

## Cyto-Chex BCT Stabilizes Whole Blood for Seven Days for Immunophenotyping by Flow Cytometry.

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### Abstract:

The purpose of this study was to compare data generated by flow cytometry from blood samples collected in Cyto-Chex Blood Collection Tubes (BCT) and stored for seven days to the data from blood samples from the same donors collected in K<sub>3</sub>EDTA tubes analyzed at six hours.

### Introduction:

The ability to generate absolute count data via single platform flow cytometry is useful for the detection of disease and infection, as well as for drug therapy and disease surveillance. However, the transportation of blood samples from the site of the draw to the flow cytometry testing facility in an acceptable time frame can be problematic. Currently, National Clinical Chemistry Laboratory Society (NCCLS) guidelines for analysis of CD4<sup>+</sup> T-cells recommend that a sample be analyzed by flow cytometry 48-72 hours post-collection. Cyto-Chex BCT contains a cellular preservation and transport reagent that stabilizes white blood cell antigens for up to seven days post-collection. The data presented in this technical manuscript clearly demonstrate that flow cytometry analysis of blood samples collected and stored in Cyto-Chex BCT for seven days are comparable to that of a freshly drawn specimen.

### Results:

Blood from 25 healthy donors was collected into K<sub>3</sub>EDTA and Cyto-Chex BCT. Absolute count data from the HIV panel cluster of differentiation (CD) markers 3, 4, 8, 19, 16+56 and 45 was collected for each donor using TruCOUNT tubes prepared in duplicate and analyzed in duplicate by flow cytometry.

Table 1 shows the average values for five normal donors obtained from blood samples collected in K<sub>3</sub>EDTA tubes analyzed at six hours post-collection and in Cyto-Chex BCT analyzed at six hours and at seven days. The percent difference was obtained by dividing the absolute count average value from the K<sub>3</sub>EDTA tubes by the average value obtained from the samples stored in Cyto-Chex BCT for seven days. The range of percent differences for the 25 donors was from -10.7 to +10.3%, with 17 of the donors falling within the +/-5% range. Donors 4 and 5 displayed high and low CD4<sup>+</sup> counts and only differed in percent recoveries from the K<sub>3</sub>EDTA tubes by -2.94 and +2.73%, respectively. These data indicate that Cyto-Chex BCT is capable of maintaining accurate CD4 measurements for samples with levels outside typical values.

Figure 1 depicts the six-hour K<sub>3</sub>EDTA CD4<sup>+</sup> absolute count results plotted against the seven-day Cyto-Chex BCT results for all donors. The slope value (y) and correlation coefficient (R<sup>2</sup>) were 0.9917 and 0.9451, respectively. Statistical data from all 25 samples collected in Cyto-Chex BCT analyzed at day seven for all other immune markers tested (CD3, 8, 19, 16+56) resulted in correlation coefficient values greater than 0.931 (data not shown).

These data demonstrate that the measurements of CD4<sup>+</sup> levels from samples stored in Cyto-Chex BCT for seven days were consistent with those obtained from samples at six hours in K<sub>3</sub>EDTA tubes.

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Table 1. Average CD4 + Counts Obtained by Flow Cytometry from Five Normal Donors.

Donor Number	K <sub>3</sub> EDTA (6 Hour) Average	Cyto-Chex BCT (6 Hour) Average	Cyto-Chex BCT (7 Day) Average	%Difference K <sub>3</sub> EDTA(6Hour)/BCT (7 Day)
1	765	775	752	-1.73%
2	871	884	883	1.36%
3	822	849	830	0.96%
4	1958	2039	1902	-2.94%
5	571	563	587	2.73%

### Discussion:

The data presented here establish that Cyto-Chex BCT maintains white blood cell antigen integrity for the HIV panel for up to seven days. This extended storage time for whole blood is a critical breakthrough for the clinical laboratory that currently has a limited timeframe in which to process samples for flow cytometry. We have shown that the absolute counts for CD markers used as indicators for immunodeficiency diseases (i.e. CD3, 4, 8, 19, 16+56 and 45) are stable for up to seven days in the Streck Cyto-Chex BCT.

### Methods:

#### *Blood Collection*

After informed consent, peripheral blood was obtained from 25 healthy donors by venipuncture (Streck, Omaha, NE). Blood from each donor was collected into K<sub>3</sub>EDTA and Cyto-Chex BCT evacuated blood collection tubes. Samples in Cyto-Chex BCT were stored at ambient temperature for seven days.

#### *Sample Preparation for Flow Cytometry*

Flow cytometric analysis was performed on a FACSCalibur system (BD Biosciences, San Jose, CA). Samples were processed and analyzed via single platform technology. Briefly, using reverse-pipetting, 50 µL of blood was placed into two 12x75 mm TruCOUNT tubes (BD Biosciences) and incubated with 20 µL MultiTEST mAb (BD Biosciences). The antibodies and fluorescent conjugates were: CD3-FITC, CD45-PerCP, CD8-PE, and CD4-APC. Samples were incubated in the dark for 20 minutes at room temperature (18-22°C), followed by RBC lysis using BD FACS Lysing Solution (BD Biosciences). Samples were incubated for an additional 20 minutes in the dark. Percent recovery values and absolute counts were recorded for lymphocyte subsets.

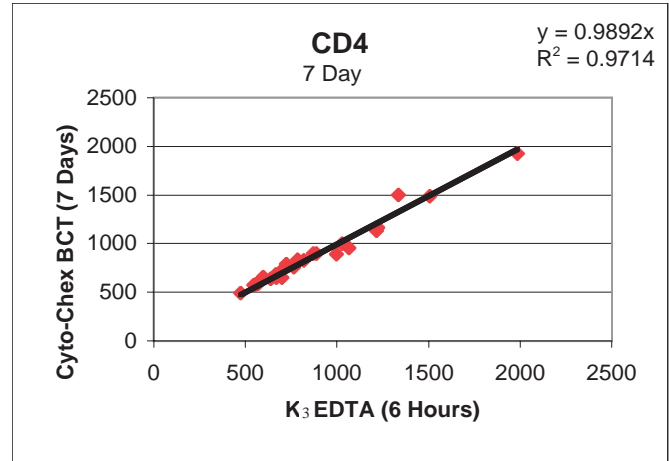


Figure 1. Percent Recovery Statistical Data from 25 Normal Donors.

#### *Flow Cytometry*

The FACSCalibur flow cytometer was calibrated daily with CaliBRITE beads and FACSComp software (BD Biosciences). Instrument settings used were those established by FACSComp software. Samples were evaluated using MultiSET software (BD Biosciences).