

Cyto-Chex BCT Stabilizes Light Scatter and Cell Morphology.

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Abstract:

The purpose of this study was to compare light scatter data generated by flow cytometry and cell morphology detail obtained by blood smears from samples collected into Cyto-Chex BCT and K₂EDTA blood collection tubes. The data presented in this technical bulletin show that light scatter patterns and cellular morphology of blood collected and stored in K₂EDTA tubes for longer than 24 hours were compromised. Samples collected into Cyto-Chex BCT display almost identical light scatter patterns and cellular morphology from six hours to seven days post-collection.

Introduction:

The CDC guidelines for analysis of CD4⁺ T-cells recommend that a sample be analyzed by flow cytometry within 48 hours, but no later than 72 hours post-collection. However, laboratories that analyze samples in standard K₂EDTA tubes after 24 hours of collection may be generating sub-optimal patient results. Cyto-Chex BCT contains a cellular preservation and transport reagent that stabilizes white blood cell antigens for up to seven days post-collection. Analysis of the FSC vs. SSC light scatter patterns of blood samples maintained in standard K₂EDTA tubes shows signs of degradation and increased cellular debris after 24 hours. In comparison, the light scatter and manual differentials of samples stored in Cyto-Chex BCT for seven days are only slightly affected when compared to a freshly drawn sample.

The ability to store a sample for seven days can significantly improve the flow cytometry laboratory's ability to manage workflow, batch samples, and reduce weekend labor. The laboratory drawing the tubes will have the ability to transport them without affecting sample integrity. Conversely, the flow cytometry laboratory can be confident that they are analyzing a stable sample and the data will be accurate.

Methods:

Blood Collection

After informed consent, peripheral blood was obtained from healthy donors by venipuncture (Streck, Omaha, NE). Blood from each donor was collected into K₂EDTA and Cyto-Chex BCT evacuated blood collection tubes and stored at ambient temperature.

Sample Preparation for Flow Cytometry

Flow cytometric analysis was performed on a FACS-Calibur™ instrument (BD Biosciences, San Jose, CA). Samples were processed and analyzed using standard flow cytometry procedures. Briefly, 100 µL of blood was placed into a 12x75 mm tube and incubated with 5 µL of CD45 and 5 µL CD14 monoclonal antibodies (BD Biosciences), labeled with FITC and PE, respectively. Samples were incubated in the dark for 30 minutes at room temperature. This was followed by the addition of 2 mL of 1X BD FACS™ Lysing Solution. Samples were incubated in the dark for an additional 10 minutes. Samples were centrifuged at 2000 rpm for 5 minutes, aspirated and resuspended in 1 mL of FACS Buffer (with 0.5% BSA and 0.1% sodium azide). The cells were washed again and resuspended in 1 mL FACS Buffer prior to analysis. Percent recovery values were recorded for lymphocyte subsets.

Flow Cytometry

The FACSCalibur flow cytometer was calibrated daily with CaliBRITE™ beads and FACSComp™ software. Instrument settings used were those established by FACSComp. Samples were evaluated using SimulSET software.

Hematology Staining

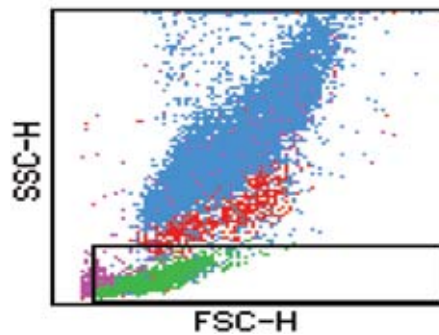
Slides were prepared by evenly smearing a drop of blood across slides. The slides were then stained with the Neat Stain Hematology Stain kit (Astral Diagnostics, Inc., Paulsboro, NJ) according to the manufacturer's instructions. The air-dried blood films were fixed by dipping the film six times for one second into the fixative, followed by six dips in the eosin solution. The slides were then dipped in azure solutions, six times for one second each, rinsed with deionized water and allowed to air dry. The slides were viewed under an oil immersion lens and cells were counted manually.

