



Clinical Laboratory Products  
You Can Count On.

# Stabilization of White Blood Cells and Immunologic Markers for Extended Analysis Using Flow Cytometry

Dominic E. Warrino\*, Louis J. DeGennaro\*, Mary Hanson\*, Sarah E. Starns†, Samuel J. Pirruccello†, Wayne L. Ryan\* and Susan Swindells†  
\*Streck Laboratories, Omaha, NE 68128 †Section of Infectious Diseases, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198

## ABSTRACT

**Background:** Accurate and reliable measures of CD4+ T lymphocytes (CD4+ T cells) are essential for the evaluation and management of human immunodeficiency virus (HIV)-infected persons. Transportation to a laboratory capable of these analyses in a timely fashion may be difficult to accomplish, especially in resource-poor settings. The Cyto-Chex® Blood Collection Tube (BCT) contains an anticoagulant and fixative to preserve blood samples prior to immunophenotyping by flow cytometry (Streck Laboratories, Omaha NE).

**Methods:** Blood samples were collected by venipuncture from 40 HIV-infected donors into both a K<sub>3</sub>EDTA tube and Cyto-Chex® BCT and evaluated for lymphocyte subsets by flow cytometry at 6h, 72h and 7days after collection. Samples were stored at ambient temperatures.

**Results:** Subjects ranged in age from 24-65 years and 15% were female. Using single platform technology linear regression analyses showed that results from 6h K<sub>3</sub>EDTA samples correlated with those from Cyto-Chex® BCT samples at 7 days for CD4, CD8, and CD3 absolute cell counts/ul. Additionally, samples collect in Cyto-Chex® BCT preserved leukocyte differentiation up to 7 days.

**Conclusion:** Using Cyto-Chex® BCT, immunophenotyping results were equivalent to 6h K<sub>3</sub>EDTA for as long as one week after collection. Importantly, samples in Cyto-Chex® BCT did not require refrigeration or other special handling. This new blood collection system may be a valuable tool for patients without ready access to a flow cytometry laboratory and particularly in resource-poor settings.

## INTRODUCTION

Extending the stability of **immune markers** used for monitoring diseases by flow cytometry would have great practical significance:

Instrument setup/maintenance and processing time can be reduced by **batching samples**.

**Personnel costs for weekend samples** can be reduced since staff need not be on-call for routine flow cytometry analysis.

Extending stability for repeat analysis **eliminates the need for redraws** if a spurious result is yielded during the first analysis.

Extended stability would likely result in cost savings due to the processing of **fewer expired samples**.

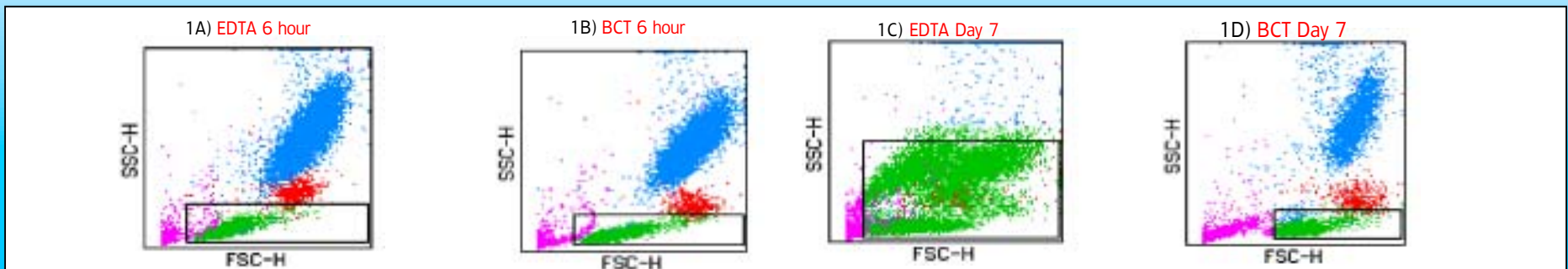
An especially important benefit of extended stability may be realized in **resource-poor settings** where patients may reside in rural areas at a **great distance from central laboratory facilities**.

In this study, we examine the use of a novel blood collection tube, **Cyto-Chex® BCT** (Streck Laboratories, Omaha, NE), which is engineered to stabilize white blood cells for up to 7 days prior to immunophenotyping by flow cytometry.

**CD4, CD3, and CD8 absolute cell counts/ul** were measured in **40 HIV-infected subjects**.

- Flowcytometry has emerged as a major tool in the diagnosis of immune system disorders (Most notably **HIV/AIDS**).
- The measurement of **CD4+** lymphocyte counts by flow cytometry is presently the gold standard for monitoring the immune status of **HIV-infected subjects**.
- CDC guidelines for the analysis of **CD4+** T cells recommend that a typical collection tube containing K<sub>3</sub>EDTA or heparin is only suitable for testing within **72 hours** of collection (MMWR 2003; 52(RR-2). (Generally, results from specimens > 48 hours old may be unreliable)
- A major obstacle for this type of clinical testing is the lack of stability of **immunologic markers** during storage and transportation.

