Streck Cyto-Chex® BCT stabilizes TRBC1 marker expression in whole blood

Stephanie Wigginton, B.S., Karl S.M. Bisselou, Ph.D., Bradford A. Hunsley, John W. Scholl
Research and Development Division, Streck, La Vista, NE 68128 USA

Introduction

The identification and stabilization of immunological markers plays a vital role in advancing our understanding of the immune system. Among these markers are T-cell receptor (TCR) β-chain constant region 1 and 2 (TRBC1 and TRBC2), which are crucial for T-cell function. During T-cell development, clonality is determined when the β-chain region of the TCR expresses either TRBC1 or TRBC2. T-cell clonality testing has become an important part of the diagnostic process for T-cell malignancies, as they are often characterized by a disproportionate number of T-cells expressing either TRBC1 or TRBC2. TRBC1 analysis by flow cytometry is essential to identifying T-cell clonality and distinguishing pathological aberrancies from normal T lymphocyte populations (1, 2).

One of the biggest challenges for flow cytometric analysis is that cells begin to deteriorate once blood has been drawn into blood collection tubes that contain anticoagulants such as EDTA (3). Therefore, the integrity of flow analysis results may be compromised if there is a delay in evaluation due to shipment and/or storage requirements. To address this issue, we determined whether Cyto-Chex® BCT, a whole blood stabilization tube, could maintain TRBC1 marker expression in whole blood during extended room temperature storage. We surmised that Cyto-Chex BCT may be an effective means of stabilization, as it has previously received FDA 510(k) clearance for consistent recovery of HIV-associated lymphocyte subsets. Our data suggest that Cyto-Chex BCT maintains TRBC1 antigen expression in whole blood for up to 14 days at room temperature, allowing laboratories the versatility to ship and process samples as scheduling permits.

Methods and Analysis

Sample Collection and Storage

Whole blood was collected from five self-proclaimed healthy donors by venipuncture into EDTA or Cyto-Chex BCT. Flow cytometric analysis was performed on blood collected into EDTA after ~2 hours and on blood collected into Cyto-Chex BCT after 3, 7 or 14 days of room temperature storage (Figure 1).

Recovery of TRBC1$^+$ Cells by Flow Cytometry

Whole blood samples were stained with the antibodies listed in Table 1 for 20 minutes at room temperature. Samples were then lysed using 1X BD FACS® Lysing Solution (BD® Biosciences) for 10 minutes at room temperature, washed and resuspended in a 1X PBS + 0.5% BSA + 0.05% sodium azide buffer. Data was collected on the BD FACSCanto® II flow cytometer using the BD FACSDiva™ software (BD Biosciences, 5 replicates per sample) and subsequently analyzed using Kaluza C Analysis Software (Beckman Coulter®).

Acquisition and analysis were performed as described by Waldon et. al ((1) and Figure 1). Briefly, stained cells were first gated for single cells to remove debris and doublets from subsequent analysis. Single cells were then plotted based on CD45 expression and side-scatter (SSC) to eliminate non-white cell debris and separate the lymphocytes, monocytes and granulocytes. TCRγδ expression of gated lymphocytes was measured, and TCRγδ$^+$ cells were excluded from analysis, as these cells can interfere with TRBC1 recoveries. The resulting CD45$^+$TCRγδ$^-$ lymphocyte population was separated into CD3$^+$ and CD3$^-$ populations. Gated CD3$^+$ cells were further stratified into CD4$^+$ and CD8$^+$ populations that were placed in separate histograms to measure CD4$^+$TRBC1$^+$ and CD8$^+$TRBC1$^+$ recoveries.

