Increased Stability and Population Resolution of ZAP-70 When Drawn into the Commercial Blood Collection Tube; Cyto-Chex® BCT

C. Moore[1], J. Alt[1], L. Brown[2], and B. Hunsley[1]

[1] Streck, Omaha, NE 68128
[2] Flow Contract Site Laboratory, LLC, Kirkland, WA 98034

Abstract

ZAP-70 is an intracellular kinase, whose level of aberrant expression in B-cell Chronic Lymphocytic Leukemia (B-CLL) is used for prognosis and treatment decisions. The varying expression, dim staining, and labile nature makes measuring ZAP-70 challenging and has prevented it from becoming more widely used in Leukemia/Lymphoma screening. Stabilizing ZAP-70 would allow for a longer analysis window and more precise results. Cyto-Chex BCT stabilizes white blood cells (WBCs) through a proprietary process and allows for accurate CD marker quantitation for up to 14 days. We hypothesized that the increased antigen stability provided by Cyto-Chex BCT would extend to intracellular markers such as ZAP-70. After a minor modification to the standard intracellular staining protocol, we found that ZAP-70 in WBCs drawn into Cyto-Chex BCT shows increased fluorescence and population resolution in comparison to other common blood collection tubes. This, combined with the increased stability of the WBCs, allowed for enhanced recovery of ZAP-70 percentages up to 96 hours after draw. The other tested blood collection tubes lost accurate recovery ≤24 hours. These results were supported by an independent lab after samples were shipped and run 48 hours after draw. Previous studies have attempted to harmonize ZAP-70 detection and have suffered from the instability of ZAP-70 expression and low population resolution due to dim staining. The use of Cyto-Chex BCT for ZAP-70 examination addresses these two main difficulties. This proof of principle study warrants the examination of B-CLL patient samples drawn into Cyto-Chex BCT to determine if stabilization can improve the ZAP-70 assay.

ZAP-70 Background

- Naturally expressed in T cells (CD3+) and NK cells (CD3-)
- Abnormally expressed in B cells (CD19+) in B-cell Chronic Lymphocytic Leukemia (B-CLL)
- Used in diagnosis and monitoring of B-CLL
  - Amount of ZAP-70 expressed in B-CLL is correlated with survival
  - Not stable in drawn blood
  - Decreases by ~10% per day in blood collection tubes
- Difficult assay to do correctly
- Opportunity for improvement of a clinically relevant assay by stabilization of ZAP-70 in whole blood

Materials and Methods

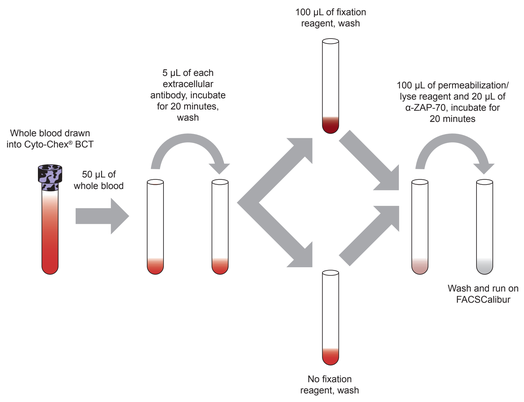
50 µLs of whole blood drawn into the indicated blood collection tubes were stained with 5 µL of α-CD3-FITC, α-CD45-PerCP, and α-CD19-APC for 20 minutes at 22 °C. The samples were fixed or not as indicated with a formaldehyde based commercial fixation reagent for 20 minutes at 22 °C and then washed in PBS with BSA. The samples were permeabilized with a saponin based commercial perm/lyse reagent and stained with 20 µL of α-ZAP-70-PE for 20 minutes at 22 °C. The samples were washed twice in PBS with BSA and fixed in 1% paraformaldehyde in PBS for 30 minutes at 22 °C before being run on a BD FACSCalibur™. Percentages of T, NK, and B cells were obtained by the BD Multitest™ HIV panel. Cyto-Chex® BCT is a product of Streck. All antibodies and other blood collection tubes were from BD. Flow cytometry (Figures 1 and 2) was performed at Streck on a BD FACSCalibur. External flow cytometry (Figure 3) was performed by Flow Contract Site Laboratory on samples sent from Omaha, NE, to Kirkland, WA, and run on a BD FACSCanto™. Data are representative from multiple experiments with multiple donors.