Figure 1. Light Scatter of EDTA 6 hour and Day 7 compared to BCT 6 hour and day 7



# Stabilization of White Blood Cells and Immunologic Markers for Extended Analysis Using Flow Cytometry

Dominic E. Warrino\*, Louis J. DeGennaro\*, Mary Hanson\*, Sarah E. Starns†, Samuel J. Pirruccello†, Wayne L. Ryan\* and Susan Swindells †

\*Streck Laboratories, Omaha, NE 68128

†Section of Infectious Diseases, Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198

## MATERIALS AND METHODS

- 5 mL of Blood was drawn by venipuncture from 40 previously identified HIV-infected subjects and collected into a K<sub>3</sub>EDTA tube and **Cyto-Chex<sup>®</sup> BCT** (Streck, Omaha, NE).
- Whole blood samples were analyzed on a Beckman Coulter EPIC XL flow cytometer at **6 hours, 3 days, and 7 days** (Samples were stored at room temperature).
- 4-color flow was performed using Beckman Coulter tetraCHROME™ reagents (CD45-FITC / CD4-RD1 / CD8-ECD / CD3-PC5)
- HIV-infected subjects 1-20 were analyzed using **dual platform technology** (4-color flow and Hematology instrument).
- HIV-infected subjects 21-40 were analyzed using **single platform technology** (4-color flow utilizing Beckman Coulter Flow-count beads).
- Microsoft Excel was used to plot and analyze cell count data for slope and R<sup>2</sup> values.

## RESULTS

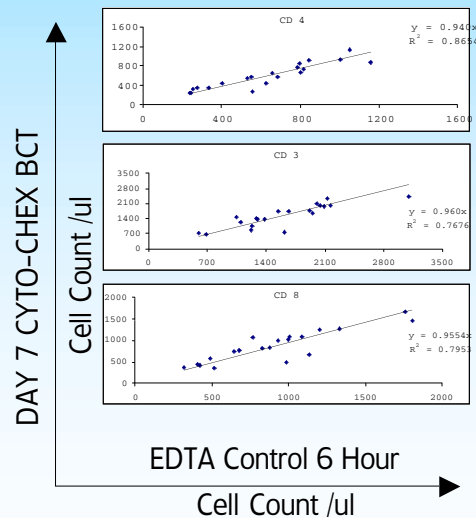
•**Cyto-Chex<sup>®</sup> BCT** preserved the differentiation of lymphocytes, monocytes, and granulocytes by light-scatter on the flow cytometer.

•HIV-infected donors 1-20, using **dual platform technology**, resulted in **R<sup>2</sup>** values of **0.8655** for **CD4**, **0.7676** for **CD3**, and **0.7953** for **CD8** when samples analyzed at 6 hours collected in K3EDTA tube were compared to samples analyzed at 7 days collected in a **Cyto-Chex<sup>®</sup> BCT**.

•HIV-infected donors 21-40, using **single platform technology**, resulted in **R<sup>2</sup>** values of **0.9892** for **CD4**, **0.9799** for **CD3**, and **0.9619** for **CD8** when samples analyzed at 6 hours collected in K3EDTA tube were compared to samples analyzed at 7 days collected in **Cyto-Chex<sup>®</sup> BCT**.

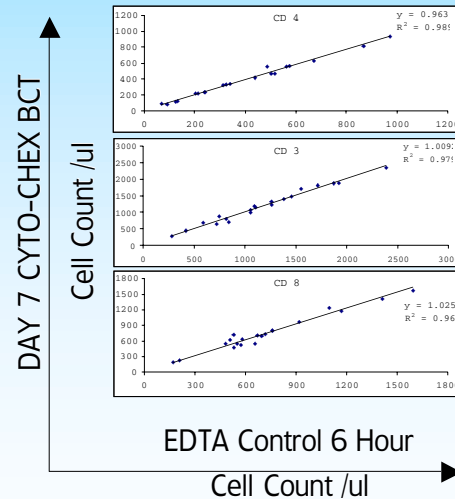
### Figure 2:

FIGURE 2: Summary of HIV patients 1-20 Day 7 Cyto-Chex BCT and 6 hour EDTA immunophenotyping using Dual Platform Analysis.



### Figure 3:

FIGURE 3: Summary of HIV patients 21-40 Day 7 Cyto-Chex BCT and 6 hour EDTA Immunophenotyping using Single Platform Analysis



## CONCLUSIONS

•Collection of **HIV-infected** donor peripheral blood into a **Cyto-Chex<sup>®</sup> BCT** tube preserves antigen epitopes for at least seven days without compromising the ability to differentiate the white blood cell populations.

