Cyto-Chex® BCT Stabilizes Cells for Minimal Residual Disease Testing

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Abstract

Recent studies and clinical trials provide evidence for the utility of minimal residual disease (MRD) testing during treatment and post-therapy monitoring of patients with hematopoietic neoplasms. The development of standardized methods has been sought for these tests and flow cytometry is utilized by many contract research organizations and hospitals to reach these goals. The purpose of this study was to determine if minimal residual disease cells are preserved and stable in whole blood collected in the blood collection device, Cyto-Chex® BCT. We demonstrate the stability of a tissue culture cell line, as a model for MRD, at levels as low as 0.01%, in normal peripheral whole blood for up to 7 days. Disease specific antigens (CD25, CD71, and CD117) were utilized for detection of the abnormal cells. This is stark contrast to the abnormal cells maintained in K_2EDTA, which show instability by day 3. Additionally, peripheral whole blood samples containing MRD cells are also stable for up to 7 days after sample shipment. This discovery shows the valuable application of rare cell stabilization with sample stress to allow more accurate identification of patients with clinically relevant minimal residual disease which may require additional treatment for their hematopoietic neoplasm.

Introduction

Evidence of minimal residual disease (MRD) during treatment and post-therapy is an important predictor of patient relapse and long-term survival in many leukemia types including acute lymphocytic leukemia (ALL) and chronic lymphocytic leukemia (CLL).[1-3] In addition to being used as an endpoint in clinical trials, the risk of MRD, as a measure of treatment efficacy or future relapse, is quickly becoming recognized as standard of care.[4] For MRD assessment, various methods can be utilized, including molecular biology (i.e. PCR techniques) and multi-dimensional flow cytometry. The use of flow cytometry compared to molecular biology techniques is growing in popularity as it is more cost effective, less labor intensive, faster, and has detection levels comparable to molecular methods.[1,3,5,6] Flow cytometry procedures are not patient specific and can also evaluate cell quality and viability.

The limits of detection (LODs) for MRD positive and MRD negative patients being treated for hematopoietic neoplasms have been previously established.[1,3,5,6] The LODs are grouped as ≤ 0.01%, > 0.01% - 0.1%, and > 0.1% MRD cells in patient samples, relative to non-diseased leukocytes, and can provide clinicians with information on how the selected treatment is performing and gauge a patient's risk for relapse.[7] Advances in these technologies continue with standardized, disease-specific practices being developed to ensure inter-laboratory consistency. To be classified as a disease cell, several criteria must be met. Testing of multiple antigenic sites (4-6) using a combination of disease relevant and lineage markers is a preferred approach.[4] Disease cells must also cluster using multi-parameter gating strategies to identify abnormal cells.[4] Current guidelines recommend detection of at least 1 positive cell per 10,000 normal white blood cells for assay sensitivity and 1 x 10^5 to 1 x 10^6 total cells should be collected per sample.[1]

Clinical trial protocols, including those being evaluated for MRD, often send precious samples to a central laboratory for evaluation to increase turnaround time and sample analysis consistency.[1,2] Optimally, shipment time should not exceed 24 hours[4], but the chance of missing MRD events even within this timeframe is unknown. Despite the advances in molecular biology and flow cytometry techniques for evaluation of minimal residual disease, the impact of sample storage and shipment has not been thoroughly investigated. In these studies, we sought to determine if MRD cells in peripheral whole blood could be identified following stabilization in Cyto-Chex BCT. Using an established leukemia cell line and normal patient blood, we show Cyto-Chex BCT stabilizes MRD cells at a 0.01% level for up to 7 days when stored at room temperature. Results are equivalent even after sample shipment.