Results:

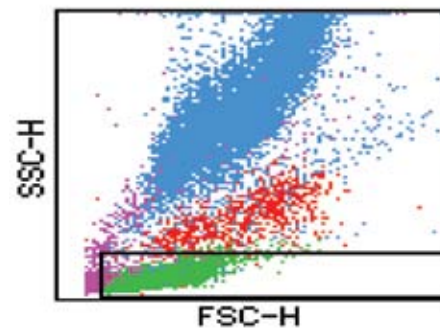
The panels to the right show the flow cytometry light scatter patterns of a sample collected into a standard K₂EDTA tube analyzed at 6, 24, 48 and 72 hours post-collection.

The light scatter began to degrade after 24 hours and by 72 hours, there was a noticeable change in the gate set by the flow cytometer. **The instrument experienced difficulty discerning the differences between the White blood cell populations.**

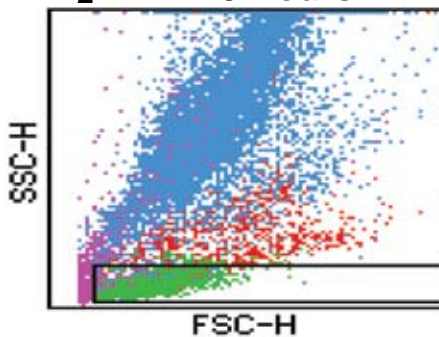
K₂EDTA 6 Hours



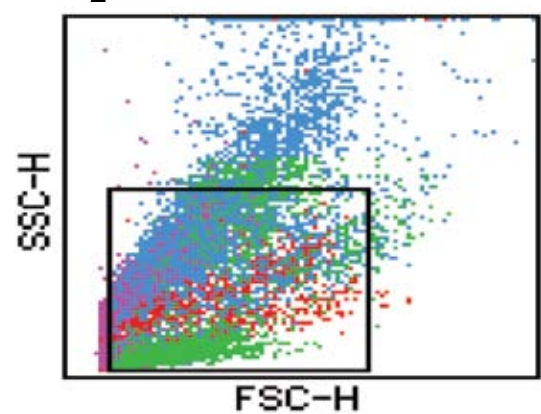
K₂EDTA 24 Hours



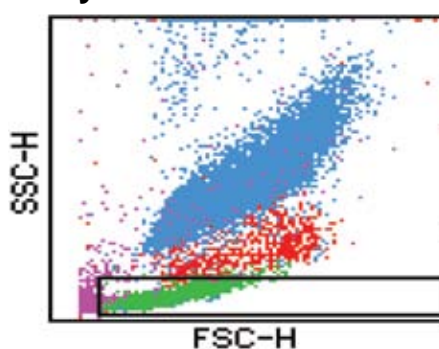
K₂EDTA 48 Hours



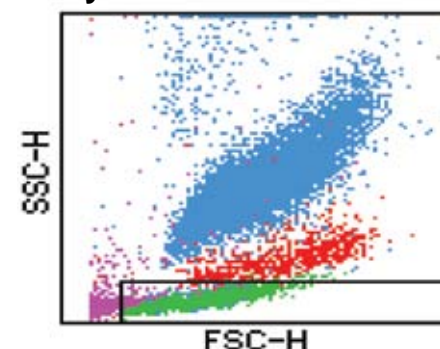
K₂EDTA 72 Hours



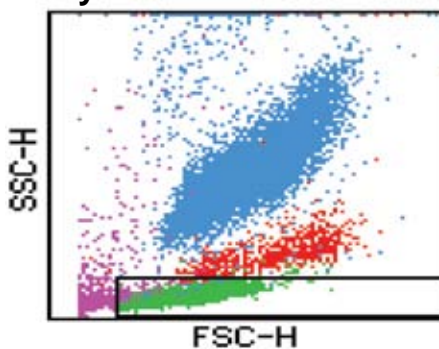
Cyto-Chex BCT 6 Hours



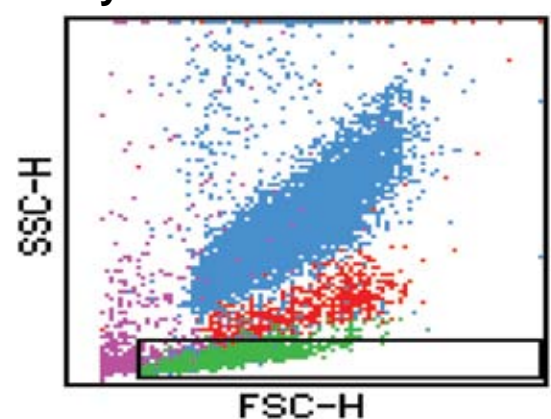
Cyto-Chex BCT 24 Hours



Cyto-Chex BCT 48 Hours



Cyto-Chex BCT 72 Hours



The panels to the right illustrate the flow cytometry light scatter patterns obtained from a blood specimen from the same individual collected into Cyto-Chex BCT analyzed at 6, 24, 48 and 72 hours post-collection.

The Cyto-Chex BCT samples analyzed at all time points are **virtually indistinguishable**. This data supports the ability of Cyto-Chex BCT to maintain the integrity of White blood cell populations.

