Evaluation of a Single Spin Protocol for Plasma DNA Isolation from Blood Collected & Stored in Cell-Free DNA BCT®

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ABSTRACT

Double spin protocols are commonly used to separate blood plasma for Cell-Free DNA isolation. This study was undertaken to evaluate a single spin protocol for use with blood samples collected into Cell-Free DNA BCT®. Blood was collected into 10 mL Cell-Free DNA BCT and divided into 3 groups for the single spin process (i.e., 1600 x g for 15 min) and two double spin processes (i.e., 300 x g for 20 min followed by 5000 x g for 10 min or 1600 x g for 10 min followed by 16000 x g for 10 min), respectively. Samples were stored at room temperature and cell-free DNA was isolated on day 0, 7 and 14. In addition to the storage study, the single spin protocol was also compared with the double spin protocol in a shaking study to simulate conditions that may occur during sample shipping. In all cases, total plasma cell-free DNA was assayed by droplet digital PCR. The comparison of the cell-free DNA concentrations in plasma revealed no statistically significant difference between "paired samples" (i.e., single spin vs. double spin protocols) in both storage and shaking studies. We conclude that the single spin protocol is compatible with the plasma separation procedure from blood collected in Cell-Free DNA BCT for isolation of plasma cfDNA.

INTRODUCTION

The discovery of cell-free nucleic acids in the circulation has opened up new opportunities for non-invasive diagnostic applications in cancer testing and prenatal diagnosis.[1] Cell-free plasma separation is the first step in specimen processing for such applications. Double spin protocols are widely considered the gold standard when separating plasma from blood for plasma cell-free DNA isolation. For example, Streck's in-house protocol specifies a low speed centrifugation of 300 x g for 20 min followed by a high speed centrifugation of 5000 x g for 10 min to effectively separate plasma from blood. The purpose of starting with a low speed spin is to minimize cell lysis during the plasma isolation step, potentially caused by centrifugation spin speed, followed by high speed centrifugation to remove the low quantity of residual cells remaining. Other pioneer researchers in this field also use a slightly different double spin protocol,[2,3] 1600 x g for 10 min followed by a microcentrifugation of 16000 x g for 10 min. The two-spin processes are time consuming in the specimen processing procedure and were originally developed for fresh unpreserved blood drawn into regular tubes, e.g., EDTA tubes. Little is known about optimization of centrifugation protocols for preserved blood samples under conditions of storage and shaking/shipping at room temperature. In this study, a single spin protocol (1600 x g for 15 min) was tested for use with blood samples collected and stabilized in Cell-Free DNA BCT. We compared the proposed single spin protocol with two commonly used double spin processes and assessed the merits of the single spin protocol for plasma cell-free DNA isolation from blood stored in Streck's Cell-Free DNA BCT without introducing cellular DNA contamination.

MATERIALS AND METHODS

Blood Collection

Blood samples were drawn into 10 mL Cell-free DNA BCT (Streck, Inc., La Vista, NE). Cell-Free DNA BCT is a blood collection device that contains a novel chemical cocktail. This cocktail stabilizes nucleated blood cells and contains K_3EDTA as an anticoagulant. Blood was mixed immediately after the draw by inverting tubes 10 times.

Sample Processing and Plasma Separation

Storage study: Blood samples were stored at room temperature and divided into three groups for various centrifugation protocols. One tube of blood was removed from each group on day 0, 7 and 14. Plasma separation centrifugation protocols included the single spin protocol (1600 x g for 15 min) and two double spin processes (300 x g for 20 min followed by 5000 x g for 10 min or 1600 x g for 10 min followed by 16000 x g for 10 min). During double spin processes, the supernatant was carefully removed without disturbing buffy coat and transferred to a new tube followed by a second spin.

Shaking study: Blood samples were secured onto the platform of an orbital shaker and shaken at 150 rpm at room temperature. After 0, 3, 6 and 24 hrs of shaking, plasma was separated by the single spin protocol (1600 x g for 15 min) and the double spin protocol (300 x g for 20 min followed by 5000 x g for 10 min).