Materials and Methods

Healthy donor 5mL whole blood samples were collected into K_2EDTA and Cyto-Chex BCT. White blood cell counts were determined on the LH-750 hematology analyzer. To simulate MRD cells present in normal samples, JURL-MK1, a chronic myelogenous leukemia (CML) cell line, was used as a model for abnormal cells. Prior to harvesting, cell counts were obtained using a hemocytometer with Trypan Blue Solution (0.04%). JURL-MK1 cells were mixed with whole blood at 0.01%. Samples were maintained at room temperature (18˚- 22˚C) for the duration of the study. Samples were prepared for flow cytometry evaluation for analysis on the BD FACSCalibur™ flow cytometer on days 1, 3, 5, and 7 post-draw using a lyse-wash protocol. Antibodies included CD45, CD25, CD71, and CD117.

CD Marker	Fluorochrome	Clone	Catalog Number	Vendor
CD45	PerCP	2D1	347464	BD Biosciences
CD25	FITC	M-A251	555431	BD Pharmingen
CD71	APC	L01.1	341029	BD Biosciences
CD117	PE	104D2	340529	BD Biosciences

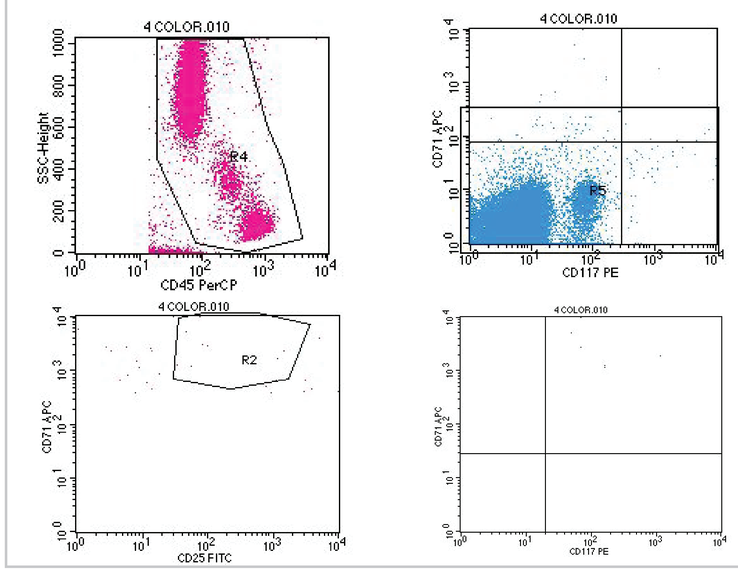
For shipping studies, samples were shipped to an off-site location via FedEx on the day of blood collection for receipt the following day (day 1 to day 2). It was returned to Streck via FedEx the following day (day 2 to day 3).

Results

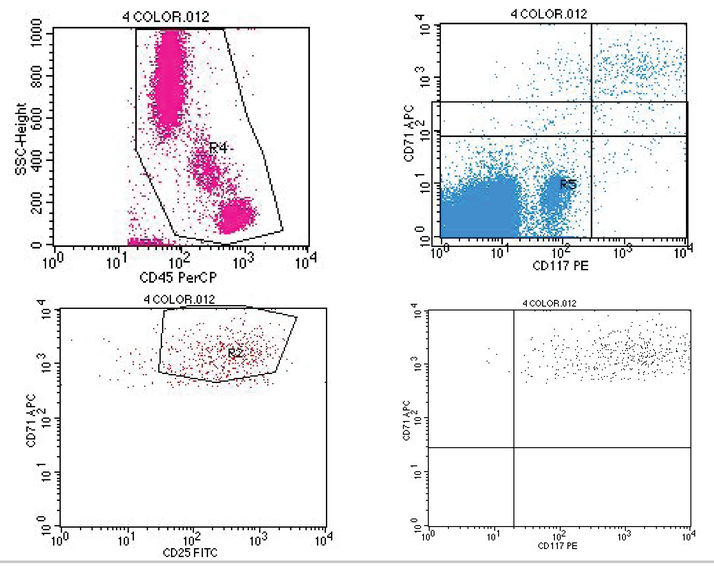
Prior to the initiation of experiments for MRD detection, a feasibility study was completed to test our panel design and detection methods. Once the panel was qualified, whole blood samples maintained in K_2EDTA and Cyto-Chex BCT were spiked with 0.01% JURL-MK1 cells to simulate MRD. Cyto-Chex BCT maintained equivalent MRD detection for the duration of the study while the MRD cells in K_2EDTA were unstable. Comparable results were seen with the shipped samples.