Results:

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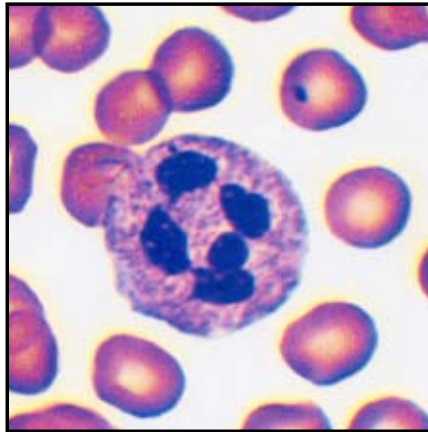
The top panels are hematology stains obtained from a sample drawn into a K₂EDTA tube initially, and at 48 hours.

The bottom panels show the hematology stains of a sample from the same donor drawn into Cyto-Chex BCT initially, and at seven days.

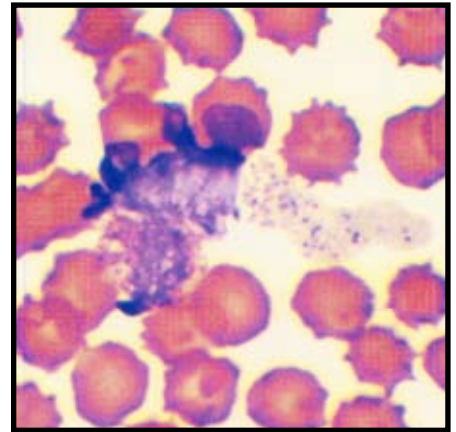
White blood cell populations from blood samples collected into K₂EDTA began to degrade at 24 hours, whereas the cells from the blood sample in Cyto-Chex BCT displayed no cellular degradation through seven days. The preservative in Cyto-Chex BCT does appear to cause some cell shrinkage; however, the neutrophils and lymphocytes continued to be readily discernible through seven days. The hematology staining patterns indicate that BCT is preserving the morphology of the white blood cells. Because the flow cytometer identifies cells by size and granularity, the instrument is more likely to obtain appropriate and accurate information from a sample that contains intact white blood cells of the correct cellular morphology.

