



Immunomagnetic isolation of PBMCs from Cyto-Chex BCT®-stabilized whole blood

Jessica Hart, MPH, MLS(ASCP)SH, and Rebecca Wilshusen, Ph.D.
Streck Research and Development, La Vista, NE

ABSTRACT

Streck's Cyto-Chex® BCT has been FDA 510(k) cleared for consistent recovery of HIV-associated lymphocyte subsets for up to 14 days at room temperature blood storage while maintaining cellular morphology and surface antigen expression. Ficoll (density-gradient) separation is one of the most common methods for isolating peripheral blood mononuclear cells (PBMCs) from whole blood samples prior to flow cytometry analysis. Although Ficoll separation is readily accomplished with whole blood collected into EDTA, the stabilization reagent in Cyto-Chex BCT is not compatible with density-gradient separation. However, blood collected into EDTA does not effectively maintain cellular counts and morphology beyond 24 hours at room temperature, severely limiting a laboratory's ability to ship and process before cellular integrity is lost. PBMC isolation of Cyto-Chex BCT-stabilized whole blood is achievable through separation of PBMCs by immunomagnetic isolation up to 7 days post blood collection, allowing laboratories to continue utilizing a collection method that allows for extended storage time. No significant differences in leukocyte population recoveries were demonstrated between this method and density-gradient separation from EDTA-collected whole blood. Moreover, resultant T- and B-cell populations are comparable to those obtained by Ficoll density-gradient method. In addition, cell count and antigen expression were maintained for classical T, B, and NK subpopulations. Separation by EDTA/Ficoll is time-dependent, whereas Cyto-Chex BCT/immunomagnetic separation eliminates this rate-limiting step and allows for PBMC isolation up to 7 days post blood draw. In conclusion, isolation of PBMCs from Cyto-Chex BCT-stabilized whole blood allows laboratories the flexibility to transport and store samples until time permits for processing.

MATERIALS AND METHODS

Tube	PBMC isolation method	Time elapsed after blood draw
EDTA	Ficoll	2 hours
Cyto-Chex BCT	EasySep Kit	24 hours
Cyto-Chex BCT	EasySep Kit	5 days
Cyto-Chex BCT	EasySep Kit	7 days

Table 1. Isolation method and blood storage time of EDTA and Cyto-Chex BCT-drawn samples.

Whole blood was collected from five separate donors by venipuncture into EDTA and Cyto-Chex BCTs. EDTA-collected whole blood was processed via Ficoll density gradient separation (Lymphoprep™, STEMCELL™ Technologies) approximately two hours after blood draw. Cyto-Chex BCT-stabilized whole blood was processed using the EasySep™ Direct Human PBMC Isolation Kit (STEMCELL™ Technologies) at approximately 24 hours, 5 days, and 7 days after blood draw (Table 1). However, due to the stabilization properties of Cyto-Chex BCT it is not necessary to add EDTA to the whole blood sample as the EasySep IFU recommends. In summary, Cyto-Chex BCT-stabilized whole blood was incubated with EasySep Isolation Cocktail and RapidSpheres before incubation on an EasySep magnet for 5 minutes. Cell suspension was poured into a new tube, RapidSpheres were added, and the tube was incubated on the magnet for 5 minutes before pouring the cell suspension into a new tube. Cell suspension was incubated on the magnet for a final 5 minutes and the supernatant, containing the PBMC cell suspension, was poured into a new tube. Isolated PBMCs were ready to process for flow cytometry. Before and after PBMC isolation, samples were stained with CD45-FITC (BD Biosciences) for enumeration of leukocyte populations by flow cytometry. A minimum of 2 x 10⁴ events per sample were acquired using a FACSCanto II™ (BD Biosciences) and analyzed using Kaluza Analysis Software (Beckman Coulter). Post-isolation T, B, and NK cell populations were characterized using BD Multitest™ antibody cocktails with BD Trucount™ tubes (BD Biosciences). Samples were analyzed using BD FACSCanto™ Clinical IVD software for enumeration of lymphocyte subsets.

