



OPTIMIZING COLLECTION OF CLINICAL BLOOD SAMPLES FOR CHIPCYTOMETRY™

HIGHLIGHTS

- **ChipCytometry™ is a high-plex imaging platform for spatially resolved single-cell immunophenotyping of clinical blood samples**
- **Cryopreservation of blood samples during shipping may compromise sample integrity, reducing quality of data analysis**
- **Cyto-Chex® Blood Collection Tubes (BCT) are a simple, easy-to-deploy solution to preserve blood samples and circumvent cryopreservation**
- **Cyto-Chex BCT preserve blood samples for up to 7 days prior to immunophenotyping in ChipCytometry assays, without any loss in sample integrity**

INTRODUCTION

Blood is a highly valuable clinical analyte for both diagnostic and research purposes. Analysis of clinical blood samples has the potential to inform the treatment of a variety of diseases, including infectious diseases and solid tumors (Cohen et al., 2018). Clinics have the resources necessary to immediately process blood samples for routine diagnostic tests, but samples must often be shipped to an off-site laboratory to gain access to special equipment used in innovative research.

Cryopreservation is commonly used to preserve biological information during shipping. Yet, this method can damage samples and significantly affect the relative frequency of viable cell subpopulations (Laskowski et al., 2020). Determining an appropriate sample preservation approach is therefore critical to generating high-quality data in immunophenotyping assays and performing subpopulation analysis. Better methods for sample preservation during shipping are necessary to support novel research.

Cyto-Chex Blood Collection Tubes (BCT) offer a simple, easy-to-use solution and are a direct draw tube for the collection and storage of whole blood specimens for immunophenotyping of white blood cells by ChipCytometry. Cyto-Chex BCT maintain cellular morphology and surface antigen expression, including cluster of differentiation markers for immunophenotyping assays with ChipCytometry.



Figure 1. *Cyto-Chex BCT is a direct draw tube for the collection and storage of whole blood specimens for immunophenotyping.*
Source: Streck

CHIPCYTOMETRY ACCELERATES CLINICAL RESEARCH

Understanding disease biology and its progression requires an unbiased and highly multiplexed approach. ChipCytometry is an iterative antibody staining method for immunophenotyping of PBMCs (Hennig et al., 2014; Roesner et al., 2015) and other cell suspensions (Hümmert et al., 2018; Teo et al., 2017). Our state-of-the-art optical system includes a proprietary HDR imaging approach, enabling the collection of precise protein expression data for high- and low-expressing proteins. With ChipCytometry, researchers can:

- Investigate distribution of cell populations
- Explore changes in cellular morphology
- Analyze disease pathophysiology
- Predict response to treatment



STUDY PURPOSE

Our objective is to find a method for blood collection that preserves the integrity of clinical blood samples during shipment. Current techniques require blood to be either immediately processed or cryopreserved, risking sample integrity. Newer methods would likely benefit researchers performing immunophenotyping analyses.

The goal of this study is to investigate the effect of blood collection method on data quality in ChipCytometry immunophenotyping assays. To do this, we assessed the data both qualitatively, by comparing cellular morphology and fluorescence signal, and quantitatively, by measuring protein expression data and cell population annotations.

EXPERIMENTAL DESIGN

Whole blood was collected from three healthy donors in either Cyto-Chex BCT or standard EDTA vacutainer tubes. The samples were stored at room temperature until isolation. Whole blood in EDTA vacutainer tubes was processed immediately by density gradient separation to isolate PBMCs (0 days, room temp). Whole blood in Cyto-Chex BCT was stored for 7 days to simulate a standard shipping period, and then PBMCs were isolated (7 days, room temp). Technical replicates were loaded onto ZellSafe™ chips for immunostaining in ChipCytometry assays, as shown in the experiment schematic (Figure 2). The results of fresh blood samples (EDTA vacutainer tubes) were compared with samples processed 7 days post-collection (Cyto-Chex BCT).

