



Omaha, NE 68128

Cyto-Chex® Blood Collection Tube Stabilizes Samples Stored at Elevated Temperatures for Flow Cytometry Analysis

Dominic E. Warrino, Jodi R. Alt, James A. Grunkemeyer, and Wayne L. Ryan Streck, Omaha, NE 68128



Abstract

We have previously demonstrated that Streck Cyto-Chex® Blood Collection Tube (BCT) preserves whole blood specimens for HIV testing by flow cytometric analysis for seven days at room temperature. Increased stability reduces the need for weekend staffing and shipping delays are not likely to require patient redraws. However, in resource poor settings sample storage, transportation time, and temperature are not reliable. The effect of elevated temperatures on stabilized blood specimens collected in Cyto-Chex BCT has not been tested. Here, we report that Cyto-Chex BCT is capable of maintaining the integrity of CD markers critical for HIV assessment at temperatures up to 37°C for three days. Whole blood samples were drawn from 25 healthy donors into K2EDTA tubes and Cyto-Chex BCT. Flow cytometric analysis was performed on samples collected and stored at 30°C or 37°C for 6 hours, 3 days, or 7 days. Cell counts for CD3, CD4, and CD8 were determined for each sample at each time point and compared to 6 hour K2EDTA samples. While K2EDTA samples fail at elevated temperatures, Cyto-Chex BCT stabilizes all three markers for seven days at 30°C. Additionally, CD3 and CD4 are stable at 37°C for 72 hours. Cyto-Chex BCT can increase the reliability of CD4 testing in resource poor areas. Monitoring CD4 counts in HIV patients is an extremely important tool enabling physicians to modulate treatment accordingly. Streck Cyto-Chex BCT stabilizes whole blood samples at elevated temperatures for extended periods of time, thereby improving the reliability of HIV monitoring.

Introduction

- Flow cytometry is currently the gold standard for the measurement of CD4+ lymphocytes for monitoring the immune status of HIV-infected subjects.
- As antiretroviral treatment becomes increasingly available in resource-limited settings, the testing necessary to monitor immune responses to therapy must also be considered a priority.
- A major obstacle for HIV clinical testing in resource-limited settings is the instability of immunologic markers during transportation and exposure to heat.

Materials and Methods

- 5 mL of blood was drawn by venipuncture from normal donors into K2EDTA tubes and Cyto-Chex BCT (Streck, Omaha, NE).
- Blood samples were incubated at either 30°C or 37°C for up to seven days, then analyzed by flow cytometry on BD FACSCalibur and Coulter instruments.
- Single platform flow cytometry was performed using Multitest™ (BD Biosciences, San Jose, CA) or tetraCHROME™ (Beckman Coulter®, Fullerton, CA) reagents and Trucount™ Tubes (BD Biosciences) or Flow-Count™ Fluorospheres (Beckman Coulter®).
- Microsoft Excel was used to analyze and plot cell count data for slope and R2 values.

