electrophoresis (1xTAE; 0.8%). L = 1 kb ladder (New England Biolabs).

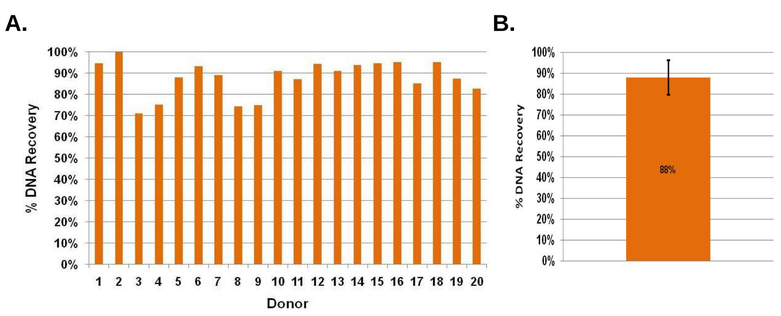


Figure 5: Recovery of genomic DNA stored dry in DNAstable *Plus*. 2 µg genomic DNA was spotted per well in 384-well PicoTube plates (TAP Biosystems) and dried. After 8 days of room temperature storage, samples were rehydrated and DNA recovery quantified by spectroscopy. **A)** Percentage of DNA recovered per donor from DNAstable *Plus*. **B)** Mean ± standard deviation of percentage of DNA recovered (n = 20).

The RNAgard Blood Tube samples were shipped overnight at ambient temperature (range 22°C - 27°C). Upon arrival these samples were subjected to a 5 day temperature cycle mimicking a worst-case shipping scenario, including exposing the samples to temperature fluctuations from freezing at -20°C to elevated temperature for 13 hours at 37°C. RNA was extracted from the RNAgard Blood samples using the RiboPure Blood kit (Ambion). Purified RNA (500 ng) from the RNAgard Blood samples was spotted onto RNAstable and dried overnight in a sterile laminar flow-hood at ambient temperature (20°C - 25°C) and stored for 10 days at ambient temperature. These RNA samples were rehydrated in 10 µl sterile DEPC-H_2O. RNA was analyzed both immediately after extraction and after rehydration from dry-storage in RNAstable.

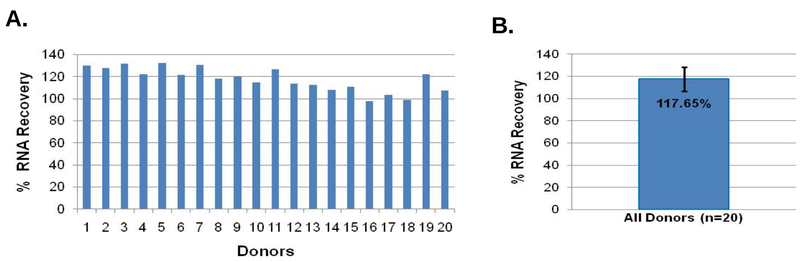


Figure 6: Recovery of RNA after dry-storage in RNAstable. A) Percentage of the 500 ng RNA spotted per RNAstable well that was recovered after 10 days of storage at room temperature. Data is shown for RNA from all 20 donors. Quantification was completed by spectrophotometry. Samples were corrected for background absorbance by subtracting out a rehydrated RNAstable well. **B)** Mean ± standard deviation of percentage of RNA recovered from the 20 donors after dry-storage in RNAstable. Mean greater than 100% recovery was likely due to large correction factors from diluting small volumes into larger volumes.

