

Technical Note

Stabilization of Circulating Tumor Cells in Blood for up To 7 Days Using Streck Cell-Free DNA BCT®

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Introduction

There is a growing interest in the use of circulating tumor cells (CTCs) in non-invasive diagnosis, prognosis and monitoring of treatment regimens. However, the low abundance of the CTCs and their fragile nature may introduce variability in the evaluation of CTCs using different assay platforms. This fragile nature of CTCs arises due to the apoptosis of CTCs which begins after separation from the tumor of origin and after removal of blood from patient. Therefore, it is necessary to address the pre-analytical issues that arise during the time between blood draw and CTC enrichment and characterization in order to effectively preserve CTCs for analysis. These include delays in blood processing, blood storage temperature, and agitation of the sample during transport and shipment of blood. Such conditions may affect the integrity of already fragile CTCs causing accurate enumeration and characterization of CTCs difficult. As a result, it is important to consider the type of blood collection device and post-phlebotomy conditions while working with CTC samples.

Streck Cell-Free DNA BCT is a blood collection device with a stabilization reagent that preserves cell-free DNA in a blood sample for up to 14 days at room temperature. It does so by stabilizing nucleated blood cells in blood and preventing cellular DNA release into plasma. Our previous study has shown that Streck Cell-Free DNA BCT is capable of stabilizing CTCs for up to 4 days at room temperature. This study was designed to investigate the effectiveness of this blood collection device for the stabilization of CTCs in blood sample for an extended period of time (i.e., 7 days) at room temperature.

Materials and Methods

Blood sample collection

Blood specimens were collected from healthy adult donors by standard phlebotomy techniques.

Cell culture

Breast cancer cell line, MCF-7, was obtained from American Type Culture Collection (Rockville, MD, USA) and routinely passaged in Eagle's MEM medium containing 10% fetal bovine serum at 37 °C in humidified atmosphere of 5% CO₂.

Recovery of spiked MCF-7 cells in blood

Blood from each donor (7 donors in total) was drawn into 10 mL BCTs (Streck Inc., La Vista, NE, USA). A known number of MCF-7 cells were then spiked into each tube

and the samples were mixed immediately by inverting 10 times each. All samples were shipped in clamshells at ambient temperature to Ann-Arbor, MI. Samples were analyzed on days 1, 4 and 7, post phlebotomy, on the Veridex CellSearch™ system in order to count the recovery rate of the MCF-7 cells. Blood samples were maintained at room temperature during the entire process.

Statistical analysis

Statistical analysis was carried out using Microsoft Excel for Office 2007. Analysis was performed using paired, two tailed Student's t-test and $p < 0.05$ was considered statistically significant.

Result

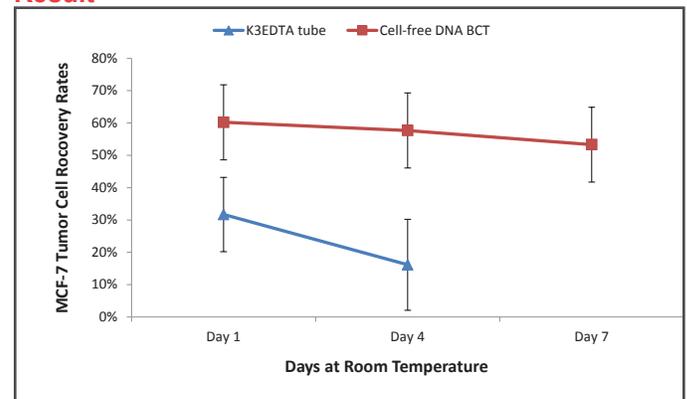


Figure: Recovery Rates of spiked MCF-7 cells in blood drawn into BCTs.

Normal donor blood (n = 7) was drawn into BCTs and a known number of breast tumor cells (MCF-7) were spiked. The whole blood samples were analyzed on CellSearch system to determine recovery of spiked MCF-7 cells at indicated time points. The tumor cell recovery from BCTs was stable. At day 1, 60% (SD = 4%) of spiked MCF-7 cells were recovered and at day 4 and 7 they were 58% (SD = 8%) and 52% (SD = 12%), respectively. This is comparable to the recovery rates for the CellSave blood collection tube. Refer to "Stabilization of Circulating Tumor cells in Blood using a Collection Device with a Preservative Reagent", Qin et al. Cancer Cell International 2014, 14:23).

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As compared to day 1, there are no statistical differences in recovery rates after shipping and 4 or 7 days storage ($p > 0.05\%$). In contrast, K₃EDTA tubes failed to preserve CTCs resulting in a much lower recovery rate for both day 1 and 4 as compared to BCTs. In K₃EDTA tubes, at day 1, recovery rate was 32% (SD = 12%) of the spiked MCF-7 cells and at day 4 it was 16% (SD = 14%). (Refer to “Stabilization of Circulating Tumor Cells in Blood Using a Collection Device with a Preservative Reagent,” Qin et al. *Cancer Cell International* 2014, 14:23).

Conclusion

In this study, we have modeled circumstances that could alter CTC detection on an FDA cleared instrument running assays that are designed to be helpful in cancer diagnosis, prognosis and the monitoring of patient response to treatments. The modeling of post-phlebotomy has shown that BCT provides preservation and stabilization of CTCs in blood samples for up to 7 days at room temperature. Using Streck Cell-Free DNA BCT, shipping or ex vivo storage of CTC blood samples for up to 7 days become possible, allowing the laboratory to better manage sample testing workflow.