RESULTS

Minimal difference in lymphocyte subset recovery after isolation

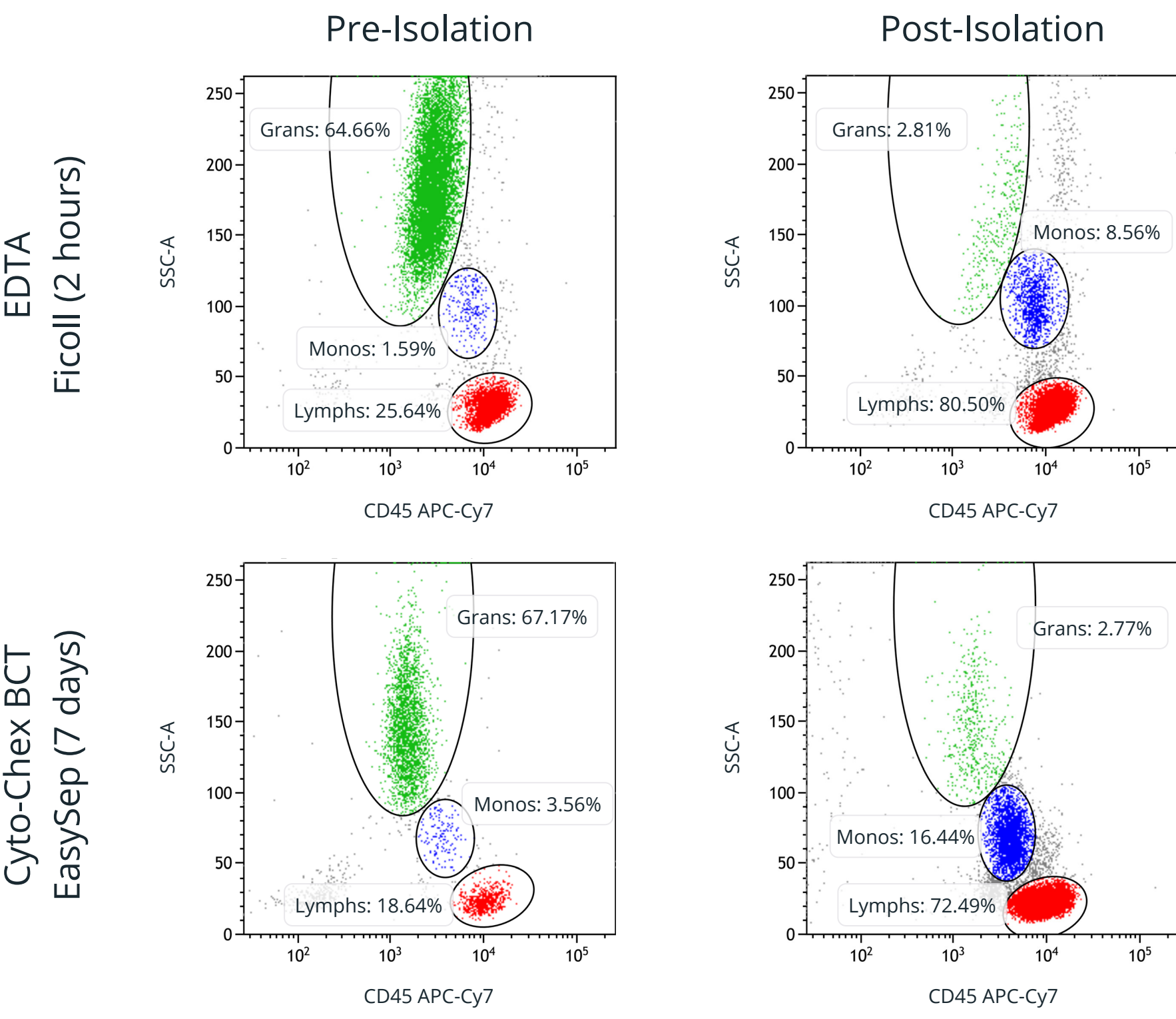


Figure 1. Lymphocyte subset recovery pre- and post-PBMC isolation. Seven days post blood collection, EasySep Kit PBMC recovery from Cyto-Chex BCT-stabilized whole blood is comparable to Ficoll density-gradient PBMC recovery from EDTA-collected whole blood. Leukocyte populations in a single donor pre- (left) and post- (right) PBMC isolation for the Ficoll separation technique (top) and the EasySep PBMC Isolation kit (bottom).