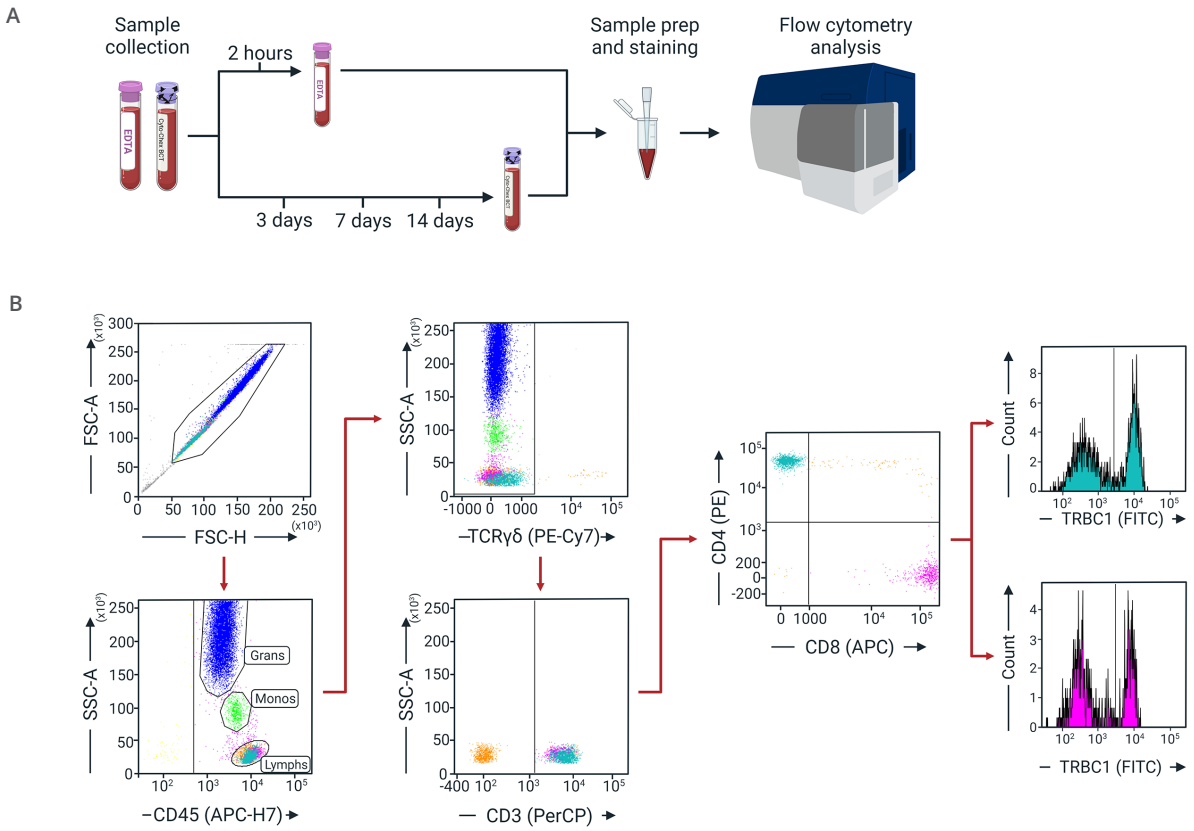

Figure 1. Overview of sample processing and the gating strategy used to obtain TRBC1 recoveries. (A) *Whole blood was collected in EDTA or Cyto-Chex® BCT and processed for flow cytometry analysis at ~2 hours post-draw (EDTA) or after 3, 7 or 14 days of room temperature storage (Cyto-Chex BCT).* ***(B)*** *Stained cells were gated for singlets before gating for CD45⁺ lymphocyte, monocyte and granulocyte populations. Lymphocytes were analyzed for TCRγδ expression and the TCRγδ⁻ population was gated for measurement of CD3 expression. CD45⁺TCRγδ⁻CD3⁺ were selected and analyzed for CD4 and CD8 expression. CD45⁺TCRγδ⁻CD3⁺CD4⁺ and CD45⁺TCRγδ⁻CD3⁺CD8⁺ cells were plotted on separate histograms to measure TRBC1 recoveries for each population.*

Table 1. List of antibodies used in this study.

Marker	Fluorochrome	Clone	Manufacturer
TRBC1	FITC	JOVI.1	Ancell
CD4	PE	SK3	BD Biosciences
CD3	PerCP	SK7	BD Biosciences
CD8	APC	SK1	BD Biosciences
CD45	APC-H7	2D1	BD Biosciences
TCRγδ	PE-Cy7	11F2	BD Biosciences

Statistical Analysis

The Bland-Altman methods of agreement were used to estimate recovery bias between Cyto-Chex BCT and EDTA at various times and storage conditions (4). To assess the proportion of variance explained by storage conditions for Cyto-Chex BCT, a regression R-squared (R^2) was used.

