Establishment of an All Ambient Automated Workflow Biobank Pilot Plant for the Purification and Archiving of Nucleic Acids Found in Blood for the U.S. Army.

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Overview

Project Aim: The development of a scalable, all ambient temperature, automated, biological sample workflow to extract and preserve nucleic acids from blood for molecular analysis.

- This ambient workflow is being developed to support:
  - large scale blood collection
  - transport and long-term archiving without the use of costly and unreliable cold-chain management using commercially available bio-preservation products

Current Status

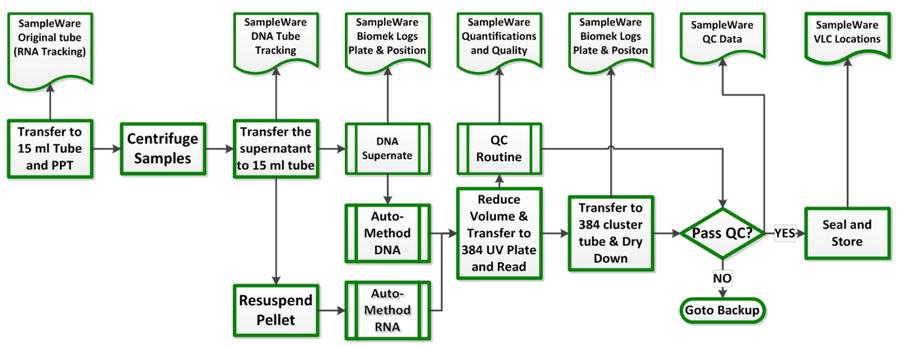
- The RNA extraction methods can process in excess of 100 specimens per day with an average yield of 16.16 ug per RNAgard® Blood (RGB) tube using the Agencourt RNAdvance® technology on the Beckman Coulter Biomek® FXP laboratory automation workstation.
- The DNA extraction methods can process in excess of 100 specimens per day with an average yield of 35 ± 1.5 ug/mL of RGB (808 uL of whole blood) utilizing the Macherey-Nagel Nucleomag® Blood chemistry on the ThermoFisher KingFisher™ Magnetic Particle Processor. Both the RNA and DNA processes continue to be optimized to ensure error free and facile handling.

Introduction

Our objective is the development of a scalable, all ambient temperature, biological sample workflow, preserving the nucleic acids present in blood for molecular analysis. This ambient workflow can be developed for large scale blood collection, transport, and long-term archiving, without costly and unreliable cold-chain management using our commercially available biopreservation product, RNAgard® Blood (RGB). Indeed, we have established a pilot plant that is capable of processing at least 100 RGB preserved specimens per day resulting in at least 4 samples each of DNA and RNA from each specimen with suitable quality and in amounts to support downstream molecular analysis. Samples are archived in a dry stabilized form using DNAStable® *Plus* and RNAstable® as excipients, respectively, and location information is stored in a SampleWare® database. This workflow can be scaled to process up to 900 samples per day with only incremental costs and no shift in paradigm. The complexity of the human genome requires large sample populations to increase the predictive value of genetic analysis. Large scale archiving of population and/or patient specimens is the key to create this knowledge base.

Methods

Process Overview



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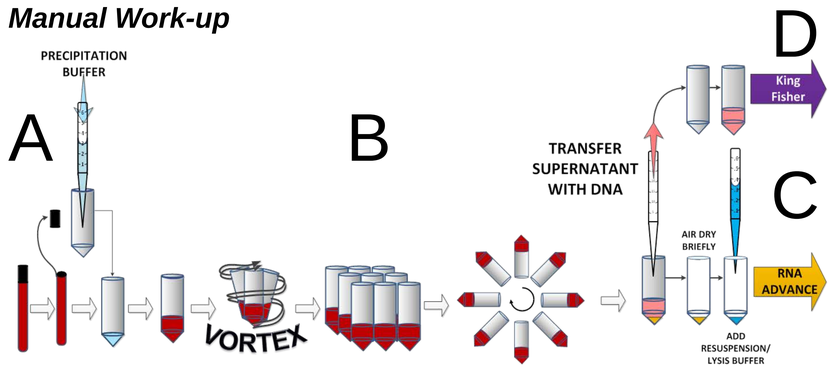
Manual Work-up

