

Streck Cyto-Chex® BCT Stabilizes TRBC1 Marker Expression in Whole Blood

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ABSTRACT

During T-cell development, the β -chain region of the T-cell receptor (TCR) will express TRBC1 (T-cell receptor β -chain constant region 1) or TRBC2 (T-cell receptor β -chain constant region 2). These genes can be an important marker for cancer screening, as T-cell malignancies often display a disproportionate number of T-cells expressing either TRBC1 or TRBC2. However, transport and storage can negatively impact the marker recovery and staining characteristics of white blood cells in patient samples. To address this issue, we evaluated whether a whole blood stabilization tube, Cyto-Chex BCT, 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. Indeed, our data suggest that 14 days of room temperature storage has minimal effect on leukocyte population distribution and CD marker expression when blood is collected into Cyto-Chex BCT. Further, our data demonstrate linear association and constant variability of TRBC1 marker expression between samples collected into Cyto-Chex BCT or EDTA with an observable bias of less than 3%. Taken together, these data suggest that TRBC1 antigen expression can be preserved in Cyto-Chex BCT-stabilized 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 for 10 minutes at room temperature using 1X BD FACS® Lysing Solution (BD® Biosciences), washed and resuspended in a 1X PBS + 0.5% BSA + 0.05% sodium azide buffer. Data were collected on the BD FACSCanto® II flow cytometer using the BD FACSDiva™ software (BD Biosciences, 5 replicate per sample) and subsequently analyzed using Kaluza C Analysis Software (Beckman Coulter®).

Acquisition and analysis were performed as described by Waldron 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. TCR $\gamma\delta$ expression of gated lymphocytes was measured, and TCR $\gamma\delta$ ⁺ cells were excluded from analysis, as these cells can interfere with TRBC1 recoveries. The resulting CD45⁺TCR $\gamma\delta$ ⁻ lymphocyte population was separated into CD3⁺ and CD3⁻ populations. Gated CD3⁺ cells were further stratified into CD4⁺ and CD8⁺ populations that were placed in separated histograms to measure CD4⁺TRBC1⁺ and CD8⁺TRBC1⁺ recoveries

