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Research paper

Stabilization of white blood cells and immunologic markers for extended analysis using flow cytometry

Dominic E. Warrino[a,*], Louis J. DeGennaro[a], Mary Hanson[a], Susan Swindells[b], Samuel J. Pirruccello[c], Wayne L. Ryan[a]

[a]*Streck, 7002 S. 109th Street, Omaha, NE 68128, United States*
[b]*Department of Internal Medicine, University of Nebraska Medical Center, Omaha, NE 68198, United States*
[c]*Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198, United States*

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Abstract

We evaluated whole blood samples drawn from 25 healthy donors and 20 HIV-infected donors into K_3EDTA and Cyto-Chex® BCT blood collection tubes for CD4, CD8, and CD3 cell counts (HIV Panel). Samples collected in Cyto-Chex® BCT were stored at room temperature and tested by 4-color flow cytometry at 6 h, 3 days, and 7 days after isolation for CD4, CD8, and CD3 absolute cell counts/μl and compared with samples collected in K_3EDTA tubes and tested at 6 h. Regardless of donor type, the samples collected in Cyto-Chex® BCT and tested on day 7 yielded results that were statistically indistinguishable (with correlation coefficients of 0.96 or greater) from samples collected in K_3EDTA tubes and tested at 6 h. We conclude that whole blood samples collected in Cyto-Chex® BCT are stabilized for their marker phenotype for at least 7 days after phlebotomy.

Keywords: Stabilization; HIV panel; Flow cytometry; Immune markers

1. Introduction

Flow cytometry has emerged as a major tool in the diagnosis of numerous immune-system disorders including immunodeficiencies and lymphoma/leukemia (Ross et al., 2003; Carlson et al., 2003; Mainou-Fowler et al., 2004; Benesch et al., 2004). Furthermore, the measurement of the levels of specific white blood cells via discriminatory markers is now routinely used in monitoring the success of drug therapy in several diseases including HIV/AIDS (Kern et al., 2004; Mandy et al., 2003). Indeed, the measurement of CD4+ T lymphocyte counts by flow cytometry is presently the gold standard for the monitoring of patients receiving antiretroviral treatment (Mandy et al., 2003). However, a major

* Corresponding author. Tel.: +1 402 333 1982; fax: +1 402 537 5352.
E-mail address: wryan@streck.com (D.E. Warrino).

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limitation currently associated with this type of clinical testing is the instability of these phenotypic markers during prolonged specimen storage and transportation. CDC guidelines for the analysis of CD4+ T-cells recommend that a typical collection tube containing K_3EDTA or heparin is only suitable for testing within 72 h of collection (Mandy et al., 2003). However, in general, specimens over 48 h old are considered suspect (Bergeron et al., 2002). Therefore, the ability to extend specimen stability, at room temperature, would limit the observed variability of clinical results and facilitate the reproducible monitoring of patients by flow cytometry. In addition, extending the stability of blood samples may reduce the overall performance cost of clinical flow cytometry. Costs associated with overnight shipment may be reduced since samples can be transported less often and at lower shipping rates. Extended stability may also reduce instrument set up, maintenance and processing time by permitting batch processing of samples and weekend personnel may be reduced since requisite staff need not be on-call for routine flow cytometry analysis. Finally, extended specimen stability would likely result in cost savings due to the processing of fewer expired samples. Perhaps the greatest practical benefit of extended specimen stability, however, would be realized in less developed countries, where patients reside in rural areas at a great distance from central laboratory facilities. The magnitude of this need is reflected in the fact that, according to the UNAIDS 2003 year-end statistics, an estimated 40 million people world-wide are infected with HIV/AIDS and approximately two-thirds of these individuals reside in Sub-Saharan Africa (UNAIDS, 2004). Recent reports indicate that resource-poor countries lack the ability to diagnose/monitor HIV-infected individuals due, in part, to expiration of samples prior to reaching regional clinical laboratories (Mwaba et al., 2003; van Dyk and van Dyk, 2003).

To circumvent sample transportation issues, the development of alternative quantitative methods to flow cytometric analysis has been necessitated, most notably the ELISA TRAx CD4 test kit (T Cell Diagnostics, Cambridge, MA, USA). This method has shown comparable results to flow cytometry in the analysis of CD4+ T lymphocytes, when sample values are greater than 200 cells per μl (Mwaba et al., 2003). However, the method excludes 38% of the patient population whose CD4+ T lymphocyte counts are less than 200 cells per μl (Shapiro et al., 2004). It has also been noted that the TRAx test kit may no longer be commercially available, making it difficult to reproduce published results (Shapiro et al., 2004). As a result, there has been great impetus to develop a device that allows for extended transport and storage of blood samples while maintaining sample integrity.

In this study, we examine the use of a novel blood collection tube, Cyto-Chex® BCT (Streck, Inc., Omaha, NE), which has been engineered to stabilize white blood cells for up to 7 days at room temperature prior to immuno-phenotyping by flow cytometry. Seven-day stabilization of specimens would significantly extend the time allowed for transporting samples to clinical laboratory facilities without compromising the quality and reproducibility of results.

In the current study, CD3+, CD4+, CD8+, CD16/56+ and CD19+ cell percentages and absolute counts in healthy donor blood and CD3+, CD4+ and CD8+ cell percentages and absolute counts in HIV-infected donor blood specimens were examined after collection in K_3EDTA and Cyto-Chex BCT. In particular, we sought to compare data obtained from K_3EDTA tube blood samples analyzed at 6 h with blood samples collected in Cyto-Chex® BCT stored at room temperature and analyzed at 6 h, 3 days and 7 days. All tests were performed with single platform flow cytometry technology (SPT).

2. Materials and methods

2.1. HIV-infected donor and healthy donor peripheral blood samples

Peripheral blood samples from 20 HIV-infected donors and 25 healthy donors were evaluated. HIV-infected donor blood was obtained with informed consent under an Institutional Review Board-approved protocol. From each donor, blood was collected by venipuncture into a K_3EDTA BD Vacutainer® tube (Becton-Dickinson VACUTAINER Systems, Franklin Lakes, NJ) and a Cyto-Chex® Blood Collection Tube (BCT) (Streck, Inc., Omaha, NE). Cyto-Chex® BCT is designed to collect a 5 ml