Minimal difference in lymphocyte population recovery over a 7-day storage period

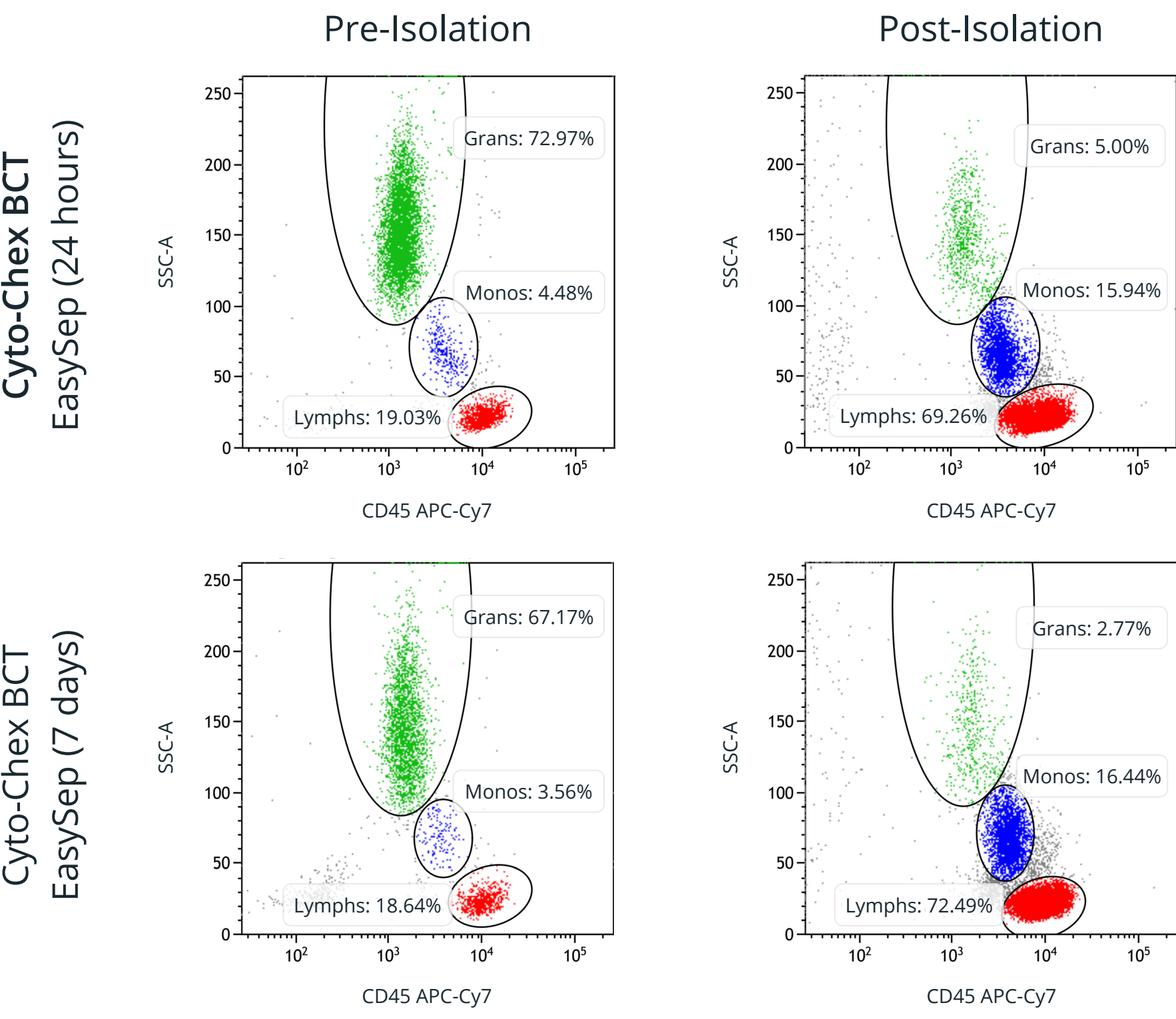


Figure 2. EasySep PBMC isolation lymphocyte subset recovery as a function of time. EasySep kit PBMC recovery from Cyto-Chex BCT-stabilized whole blood is appropriate as early as 24 hours, and as late as 7 days post blood collection. Leukocyte populations in a single donor pre- (left) and post- (right) PBMC isolation at 24 hours (top) and 7 days (bottom) after blood collection.