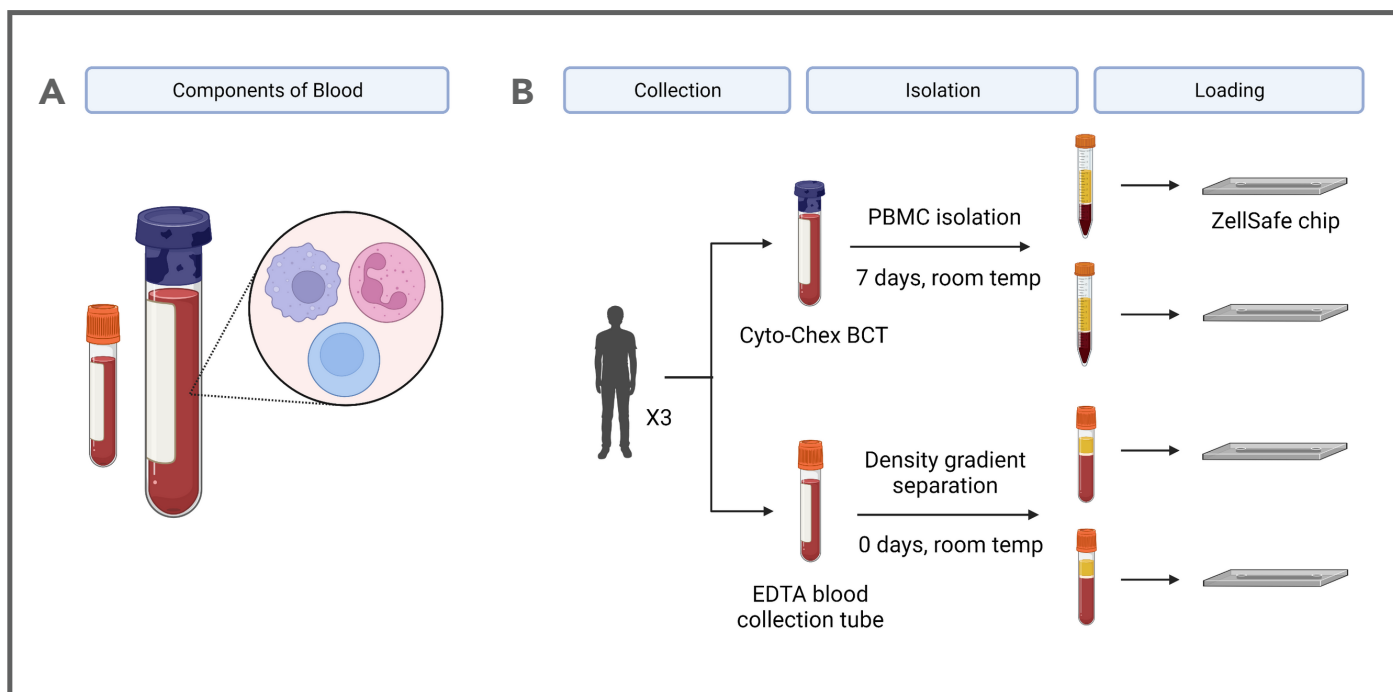


Figure 2. Schematic of experimental design to compare blood collection methods. A) The components of blood must be separated before profiling with ChipCytometry. B) Blood from three donors was collected in either Cyto-Chex BCT, and stored for 7 days, or EDTA vacutainer blood collection tubes, and processed immediately. After PBMC isolation, cells are mounted onto ZellSafe chips for ChipCytometry analysis.

EFFECT OF COLLECTION TUBE ON DATA QUALITY

Qualitatively, we note cellular morphology and fluorescence signal was the same in both Cyto-Chex BCT and EDTA vacutainer blood collection tubes (Figure 3). These observations were confirmed quantitatively by applying hierarchical gating strategy to identify cell populations. There was virtually no difference in the percent of cell populations identified. For example, helper T cells comprised of 59% of all T cells in Cyto-Chex BCT compared to 55% of all T cells in EDTA vacutainer tubes (Figure 4).