Results

The current standard for evaluation of whole blood samples requires flow cytometric analysis to be carried out within 48 hours of collection into a tube containing an anticoagulant (typically EDTA). As such, we compared TRBC1 recoveries of samples collected into Cyto-Chex® BCT after 3, 7 or 14 days of room temperature storage to recoveries of samples collected into EDTA and analyzed within the 48-hour window, at ~2 hours post-draw (Table 2). We found that mean TRBC1 recoveries in Cyto-Chex BCT after 3, 7 and 14 days of room temperature storage were similar to the mean recovery from samples collected into EDTA after ~2 hours, suggesting that Cyto-Chex BCT effectively maintains TRBC1 surface expression for up to 14 days of room temperature storage (Table 2 and Figure 2A).

Table 2. Numerical representation of TRBC1 recoveries for $CD4^+$ and $CD8^+$ cells.

	Collection Tube	Time After Draw	Avg % Recovery	Std Dev	CV%
$CD4^+$ $TRBC1^+$ Recovery	EDTA	2 hours	44.20	3.59	8.13
	Cyto-Chex BCT	3 days	44.09	3.49	7.91
		7 days	43.23	4.43	10.24
		14 days	43.75	4.09	9.35
$CD8^+$ $TRBC1^+$ Recovery	EDTA	2 hours	33.28	6.84	20.56
	Cyto-Chex BCT	3 days	32.28	6.99	21.64
		7 days	32.69	7.48	22.8
		14 days	32.52	6.42	19.74

Given that mean TRBC1 recoveries for samples collected into Cyto-Chex BCT did not substantially change from day 3 to day 14, we chose to carry out linear regression and Bland-Altman analysis comparing the samples that were stored in Cyto-Chex BCT for 14 days to those collected into EDTA and processed on day 0 (Figure 2). Linear regression data demonstrated R^2 values for $CD4^+TRBC1^+$ and $CD8^+TRBC1^+$ of 0.9337 and 0.9483, respectively, suggesting strong linear association between Cyto-Chex BCT and EDTA TRBC1 recoveries (Figure 2B). Bland-Altman analyses further support that agreement by demonstrating constant variability between Cyto-Chex BCT day 14 and EDTA day 0, with 95% of all data points falling within 2 standard deviations, and a bias of 0.447 and 0.758 (i.e., bias less than 3%) for $CD4^+TRBC1^+$ and $CD8^+TRBC1^+$, respectively (Figure 2C).