Figure 1. Peripheral blood at ambient temperature

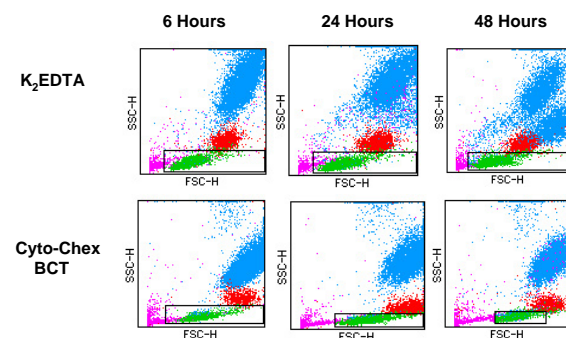


Figure 2. Peripheral blood at 30°C

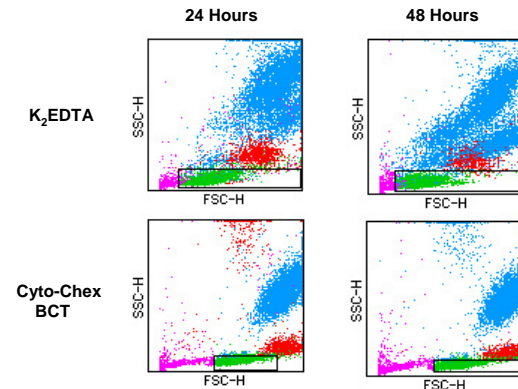


Figure 3. Peripheral blood at 37°C

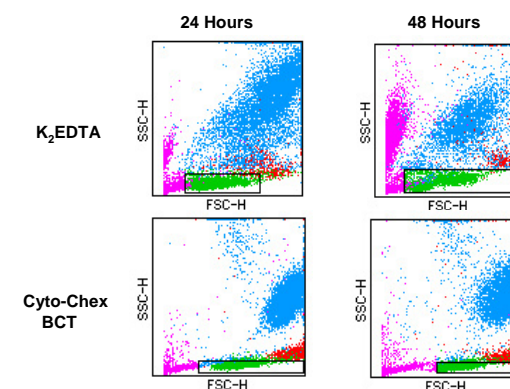


Figure 4. Statistical correlation data

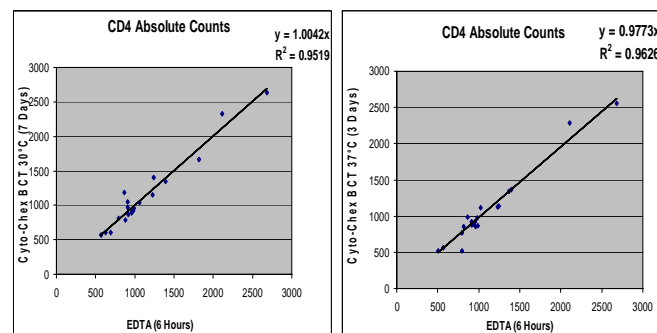


Figure 4A: Cyto-Chex BCT stored at 30°C for 7 days.

Figure 4B: Cyto-Chex BCT stored at 37°C for 3 days.

Donor	CD4+ Counts (cells/ul)		
	EDTA (6 Hours)	Cyto-Chex BCT (30°C for 7 Days)	Percent Difference (%)
1	1815	1661	-8.5
2	692	610	-11.9
3	1058	1034	-2.2
4	2110	2332	10.5
5	865	1183	36.6
6	2684	2635	-1.8
7	631	606	-4
8	916	864	-5.6
9	878	788	-10.2
10	909	973	7
11	1242	1399	12.7
12	569	572	0.6
13	795	806	1.5
14	957	889	-7.1
15	982	921	-6.2
16	962	906	-5.8
17	989	958	-3.2
18	1395	1346	-3.5
19	1228	1152	-6.2
20	911	1052	15.5
AVERAGE	1129.4	1134.4	0.4

Table 1. CD4 cells/μL from fresh, K2EDTA samples and matched donor samples in Cyto-Chex BCT stored at 30°C for seven days.

Donor	CD4+ Counts (cells/ul)		
	EDTA (6 Hours)	Cyto-Chex BCT (37°C for 3 Days)	Percent Difference (%)
1	2110	2288	8.4
2	865	984	13.7
3	2684	2558	-4.7
4	505	515	2
5	795	516	-35.1
6	819	851	3.8
7	909	924	1.6
8	1242	1141	-8.1
9	569	561	-1.3
10	795	765	-3.7
11	957	917	-4.2
12	982	976	-0.6
13	962	851	-11.5
14	989	863	-12.7
15	1395	1363	2.3
16	1228	1124	-8.5
17	911	873	-4.2
18	1369	1342	-2
19	935	889	-5
20	1024	1113	8.8
AVERAGE	1102.25	1070.7	-2.9

Table 2. CD4 cells/μL from fresh, K2EDTA samples and matched donor samples in Cyto-Chex BCT stored at 37°C for three days.

Results

- Streck Cyto-Chex BCT preserved the differentiation of lymphocytes, monocytes and granulocytes by flow cytometric light-scatter analysis through seven days at 30°C and three days at 37°C. White blood cell differentiation degrades rapidly in samples collected in standard K2EDTA tubes.
- Regression analysis resulted in R² values of 0.9519 and 0.9626 for CD4 samples stored at 30°C for seven days and at 37°C for three days, respectively (Figure 4).
- The differences in absolute counts from samples stored in Cyto-Chex BCT at 30°C for seven days to 6 hour K2EDTA samples ranged from -11.9 to 15.5%, with the majority of the samples (16/20) within +/-10% (Table 1).
- The differences in absolute counts from samples stored in Cyto-Chex BCT at 37°C for three days to 6 hour K2EDTA samples ranged from -12.7 and 13.7%, with 16/20 donor samples within +/-10% deviation (Table 2).
- Regression analysis resulted in R² values of 0.9529 and 0.9309 for CD3 samples stored at 30°C for seven days and at 37°C for three days, respectively (Data not shown).

Conclusions

Immune markers CD4 and CD3 absolute cell counts/μl from samples collected in Cyto-Chex BCT were accurately measured after exposure to 30°C for seven days and 37°C for three days, as reflected by R2 values

Samples that are collected in standard K2EDTA tubes and exposed to heat undergo rapid degradation (Fig.2&3) resulting in poor separation of lymphocytes, monocytes, and granulocytes.



Use of Cyto-Chex BCT greatly extends the time interval and temperature storage between specimen collection and analysis, offering resource-limited regions a solution to a major obstacle of sample transportation.

Additional uses for Cyto-Chex BCT include shipping samples internationally, shipping during the summer months, and shipping of precious/rare samples that if delayed or lost can still be used for flow cytometric analysis.