Panel Design

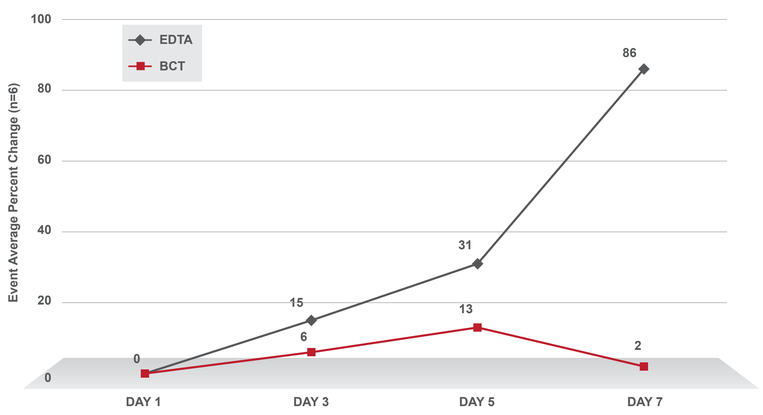
No Cells



0.3% MRD Cells



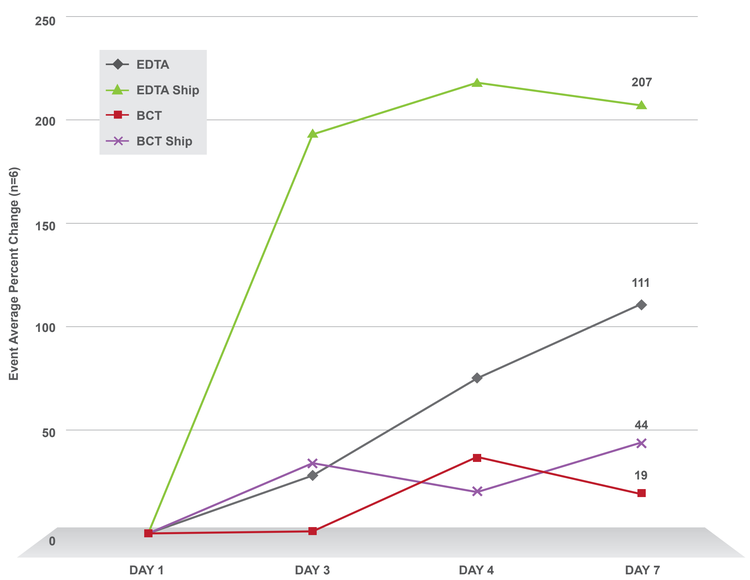
Cyto-Chex BCT stabilizes MRD cells for 3, 5, and 7 days.



JURL-MK1 event number average tested by flow cytometry. Percent difference realized from day 7 vs. day 1 tests. 100,000 WBC events were collected.

	6-Hour EDTA Average	7-Day EDTA Average	Average Percent Difference	6-Hour BCT Average	7-Day BCT Average	Average Percent Difference
Donor 1	15	28	46	17	16	6
Donor 2	25	44	43	18	17	6
Donor 3	22	63	65	16	18	11
Donor 4	11	17	35	11	12	8
Donor 5	19	23	17	17	16	6
Donor 6	17	27	37	18	19	5

Cyto-Chex BCT stabilizes shipped samples containing 0.01% MRD cells for up to 7 days.



Discussion

Cyto-Chex BCT maintains abnormal cells in peripheral whole blood samples for detection by flow cytometry at levels consistent with clinical benchmarks for minimal residual disease. MRD cells are also stable in Cyto-Chex BCT after sample shipment, which can frequently occur when samples are tested off-site at a reference laboratory. This discovery shows the valuable application of rare cell stabilization with sample stress for detection of hematopoietic neoplasm MRD cells from a peripheral blood sample.

References

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