

## Cyto-Chex<sup>®</sup> BCT Allows for Accurate T-Cell Counts by Flow Cytometry 14 Days Post Sample Collection

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### Abstract:

The goal of this study was to demonstrate that whole blood specimens collected in Streck Cyto-Chex<sup>®</sup> Blood Collection Tube (BCT) are preserved at room temperature for HIV testing by flow cytometry for a period beyond seven days. Previous studies have demonstrated that whole blood samples collected in Cyto-Chex BCT are stabilized, yielding reliable and accurate CD4+, CD8+, and CD3+ counts for seven days at room temperature (Warrino D.E. et al. (2005). Stabilization of White Blood Cells and Immunologic Markers for Extended Analysis Using Flow Cytometry. *J. Immunol. Methods.* 305(2): 107-119.).

### Introduction:

The accurate measurement of CD4+ T-cells is critical for effective HIV treatment and care. Decisions such as clinical diagnosis, the use of preventive treatment against opportunistic infections and the initiation of anti-HIV treatment are all dependent on accurate and reliable measurement of CD4+ T-cells. Flow cytometry is the current gold standard for the measurement of CD4+ T-cells.

Collection of whole blood is the first step in assessment of HIV patient status by flow cytometry. The CDC guidelines for CD4+ T-cell analysis require that blood specimens collected in a standard K<sub>2</sub>EDTA tube must be analyzed within 48-72 hours of collection. However, situations often occur in which analysis of samples within this post-collection timeframe is logistically difficult or even impossible. For example, large international clinical trial studies requiring transport of patient samples from multiple global collection sites to a single central analysis location. Clinical trial studies, which are research studies designed to answer specific questions about the safety and/or efficacy of drugs, vaccines, other therapies, or new ways of using existing treatments for a number of diseases or conditions including cancer and HIV, can vary in size from a single clinical site with fewer than 50 patient participants to large, randomized international studies with patient numbers in the thousands. Considering that the time required for sample collection, processing, and shipping in these large international clinical trials could easily approach 72 hours, it is not hard to imagine that CD4 testing within the 48-72 hour post-collection timeframe could be problematic. Preserving cell stability and cellular antigenicity until sample analysis can be performed is an effective way to address the difficulty posed by long distance transportation of patient samples.

Cyto-Chex BCT is a blood collection tube cleared by the FDA for the collection, transport and storage of blood specimens from HIV-infected individuals. Previous studies have established that data collected from patient samples stored in Cyto-Chex BCT for seven days is consistent with data obtained from freshly drawn patient samples and that the cellular preservative contained within the Cyto-Chex BCT maintains the antigenic Cluster of Differentiation (CD) markers on white blood cells for HIV testing by flow cytometry.

The data presented in this technical bulletin clearly demonstrate that CD4 testing of samples collected and stored in Cyto-Chex BCT for 14 days at room temperature is comparable to that of freshly drawn patient samples.

### Methods:

#### *Blood Collection*

After informed consent, peripheral blood was collected from ten healthy donors by venipuncture in K<sub>2</sub>EDTA and Cyto-Chex BCT (Streck, Omaha, NE). These samples were stored at 18-22°C.

#### *Sample Preparation for Flow Cytometry*

Samples were analyzed on a FACSCalibur<sup>™</sup> flow cytometer (BD Biosciences). Samples were processed and analyzed on day 7, 10, 12 and 14 using standard flow cytometry procedures. Briefly, using reverse-pipetting, 50µl of blood was placed into 12x75 mm TruCOUNT tubes (BD Biosciences) and incubated with 20µl of MultiTEST monoclonal antibodies (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 BDFACSLysing Solution (BD Biosciences). Samples were incubated for an additional 20 minutes in the dark. Percent recovery values and absolute counts in cells/µl were recorded for lymphocyte subsets.

#### *Flow Cytometry*

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

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### Results:

Blood from ten healthy donors was collected in K<sub>2</sub>EDTA and Cyto-Chex BCT (Streck, Omaha, NE). Absolute count and % recovery data was collected from each donor using TruCOUNT tubes (BD Biosciences), analyzed in duplicate by flow cytometry for the standard HIV panel of CD markers (CD3, 4, 8, 16+56, 19 and 45).

Table 1 shows the average CD4+ absolute count values for ten normal donors obtained from blood samples collected in K<sub>2</sub>EDTA analyzed at six hours post-collection and in Cyto-Chex BCT analyzed at 14 days post collection.

### Discussion:

The CDC guidelines for HIV/AIDS testing or disease surveillance via CD4+ T-cell analysis by flow cytometry require that blood specimens collected in a standard K<sub>2</sub>EDTA tube must be analyzed within 48-72 hours of collection. The data presented here establish that Cyto-Chex BCT maintains white blood cell antigenicity of the HIV panel of CD markers for up to 14 days. This extended stability allows for collection of accurate and reliable data from patient samples despite the additional time required for transport from the site of blood draw to specialized, central laboratories for analysis thereby addressing a major obstacle encountered during large, global clinical trial studies.

**Table 1.** CD3+, CD4+ and CD8+ Cell Counts Obtained by Flow Cytometry for Ten Normal Donors

Donor #	CD3			CD4			CD8		
	6 Hour		% Difference	6 Hour		% Difference	6 Hour		% Difference
	K <sub>2</sub> EDTA	Cyto-Chex BCT		K <sub>2</sub> EDTA	Cyto-Chex BCT		K <sub>2</sub> EDTA	Cyto-Chex BCT	
1	967	978	1.1	610	607	-0.5	326	315	-3.5
2	1354	1367	0.9	808	901	11.5	443	427	-3.5
3	1590	1530	-3.8	854	808	-5.4	706	650	-7.9
4	2678	2619	-2.2	2043	1961	-4.0	563	534	-5.2
5	1280	1114	-10.6	845	742	-12.2	407	340	-16.5
6	951	938	-1.3	597	641	7.4	279	285	2.3
7	1956	1863	-8.6	1275	1267	-0.6	609	582	-4.4
8	743	760	2.3	559	563	0.7	163	169	4.0
9	1197	1093	-8.6	820	758	-7.6	337	294	-12.9
10	1805	1626	-9.9	1175	1027	-12.6	602	544	-9.6

Note: Additional tests were performed on Cyto-Chex BCT samples at 7, 10, and 12 days. Results were similar to 14 day results. This data and % recovery data is available upon request.