•Immune markers **CD4**, **CD3** and **CD8** absolute cell counts/ul were accurately measured after seven days of storage at ambient temperature in samples collected in a **Cyto-Chex<sup>®</sup> BCT** tube and analyzed by **single platform technology** as reflected by R<sup>2</sup> values.

•Use of **Cyto-Chex<sup>®</sup> BCT** tubes greatly extends the time interval between specimen collection and analysis, offering improved patient convenience, less expired samples and potential utility in resource-limited settings.

**Streck**

800-843-0912



TABLE 1  
 Analysis of Lymphocyte Subsets in HIV Positive Subjects  
 Cell Count?1  
 Number of Lymphocytes Positive for CD

Patient Information			CD 4				CD 3				CD 8			
HIV+ Donor	Age	Gender	Control (EDTA) 6 hour	Cyto-Chex BCT 6 hour	Cyto-Chex BCT 3 day	Cyto-Chex BCT 7 day	Control (EDTA) 6 hour	Cyto-Chex BCT 6 hour	Cyto-Chex BCT 3 day	Cyto-Chex BCT 7 day	Control (EDTA) 6 hour	Cyto-Chex BCT 6 hour	Cyto-Chex BCT 3 day	Cyto-Chex BCT 7 day
1	45	MALE	551	524	490	572	1272	1298	1146	1446	674	718	612	780
2	41	MALE	1047	1066	1088	1134	2121	2222	2271	2325	999	1060	1076	1090
3	46	MALE	659	649	676	649	1660	1816	1873	1754	993	1079	1124	1035
4	41	MALE	244	249	246	248	2084	1986	1944	1987	1760	1676	1644	1678
5	40	MALE	238	275	259	239	680	778	771	696	414	501	428	428
6	32	FEMALE	1158	1089	1118	884	3099	3057	3013	2430	1803	1838	1793	1470
7	58	MALE	272	324	355	340	597	680	756	744	315	353	391	391
8	37	MALE	815	708	720	733	2160	1987	2030	2037	1331	1287	1310	1286
9	56	MALE	801	777	833	665	1953	1920	2044	1666	1134	1125	1197	687
10	24	FEMALE	558	509	549	394	1608	1543	1682	1205	978	963	1027	734
11	38	MALE	683	667	735	580	1227	1352	1373	1075	404	526	568	448
12	56	MALE	401	501	669	444	1379	1450	1946	1385	828	901	1195	833
13	57	FEMALE	255	314	186	323	1046	1355	1391	1459	767	986	1013	1068
14	39	MALE	333	312	319	349	1289	1287	1303	1386	927	948	957	1012
15	27	MALE	796	813	785	846	1996	2119	2037	2098	1202	1329	1218	1266
16	39	MALE	1003	937	981	931	1909	1891	1886	1817	872	862	831	846
17	38	MALE	842	903	802	915	1534	1702	1533	1761	641	740	671	763
18	36	MALE	624	610	515	449	1217	1199	992	890	510	486	414	357
19	35	MALE	531	486	460	561	1090	1088	1007	1228	484	519	482	582
20	41	MALE	786	679	663	783	2034	1699	1654	2047	1083	902	659	1089
21	41	MALE	240	232	251	235	1876	1792	1856	1864	1596	1516	1567	1578
22	54	MALE	124	106	115	108	842	704	699	704	657	549	530	540
23	46	MALE	671	642	609	628	1921	1882	1751	1881	1172	1176	1068	1184
24	36	MALE	90	91	100	82	283	300	312	284	171	184	189	183
25	30	MALE	562	442	563	551	1459	1285	1458	1475	717	660	713	735
26	38	FEMALE	311	269	298	321	1262	1183	1275	1317	920	888	942	963
27	33	MALE	438	350	420	413	1058	909	1055	987	573	518	598	527
28	34	MALE	133	109	130	115	724	625	714	644	534	462	511	468
29	32	FEMALE	869	738	806	814	2386	2145	2290	2332	1413	1305	1375	1408
30	65	MALE	70	72	86	84	593	597	626	673	483	481	498	544
31	58	MALE	203	241	204	215	418	502	429	447	209	249	218	226
32	56	MALE	238	186	206	227	815	661	741	793	550	456	505	546
33	43	MALE	340	365	351	338	1056	1110	1081	1078	669	696	692	706
34	39	FEMALE	488	493	586	551	1094	1082	1318	1194	583	583	707	629
35	41	MALE	504	525	475	460	1267	1323	1300	1215	698	734	750	690
36	44	MALE	323	293	316	328	1107	1042	1091	1148	762	732	760	802
37	39	MALE	574	577	589	563	1382	1426	1477	1399	761	785	822	783
38	44	MALE	973	799	1021	936	1550	1509	1821	1713	531	665	758	724
39	27	MALE	214	217	224	217	748	843	864	879	510	595	602	623
40	39	MALE	518	481	525	461	1716	1866	1991	1810	1097	1287	1339	1241