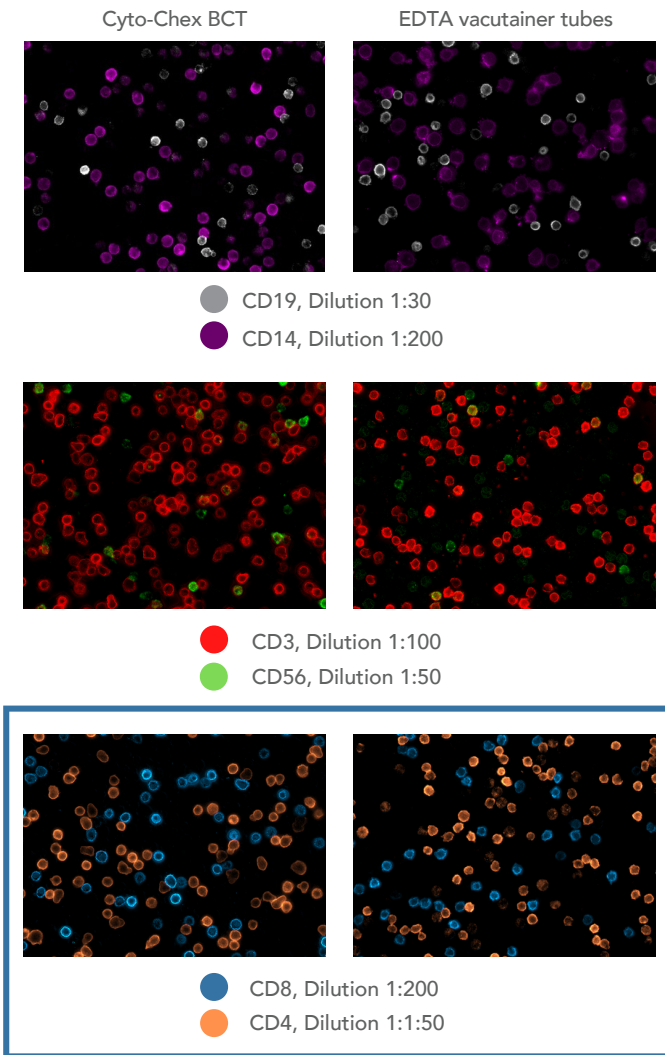


Figure 3. Immunostaining in human PBMCs collected in Cyto-Chex BCT and EDTA vacutainer tubes. Cellular morphology and fluorescence signal was similar across samples.

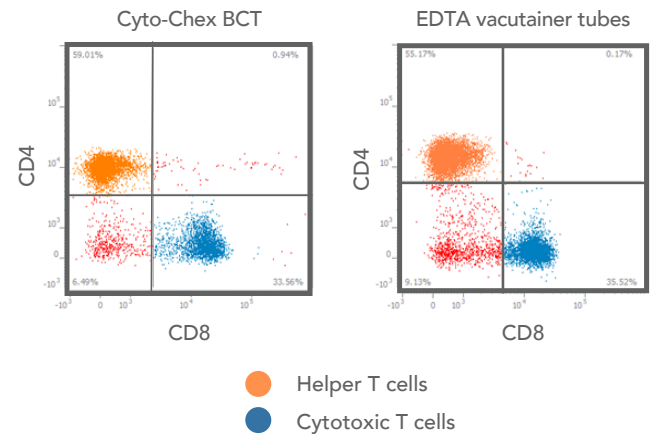


Figure 4. A hierarchical gating strategy was used to annotate and quantify T cell subpopulations. The percent of helper T cells (CD8+/CD4+, orange) and cytotoxic T cells (CD8+/CD4-, blue) is virtually the same in both collection methods.

Figure 5 shows the correlation between all immune cell subpopulations between Cyto-Chex BCT and EDTA vacutainer tubes in all three donors. There was good correlation of cell populations between collection methods, demonstrating the capability of Cyto-Chex BCT to preserve the expression of surface antigen markers in blood samples 7 days post-collection.