RESULTS cont.

Lymphocyte and monocyte populations are efficiently isolated from Cyto-Chex BCT-stabilized blood

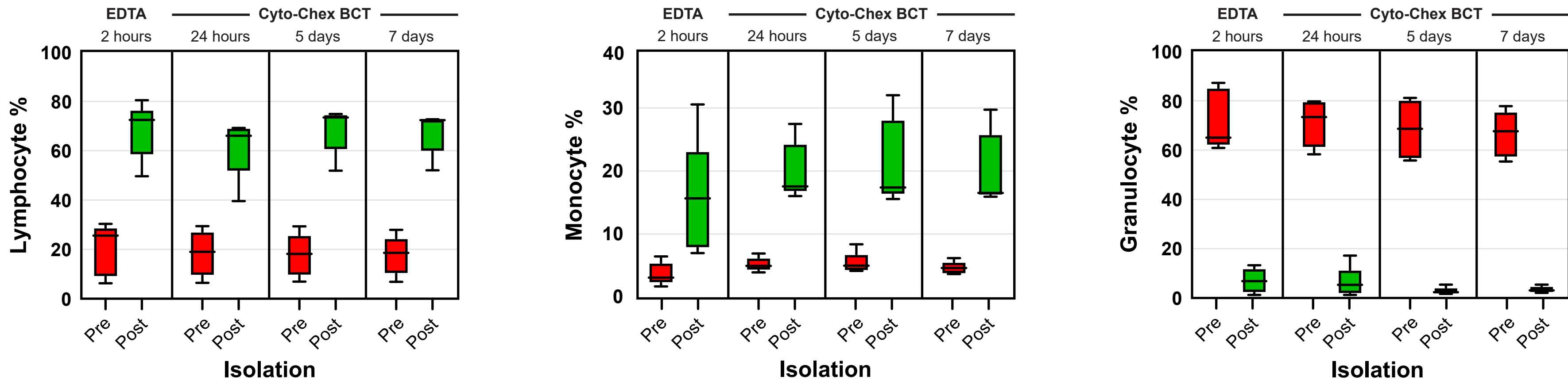


Figure 3. Efficient lymphocyte and monocyte isolation from Cyto-Chex BCT-stabilized blood as a function of blood storage time. Leukocyte recovery is shown pre- (red) and post- (green) PBMC isolation for each method and isolation time point. Granulocyte recovery was reduced in all samples after PBMC isolation whereas monocyte and lymphocyte recoveries increased.

No difference in leukocyte populations subset recovery between the Cyto-Chex BCT-stabilized/ EasySep and EDTA/Ficoll isolation.

	Tube	Method	Time after draw	Lymph%	Mono%	Gran%
Pre-isolation	EDTA	Ficoll	2 hours	20.3	3.6	71.4
	Cyto-Chex BCT	EasySep Kit	24 hours	18.4	5.1	70.6
	Cyto-Chex BCT	EasySep Kit	5 days	17.7	5.3	68.0
	Cyto-Chex BCT	EasySep Kit	7 days	17.6	4.5	66.1
Post-isolation	EDTA	Ficoll	2 hours	68.5	15.4	6.7
	Cyto-Chex BCT	EasySep Kit	24 hours	61.6	19.9	6.0
	Cyto-Chex BCT	EasySep Kit	5 days	68.8	21.2	2.6
	Cyto-Chex BCT	EasySep Kit	7 days	67.5	20.0	3.1

Table 2. Comparison of Cyto-Chex BCT-stabilized EasySep PBMC isolation and the gold standard Ficoll method demonstrates no statistically significant differences. Numerical representation of leukocyte population recoveries for each method, pre- and post-isolation. No significant difference was observed between samples (n=5 donors, p≤0.05).