Figure 6 continuation

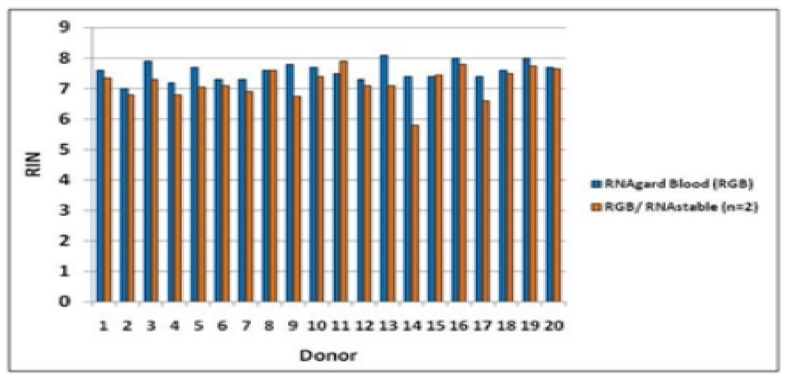


Figure 7: RNA RIN Score Pre- vs. Post-Storage RIN scores for RNA from each donor isolated from the post-shipping simulation RNAgard Blood samples compared with the RIN scores after 17 days of storage in RNAstable.

Automated Workflow Process Overview

The following Figure 8 shows the actual instrumentation or vessels used in storage of nucleic acids at each of the respective modules, as well as a list of what actually transpires at each module during the workflow. The figure is for DNA isolation and storage. For RNA, the layout is very similar but 1) different number of collection tubes are loaded in module 1, 2) the isolation chemistry is different in module 2, and 3) the isolated RNA is added to PicoTubes that have been pre-coated with RNAstable.

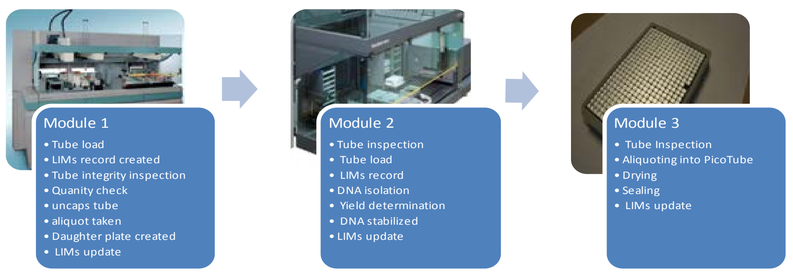
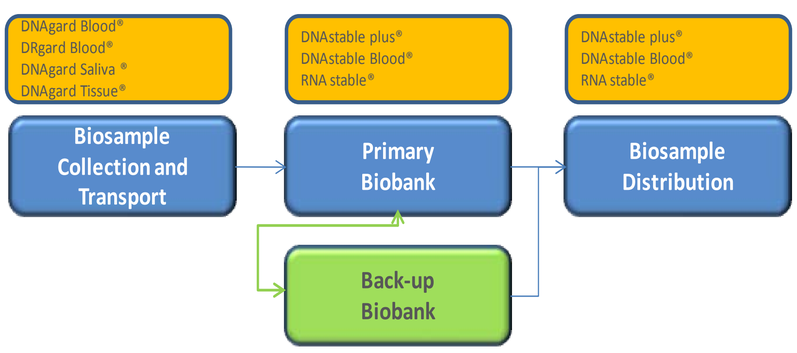


Figure 8: Modules & Processes Used in Isolation & Storage of DNA



The process is scalable to 800 patients per day. The all ambient workflow can be implemented for primary, back-up, or side biobanking for unpurified blood spots, purified DNA and purified RNA.

Summary

Biomatrica's workflow allows high volume sample collection, processing and storage under all ambient conditions.

Collection:

Biomatrica's blood collection tubes allow blood collection and sample transport for DNA and RNA at ambient temperature without loss of sample quality. DNA can be also collected from Saliva samples using Biomatrica's DNAgard® Saliva product (data not presented).

Processing:

High volume sample processing can be automated and scaled to more than 800 blood samples per day using standard liquid handling workstations and SpeedVac drying.

Storage:

Blood samples for DNA analysis can be stored in the DNAgard Blood® collection tubes or spotted directly without processing as blood spots in DNAstable Blood® The preferred storage of DNA for long-term archiving is after purification, protected in DNAstable® *Plus*

The preferred long-term storage of the RNA is after purification, stabilized in RNAstable® at ambient temperature, eliminating all freeze/thaw cycles.