Stabilization of Cellular RNA in Blood Samples for Noninvasive Diagnosis and Prognosis

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Abstract

Background: Messenger RNA molecules (mRNA) in blood cells are indicators of the activity of their genomes, illustrating what genes are expressed and to what extent. Profiling of cellular mRNA expression patterns has been demonstrated using microarrays, quantitative reverse transcription real-time PCR (RT-qPCR) and molecular beacons (1, 2, 3). Such profiling methods have become increasingly important for both disease characterization and biomarker discovery, but also carry a risk of data misinterpretation (3). A potential issue relates to the handling of blood samples *ex vivo* prior to the extraction of mRNA. Microarray data has shown that clusters of genes were strongly up- and down-regulated in as little as 2 hours post-phlebotomy. Profiles of fresh samples compared to samples shipped overnight were strikingly different, particularly with genes that are known to participate in stress-induced pathways (3). This evidence emphasizes the value of developing blood collection devices that are capable of stabilizing mRNA expression immediately after a blood draw.

Objective: To develop a standardized blood collection device capable of stabilizing mRNA expression patterns immediately after a blood draw and prevent blood cell lysis for an extended period of time at ambient temperature.

Methods: Blood samples were drawn from healthy donors into both K_3EDTA (BD Vacutainer®) and Cell-Free RNA™ BCT collection tubes developed by Streck Inc. White blood cells (WBCs) were separated, total cellular RNA was extracted and mRNAs for RASSF1A, c-fos and glyceraldehyde-3-phosphate dehydrogenase (G3PD) were quantified by RT-qPCR at different time points post-collection. Molecular beacon technology was used to detect G3PD mRNA within intact cells using flow cytometry. All blood samples were kept at ambient temperature until further processed.

Results: While blood drawn into K_3EDTA tubes showed significant changes in cellular mRNA copy numbers for c-fos, G3PD, and RASSF1A, copy number of these biomarkers were unchanged in blood stabilized with Streck's Cell-Free RNA BCT. Moreover, stabilization was also illustrated using a molecular beacon detected by flow cytometry. While an increase in G3PD mRNA levels is found in WBCs stored in K_3EDTA tubes, no change in mRNA levels is detected in samples stored in Cell-Free RNA BCT.

Conclusion: Cell-Free RNA BCT blood collection tubes developed by Streck, Inc. provide preservation and stabilization of cellular mRNAs for at least three days at ambient temperature. This technology preserves the genetic expression patterns of specific genes allowing for the use and development of non-invasive diagnosis and prognosis methodologies based on cellular RNA in blood.

Introduction

Messenger RNA molecules (mRNA) in blood cells are indicators of the activity of their genomes, illustrating what genes are expressed and to what extent. Profiling of cellular mRNA expression patterns has been demonstrated using microarrays, quantitative reverse transcription real-time PCR (RT-qPCR) and molecular beacons (1, 2, 3). Such profiling methods have become increasingly important for both disease characterization and biomarker discovery, but also carry a risk of data misinterpretation (3). A potential issue relates to the handling of blood samples *ex vivo* prior to the extraction of mRNA. Microarray data has shown that clusters of genes were strongly up- and down-regulated in as little as 2 hours post-phlebotomy. Profiles of fresh samples compared to samples shipped overnight were strikingly different, particularly with genes that are known to participate in stress-induced pathways (3). This evidence emphasizes the value of developing blood collection devices that are capable of stabilizing mRNA expression immediately after a blood draw.

Results

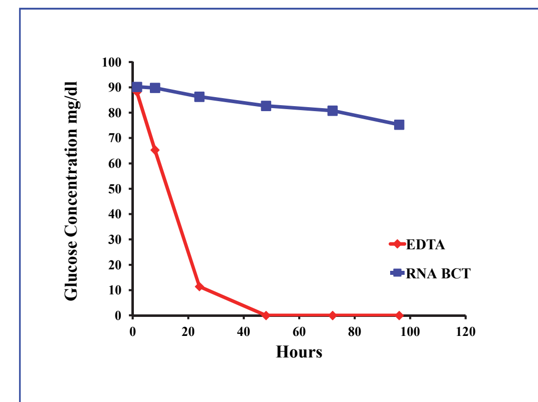

Figure 1. Total plasma glucose concentration in blood samples drawn into regular K_3EDTA and Cell-Free RNA™BCT blood collection tubes and stored at ambient temperature. Preservation of glucose in blood samples indicates inhibition of metabolism in blood cells.

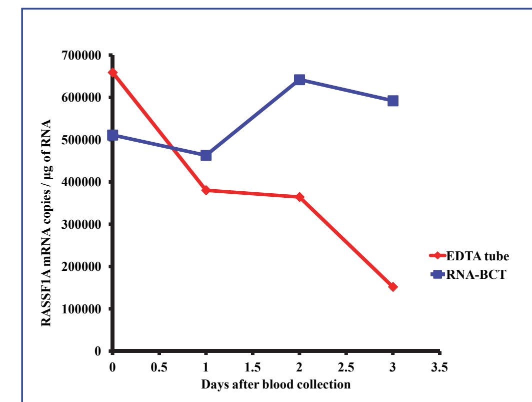

Figure 2. Effect of K_3EDTA and Cell-Free RNA™BCT blood collection devices on cellular RASSF1A mRNA concentration at ambient temperature.