T- and B-cell lymphocyte subsets are stable over 7-days blood storage time

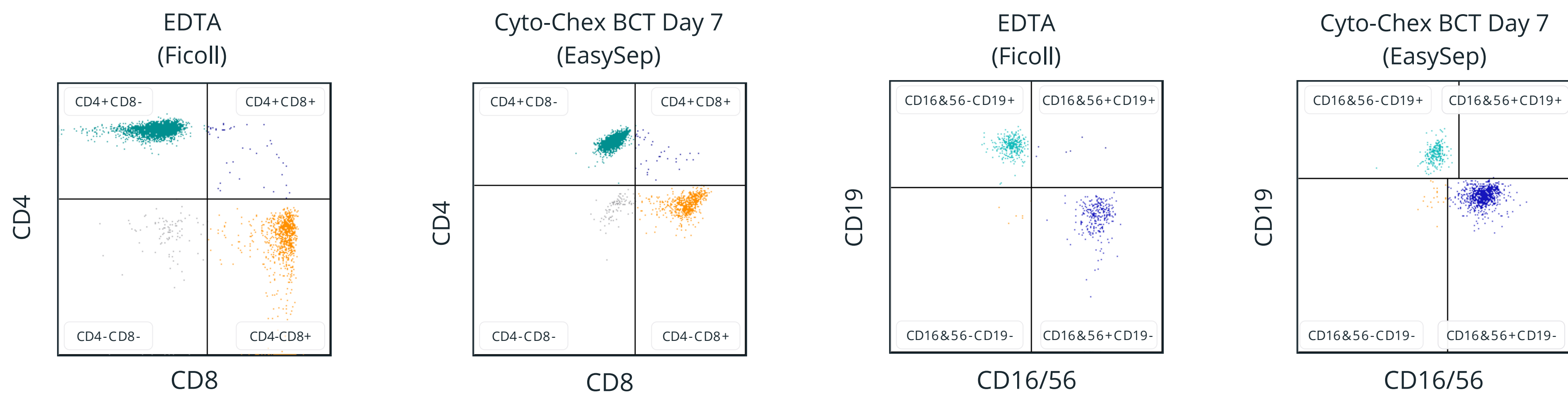


Figure 4. Lymphocyte subset stability post Cyto-Chex BCT/EasySep PBMC isolation. Cell counts, percentages, and antigen expression were maintained for T-cells, B-cells, and natural killer cells after Cyto-Chex BCT/ EasySep PBMC isolation, up to 7 days post blood collection. CD4/CD8 and CD16&56/CD19 plots are shown after EDTA-collected whole blood Ficoll isolation (left) or EasySep PBMC isolation 7 days post blood collection in Cyto-Chex BCT (right).

Taken together, this data suggests recoveries from Cyto-Chex BCT/EasySep separation between 24 hours and 7 days post blood draw are comparable to results from EDTA/Ficoll separation. Numerical results for average leukocyte population results are detailed in Table 2. Figure 4 illustrates the stability of T-cell, B-cell, and natural killer cell antigen expression after PBMC isolation with the EasySep PBMC Isolation Kit, up to 7 days post blood collection in Cyto-Chex BCT. Utilization of this technique can provide a method to isolate PBMCs from Cyto-Chex BCT-stabilized whole blood, a process which was previously incompatible with this type of stabilization.

CONCLUSIONS

- » Magnetic separation with the EasySep Direct Human PBMC Isolation Kit from STEMCELL Technologies successfully isolates PBMCs stabilized by Cyto-Chex BCT up to 7 days post blood collection.
- » No statistically significant differences seen between results obtained from the EasySep immunomagnetic separation method using Cyto-Chex BCT-stabilized whole blood and those obtained from Ficoll density-gradient separation using EDTA-collected whole blood (the gold standard for PBMC isolation).
- » Cyto-Chex BCT/EasySep eliminates the immediate need to isolate PBMCs (EDTA/Ficoll separation within 24-hours of draw) and allows for PBMC isolation up to 7 days post blood draw, offering the versatility to ship and process samples as scheduling permits with no appreciable loss to antigen integrity.
- » Cyto-Chex BCT maintains PBMC characteristics including cell size, complexity, and surface antigen expression until magnetic isolation may be performed up to 7 days room temperature storage post blood collection.