ZAP-70 Experimental Questions

- Can Cyto-Chex BCT be used for intracellular staining of ZAP-70?
- How does Cyto-Chex BCT stabilization of ZAP-70 compare with other blood collection tubes?
- How does shipping affect ZAP-70 stabilization?

Can Cyto-Chex BCT be used for Intracellular Staining of ZAP-70?

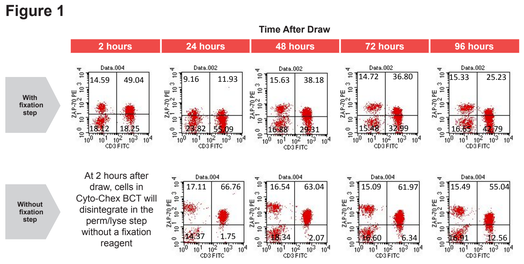
Cyto-Chex BCT contains a preservative; therefore, it may not be necessary to include the harsh formaldehyde fixation normally used in intracellular staining.



ZAP-70 will be followed over 96 hours

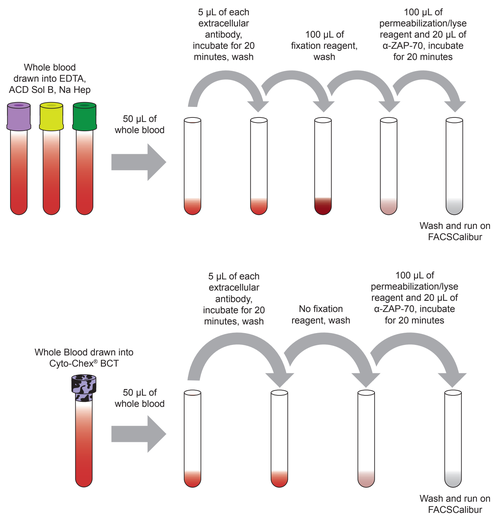
Cyto-Chex BCT gives superior ZAP-70 staining after omission of the fixation step in standard intracellular staining

Figure 1



Correct percentage of cells based on HIV panel:
- 62% T cells
- 14% NK cells

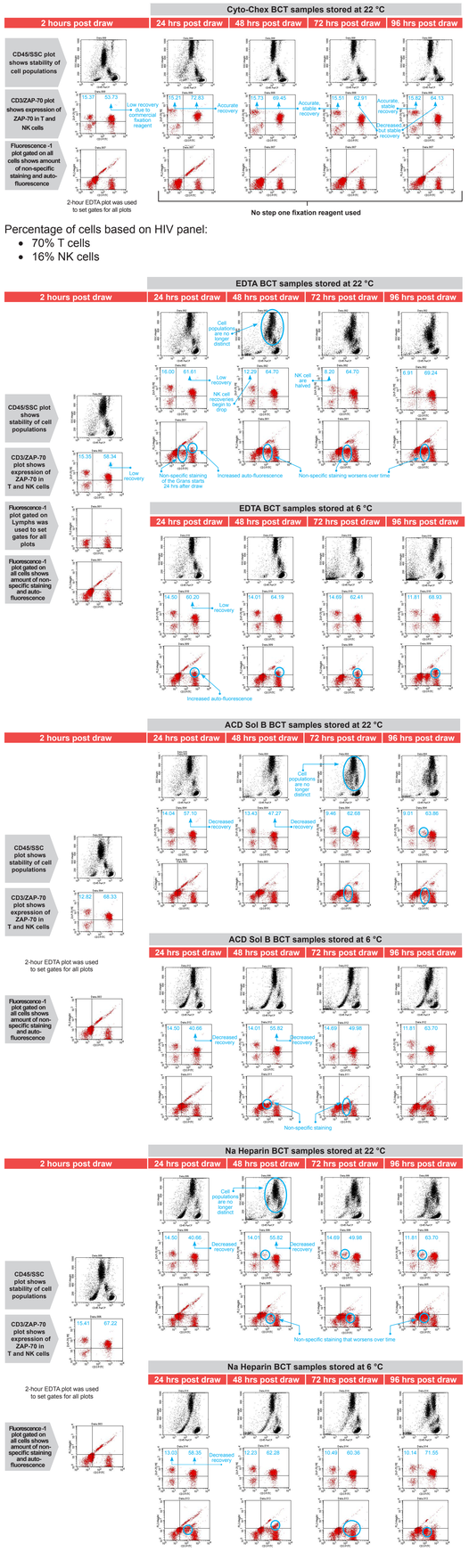
How does Cyto-Chex BCT stabilization of ZAP-70 compare with other blood collection tubes?