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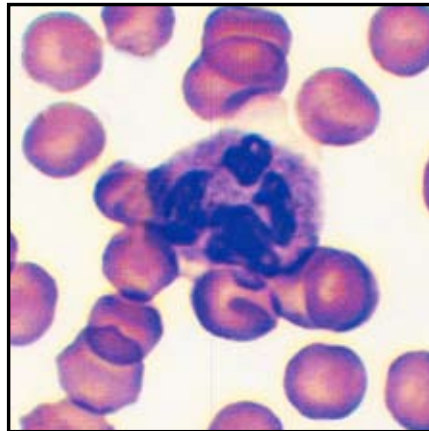
K₂EDTA Initially



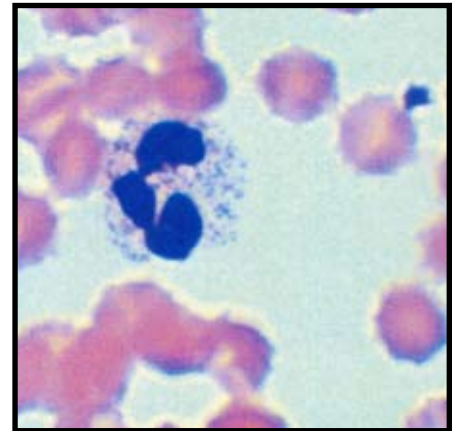
K₂EDTA 48 Hours



Cyto-Chex BCT Initially



Cyto-Chex BCT 7 Days



The flow cytometer is able to obtain more accurate data from a sample stored in Cyto-Chex BCT.

For more information about Cyto-Chex BCT and other innovative Flow Cytometry Products, visit our web site at www.streck.com

Results:

(Continued from preceding)

Differentials obtained by manual counting methods for a sample collected into K₂EDTA and Cyto-Chex BCT are shown in Table 1. The lymphocyte population in the sample collected into K₂EDTA began to drift after 24 hours and was significantly altered by 48 hours. Moreover, the sample was degraded by 24 hours with 21 degraded cells identified by manual count. No degraded

cells were detected in the sample stored in Cyto-Chex BCT after seven days, and the lymphocyte percentages fluctuated only slightly. Even at seven days in Cyto-Chex BCT, the sample only experienced a marginal shift in the lymphocyte percentage. The shift in the lymphocyte population detected in the Cyto-Chex BCT sample at day seven is less dramatic than the shift in the sample stored in K₂EDTA at 48 hours. This data indicates that Cyto-Chex BCT is capable of maintaining white blood cell morphology comparable to a fresh sample through seven days.

EDTA

Time	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil	Degraded
Initial	48	43	6	3	0	0
24 Hours	27	40	6	6	0	21
48 Hours	8	28	3	0	0	61
72 Hours	3	29	0	2	0	66
96 Hours	1	19	0	0	0	80
Day 7	0	13	0	0	0	87

Cyto-Chex BCT

Time	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil	Degraded
Initial	51	42	5	2	0	0
24 Hours	49	43	6	2	0	0
48 Hours	54	41	3	2	0	0
72 Hours	51	42	6	1	0	0
96 Hours	48	44	5	3	0	0
Day 7	50	38	8	4	0	0

Table 1. Manual White blood cell 5-part differential from blood specimens from one donor collected into Cyto-Chex BCT and K₂EDTA blood collection tubes. Manual differential data was obtained initially and at 24, 48, 72, 96 hours and 7 days.

Discussion:

The data presented in this technical paper demonstrate that samples stored in K₂EDTA tubes longer than 24 hours can become compromised. In comparison, samples stored in Cyto-Chex BCT display stable light scatter and cell morphology well beyond the capability of K₂EDTA tubes. The ability to preserve peripheral blood samples for seven days with the assurance that the integrity is maintained is an important advance for the clinical laboratory. Each medical center works to provide the best patient care possible at the most reasonable cost, and Cyto-Chex BCT can contribute to this goal. **Cyto-Chex BCT ensures that the physician or hospital reports accurate patient data** and avoids the inconvenience and added expense of patient redraws due to compromised samples.

The data presented demonstrates that the preservative in Cyto-Chex BCT maintains white blood cell integrity, thus allowing the data generated by flow cytometry to accurately reflect that of a fresh sample. The seven-day stability of Cyto-Chex BCT increases the laboratory's options for transport and workflow management. In addition, these tubes can be integrated into a health care facility with ease, as the only requirement is that a phlebotomist draws the blood sample into Cyto-Chex BCT. The flow cytometry laboratory can then be assured that the sample for analysis is stable and the data will be accurate.