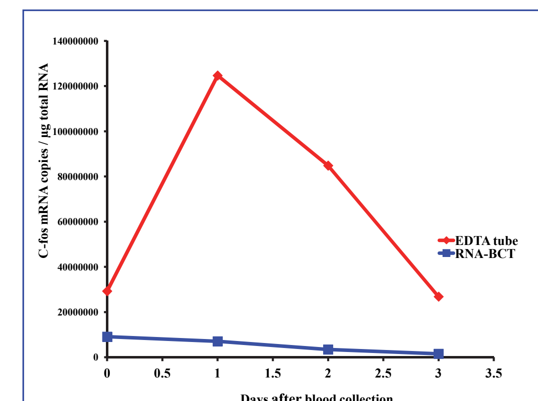

Figure 3. Effect of K_3EDTA and Cell-Free RNA™BCT blood collection devices on cellular c-fos mRNA concentration in at ambient temperature.

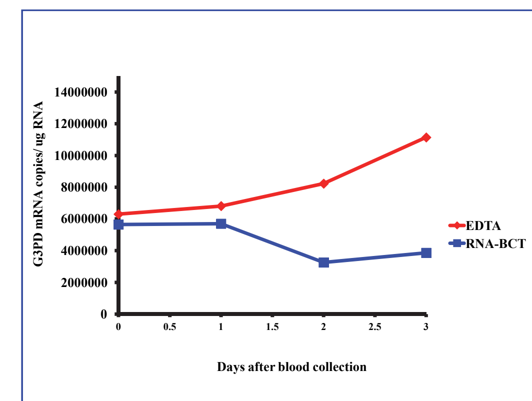

Figure 2. Effect of K_3EDTA and Cell-Free RNA™BCT blood collection devices on cellular G3PD mRNA concentration in at ambient temperature.

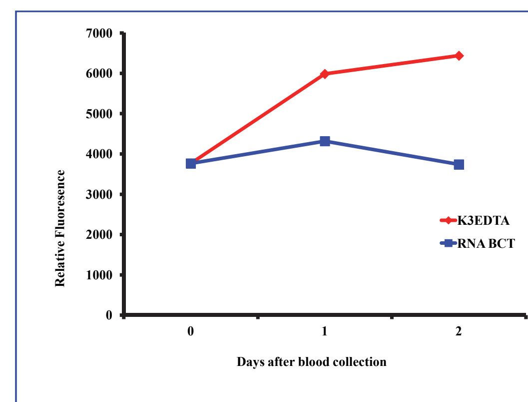

Figure 6. Effect of K_3EDTA and Cell-Free RNA™BCT blood collection devices on G3PD mRNA concentration in intact white blood cells. Molecular beacon for G3PD was introduced Into intact cells and fluorescence was detected by flow cytometry.

Materials and Methods

Healthy donor recruitment
Healthy donors were recruited with informed consent from Streck Inc., Omaha NE, 68128, USA. Donors were both male and female.

Blood collection
Blood samples were drawn into two different blood collection tubes from each donor. One tube was a 7 ml K_3EDTA blood collection tube (BD Vacutainer) and the other was a vacuum blood collection tube developed by Streck Inc. (Cell-free RNA™ BCT) which contained chemical compounds that stabilize nucleated blood cells and inhibit cell metabolism and plasma nucleases. Blood was mixed well immediately after drawing into the tubes by inverting the tube several times.

Sample processing
Blood drawn into both types of tubes was processed at 3 hours, 24 hours, 48 hours and 72 hours. Blood samples were centrifuged at ambient temperature at 400g for 20 minutes. After removing the upper plasma layer using a Pasteur pipette the buffy coat was transferred to a new tube using a Pasteur pipette and then treated with ACT reagent to remove contaminating red blood cells. Then the cells were washed once with PBS containing 0.9% EDTA and the cell pellet was used for total RNA extraction.

Plasma cell-free RNA isolation
Total cellular RNA was purified using a commercially available RNeasy® Mini RNA isolation kit (Qiagen, Santa Clarita, CA). The manufacturer's recommended protocol was modified by including a Proteinase K treatment (at 60^0C) for 2 h following the shredding step.

Real Time quantitative PCR
Primers for the real time PCR quantification of mRNAs for RASSF1A, c-fos, and (G3PD) were purchased from Integrated DNA Technologies (Coralville, IA). TaqMan probes were purchased from Applied Biosystems (Foster City, CA).

Conclusions

Cell-Free RNA™ BCT blood collection tubes developed by Streck Inc. provide preservation and stabilization of cellular mRNAs for at least for three days at ambient temperature. This technology preserves the genetic expression patterns of specific genes allowing for the use and development of non-invasive diagnosis and prognosis methodologies based on cellular RNA in blood.

References

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