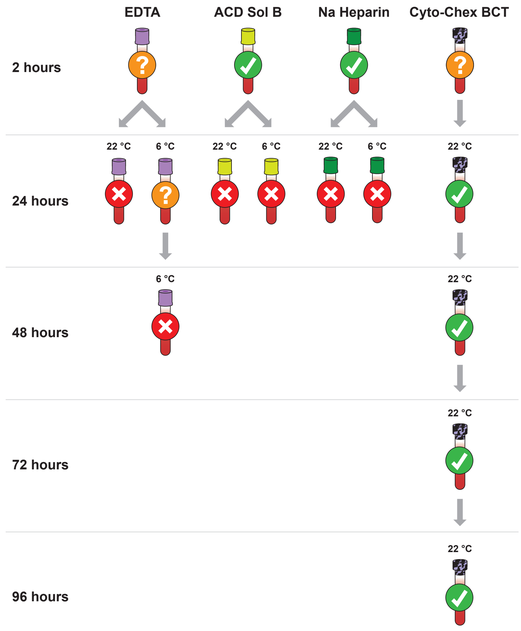
ZAP-70 will be followed over 96 hours at room and refrigerated temperatures.

Cyto-Chex BCT provides superior stabilization of ZAP-70 and population resolution in comparison to other BCTs

Figure 2



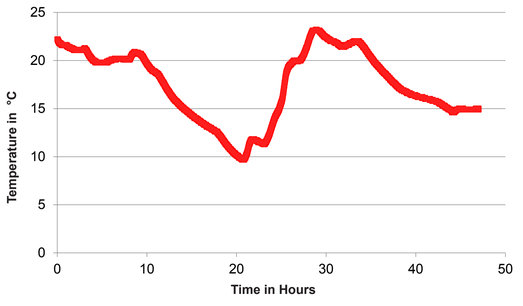
Summary



How does shipping affect ZAP-70 stabilization?

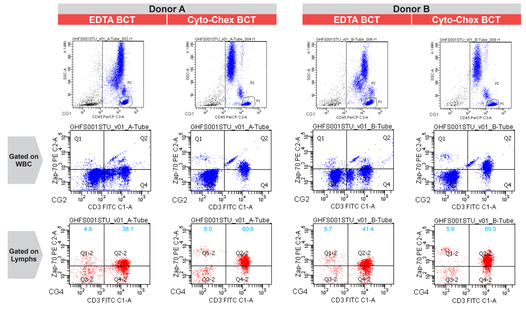
Samples were shipped via FedEx® two-day shipping from Omaha, NE, to Kirkland, WA, and run by Flow Contract Site Laboratory.

Temperature Variation of Samples During Shipping



ZAP-70 is stable after shipping in Cyto-Chex BCT

Figure 3



Conclusions and Future Directions

- Cyto-Chex BCT stabilizes ZAP-70 up to 96 hours after draw.
- A minor modification to the standard intracellular staining protocol when using samples from Cyto-Chex BCT increases ZAP-70 fluorescence and population resolution.
- Preliminary experiments show similar improvements in staining for CD79a, TdT, and BCL-2.
- Future experiments should examine B-CLL patient samples.