cfDNA Isolation From Plasma

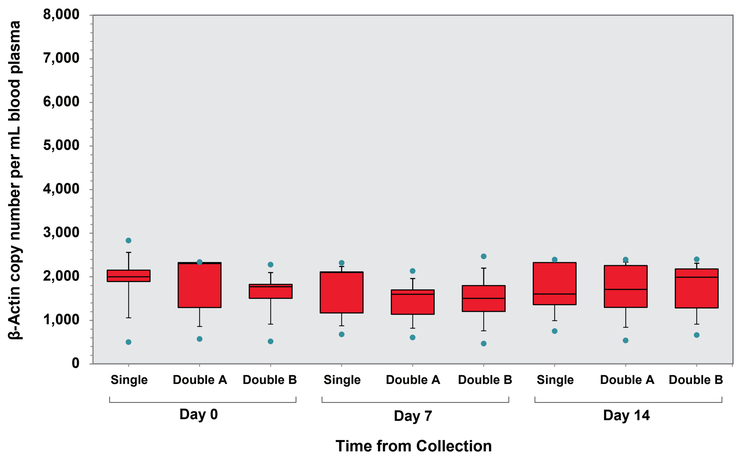
Plasma cfDNA was purified using the commercially available QIAamp® Circulating Nucleic Acid Kit (QIAGEN®, Santa Clarita, CA). For optimal results, the manufacturer's recommended protocol was modified slightly by increasing the duration of the proteinase K treatment from 30 min to 1 hour at 60 ºC. cfDNA samples were stored at -80 ºC until analysis by PCR.

Droplet Digital PCR (ddPCR)

Absolute quantification of cfDNA by PCR was performed using the QX100 Droplet Digital PCR system (Bio-Rad, Hercules, CA). Primers and the probe for the ddPCR quantification of human β-actin were purchased from Integrated DNA Technologies (Coralville, IA).

RESULTS

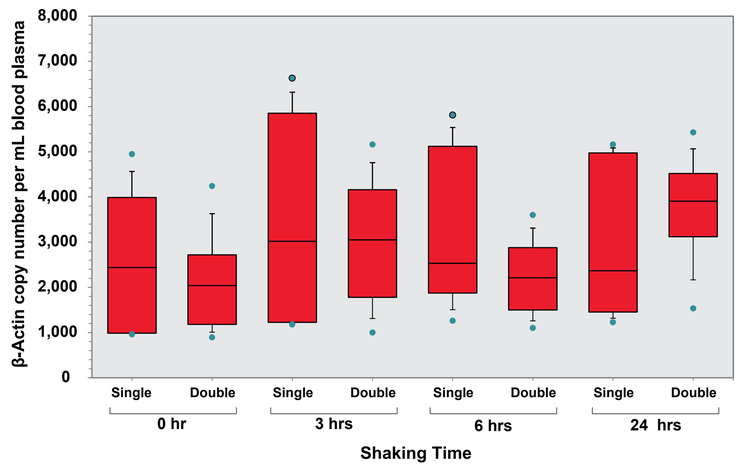
Figure 1 - Storage Study



No significant difference in plasma cell-free DNA concentrations was observed among the single and double spin protocols at each time point. Blood samples collected into 10 mL Cell-Free DNA BCT were stored at room temperature. Plasma was separated on day 0, 7 and 14 using three centrifugation protocols: the single spin process (1600 x g for 15 min), the double spin protocol "A" (300 x g for 20 min followed by 5000 x g for 10 min) and the double spin protocol "B" (1600 x g for 10 min followed by 16000 x g for 10 min). Plasma cfDNA was isolated and quantified by ddPCR.

Box plots show the median (line inside the box) and 75th and 25th percentiles (limits of the box). The upper and lower error bars indicate the 90th and 10th percentiles, respectively. The upper most and lower most dots indicate the maximum and minimum values (n=5, $p < 0.05$ was considered statistically significant).

Figure 2 - Shaking Study



No significant difference in plasma cell-free DNA concentrations was observed between single and double spin protocols at each time point. Blood samples collected into 10 mL Cell-Free DNA BCT were shaken at room temperature. Plasma was separated at 0, 3, 6 and 24 hrs using two centrifugation protocols: the single spin process (1600 x g for 15 min) and the double spin protocol A (300 x g for 20 min followed by 5000 x g for 10 min). Plasma cfDNA was isolated and quantified by ddPCR (n=5, $p < 0.05$ was considered statistically significant).

CONCLUSION

Our results demonstrate that the proposed single spin protocol (i.e., 1600 x g for 15 min) is effective in isolating cell-free plasma from blood collected, stabilized and stored in Cell-Free DNA BCT for downstream applications such as PCR. Stabilization of blood cells by the Cell-Free DNA BCT reagent serves to prevent cell lysis during centrifugation even after being stored for up to 14 days at room temperature. Considerable reductions in laboratory hands-on time and turnaround time are achieved by using the abbreviated single spin process as compared to double spin protocols.

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