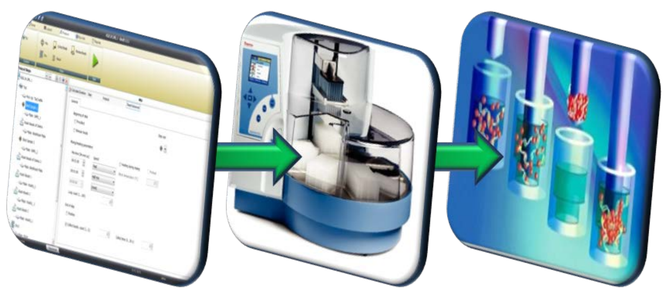


(A) RGB tubes arrive and are transferred into pre-barcoded 15 mL conical tubes pre-filled with precipitation buffer. **(B)** The samples are mixed and centrifuged to attain the RNA pellet. **(C)** The pellets are homogenized in resuspension buffer and formatted into multi-well plates for entry into the automated purification methods. **(D)** The supernatant containing DNA is transferred to a separate barcoded tube from which samples of the specimen are drawn and transferred into a multi-well plate for entry into the automated purification methods.

Automated RNA Extraction

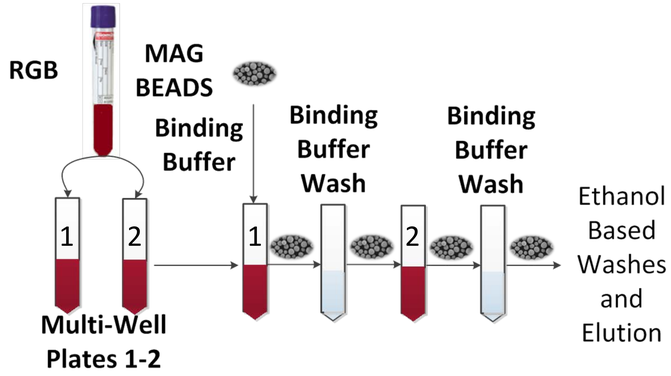
- User calls up method or changes settings in method interface, which results in operating instructions that are executed by the robot.
- RNA separation is achieved via magnetic bead technology: Binding reactions, bead washing, and elution.
- All samples are processed in 96-well plates.
- All transfer and mix steps are via pipetting.
- Tips use and labware positions are managed by the robot; no user intervention is required after the initial set up.
- Immediately following elution, the RNA is sampled for QC. Remaining eluates are combined with RNAstable® and dried in a Genevac HT-12 evaporator.

Automated DNA Extraction



- The user creates or calls a method through the easy to use Bindit® software.
- The user follows prompts to load plates containing samples, wash buffers, and elution buffers.
- The KingFisher instrument processes plates via a turntable mechanism.
- All washes, bead transfers, binding, and elution steps are conducted using disposable magnet tip shields. No liquid transfers occur.
- Generally, no user intervention is required after set up and prior to finish. DNA is eluted, analyzed, and dried.

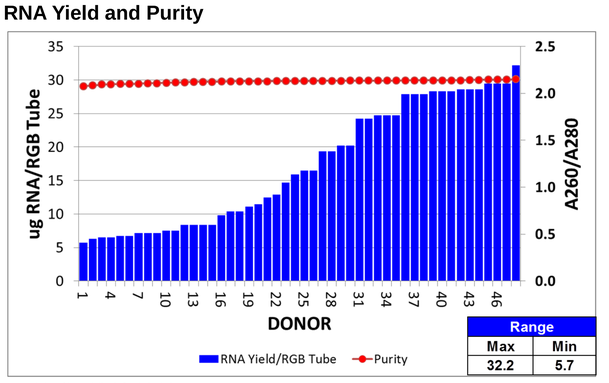
Sample Splitting to Maximize DNA Yield



RGB sample is aliquoted into 2 corresponding wells of 2 separate multi-well plates (e.g., well A1 on each plate). Beads and binding buffer are added to the sample first plate and agitated; only binding buffer is added to the second sample plate. The binding reaction occurs in the first plate. The beads are then collected and washed in a solution of dilute binding buffer to remove debris. The beads are then transferred to the wells of the second plate, and the second binding reaction is allowed to proceed with agitation. After the second binding reaction occurs, the beads are washed in ethanol-based buffers in the typical fashion. Finally, the samples are eluted into TE or water, combined with DNAStable® *Plus,* evaporated to dryness in 384-well cluster tubes, sealed, and stored in a humidity controlled chamber.

Results

RNA Yield and Purity



Median Yield: 16.16 ug
Median Purity: 2.13

DNA Yield and Purity