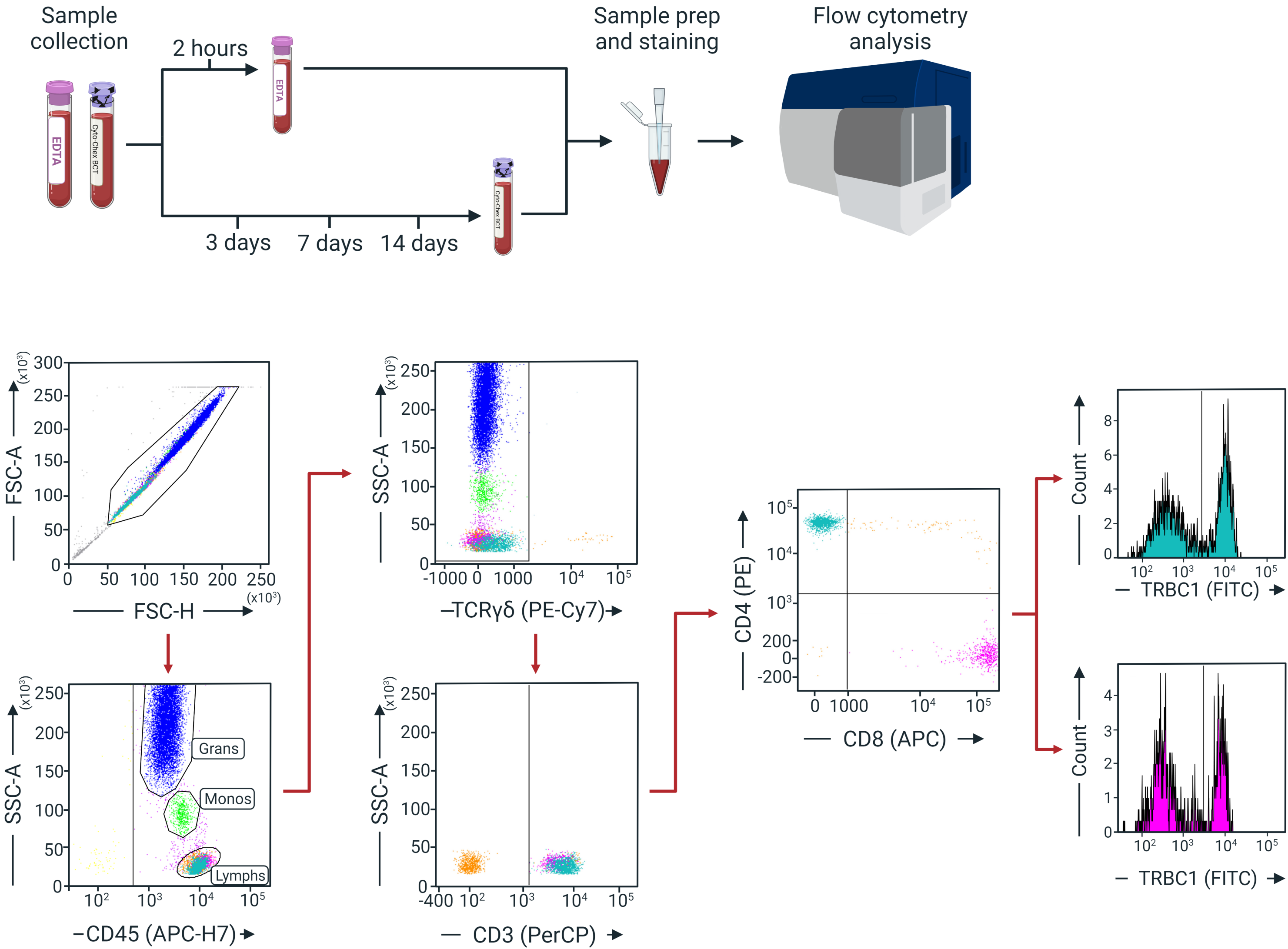


Figure 1. Overview of sample processing and the gating strategy used to obtain TRBC1 recoveries. (A) Whole blood was collected in EDTA or Cyto-Chex BCT and processed for flow cytometry analysis at ~2 hours post-draw (EDTA) or after 3, 7, or 14 days of room temperature storage (Cyto-Chex BCT). (B) Stained cells were gated for singlets, then gated for CD45⁺ granulocyte, monocyte, and lymphocyte populations. Lymphocytes were analyzed for TCR $\gamma\delta$ expression and the TCR $\gamma\delta$ ⁺ population was gated for measurement of CD3 expression. CD45⁺TCR $\gamma\delta$ ⁻CD3⁺ were selected and analyzed for CD4 and CD8 expression. CD45⁺TCR $\gamma\delta$ ⁻CD3⁺CD4⁺ and CD45⁺TCR $\gamma\delta$ ⁻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	JOV1.1	Ancell
CD4	PE	SK3	BD Biosciences
CD3	PerCP	SK7	BD Biosciences
CD8	APC	SK1	BD Biosciences
CD45	APC-H7	2D1	BD Biosciences
TCR $\gamma\delta$	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. To assess the proportion of variance explained by storage conditions for Cyto-Chex BCT, a regression R-squared (R²) was used.

RESULTS

Whole blood collected into Cyto-Chex BCT has stable TRBC1 expression for up to 14 days of room temperature storage.

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 2).

Table 2. Numerical representation of the 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
		3 days	44.09	3.49	7.91
	Cyto-Chex BCT	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
		3 days	32.28	6.99	21.64
	Cyto-Chex BCT	7 days	32.69	7.48	22.8
		14 days	32.52	6.42	19.74