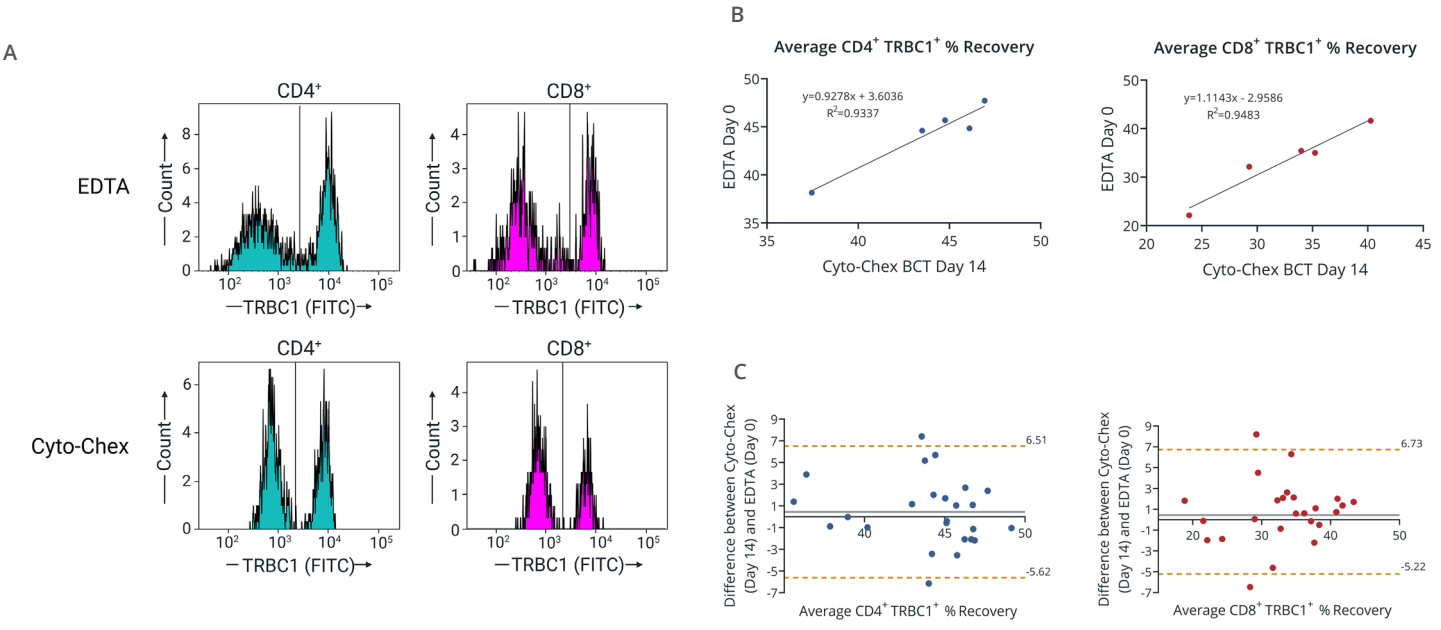

Figure 2. Cyto-Chex BCT maintains TRBC1 expressing lymphocyte populations for up to 14 days post-draw. (A) *Blood stored in Cyto-Chex BCT for 14 days at room temperature has comparable TRBC1 surface antigen expression to blood collected into EDTA and processed on day 0 (after ~2 hours).* ***(B)*** *Linear regression plots show $CD4^+TRBC1^+$ recoveries and $CD8^+TRBC1^+$ recoveries for samples stored in Cyto-Chex BCT for 14 days at room temperature and samples collected in EDTA and analyzed on day 0. Each dot on the two plots represents the average of five runs for each of the five donors.* ***(C)*** *Bland-Altman plots demonstrate agreement between recoveries from samples collected in EDTA and analyzed on day 0 and recoveries from samples stored in Cyto-Chex BCT for 14 days at room temperature. The yellow dash lines represent the 95% confidence interval of the differences between EDTA and the Cyto-Chex BCT recoveries.*

Conclusions

Overall, our data demonstrate Cyto-Chex® BCT maintains important cellular characteristics such as size, complexity, and surface antigen expression, including TRBC1, for up to 14 days when stored at room temperature. This stabilization of cellular characteristics expands the time allowed between sample collection and analysis, giving laboratories the versatility to ship and process samples as scheduling permits.

References:

1. Waldron D., O'Brien D., Smyth L., Quinn F., Vandenberghe E. Reliable Detection of T-Cell Clonality by Flow Cytometry in Mature T-Cell Neoplasms Using TRBC1: Implementation as a Reflex Test and Comparison with PCR-Based Clonality Testing. Lab Med. 2022 Jul 1;53(4):417–25.
2. Maciocia P.M., Wawrzyniecka P.A., Philip B., Ricciardelli I., Akarca A.U., Onuoha S.C., et al. Targeting the T cell receptor β-chain constant region for immunotherapy of T cell malignancies. Nat Med. 2017 Dec 1;23(12):1416–23.
3. van der Strate B., Longdin R., Geerlings M., Bachmayer N., Cavallin M., Litwin V., et al. Best practices in performing flow cytometry in a regulated environment: feedback from experience within the European Bioanalysis Forum. Bioanalysis. 2017 Aug 1;9(16):1253–64.
4. Altman D.G., Bland J.M. Measurement in Medicine: The Analysis of Method Comparison Studies. J R Stat Soc Ser Stat. 1983 Sep 1;32(3):307–17.

Figure 1A was created with BioRender.com.