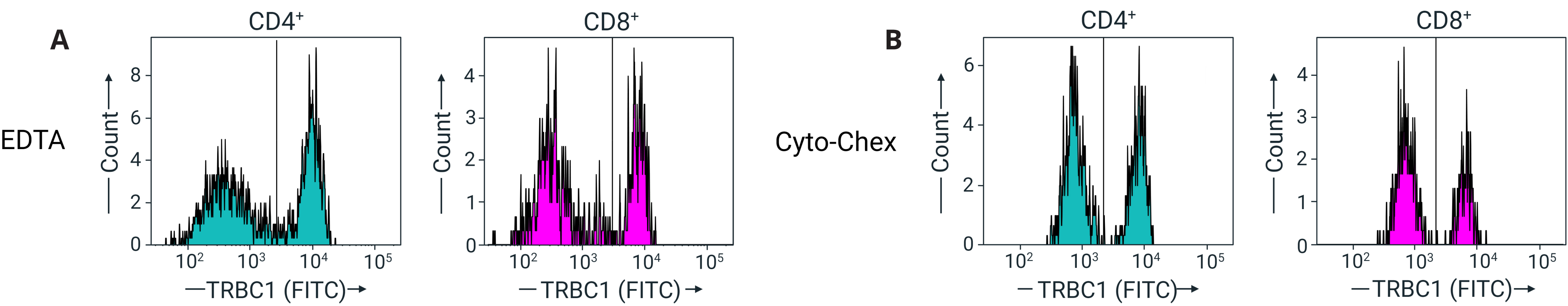


Figure 2. Cyto-Chex BCT maintains TRBC1 expressing lymphocyte populations for up to 14 days when stored at room temperature. Blood collected into EDTA (A) and processed on day 0 (after ~2 hours) has comparable TRBC1 surface antigen expression to blood stored into Cyto-Chex BCT (B) for 14 days at room temperature.

TRBC1 recoveries following 14 days of room temperature storage in Cyto-Chex BCT are statistically equivalent to draw time.

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. Linear regression data demonstrated R² 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 3A). 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 3B).

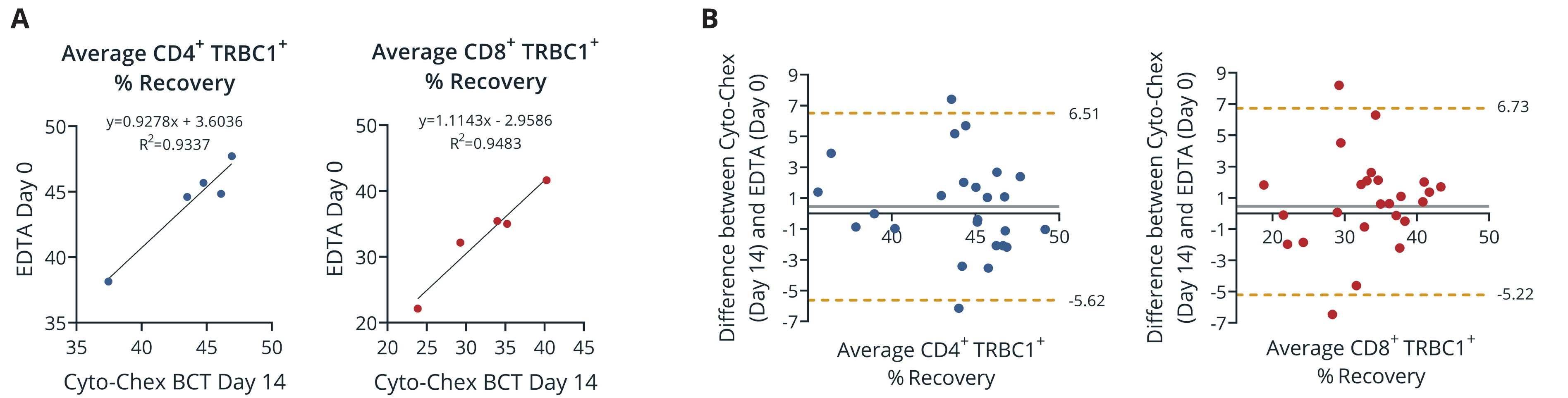


Figure 3. CD4⁺TRBC1⁺ and CD8⁺TRBC1⁺ recoveries from samples stored in Cyto-Chex BCT for 14 days at room temperature display statistical agreement to recoveries from samples collected into EDTA and analyzed at day 0. Linear regression (A) and Bland-Altman plots (B) of CD4⁺TRBC1⁺ recoveries and CD8⁺TRBC1⁺ recoveries from samples collected in EDTA and analyzed at day 0 or samples analyzed after 14 days of room temperature storage in Cyto-Chex BCT. Each dot on the linear regression plots (A) represents the average of five runs for each of the five donors. The yellow dash lines (B) represent the 95% confidence interval of the differences between EDTA and Cyto-Chex BCT recoveries.

CONCLUSIONS

Overall, our data demonstrate that 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., Vandenbergh 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.
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