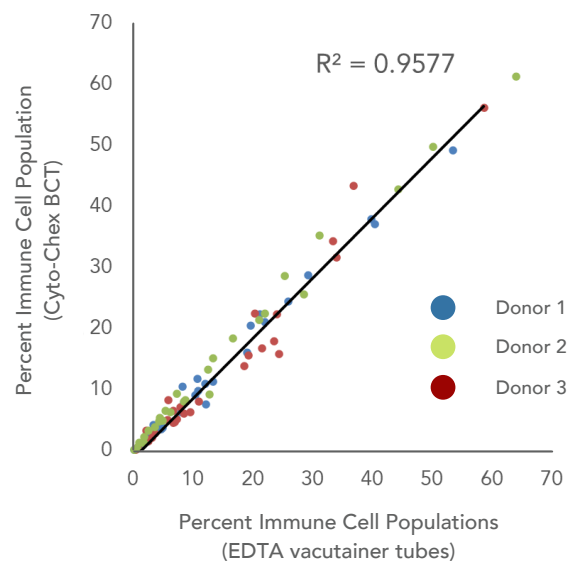


Figure 5. Scatter plot comparing the percent of immune cell populations found in blood samples collected in either Cyto-Chex BCT or EDTA vacutainer tubes.

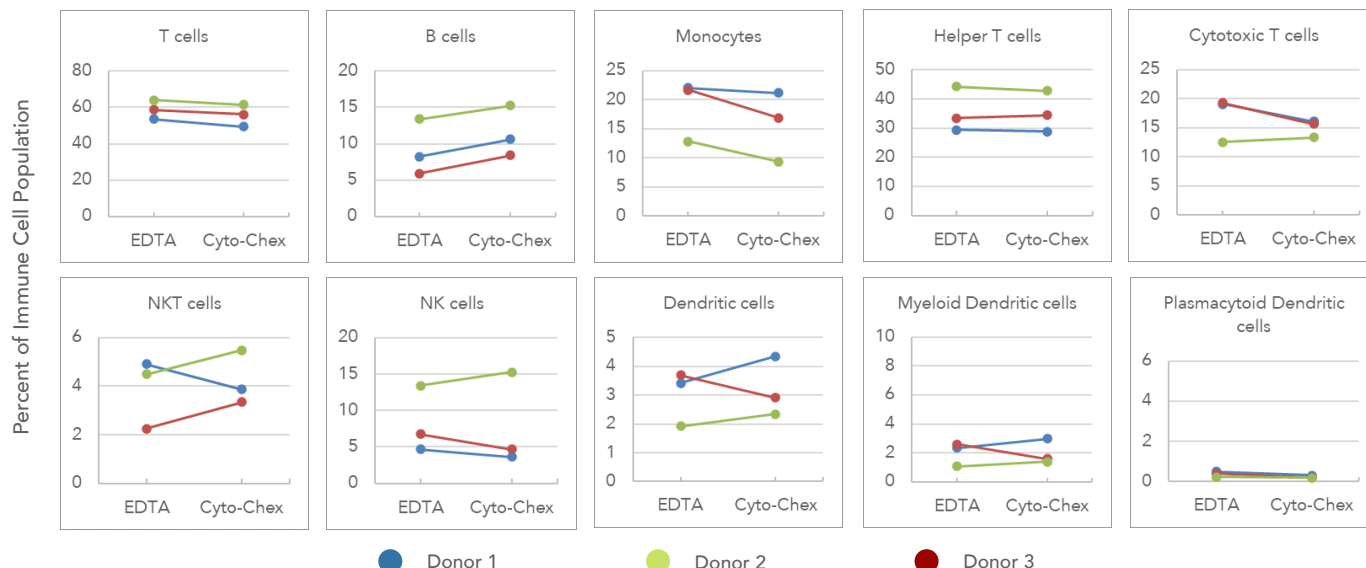


Figure 6. Paired data plot comparing the percent of immune cell populations found in blood samples collected in Cyto-Chex BCT or EDTA vacutainer tubes.

CONCLUSION

Clinical research samples must be properly handled to ensure the highest quality data for analysis. Here, we investigate an alternative to cryopreservation and compare blood stored in Cyto-Chex BCT for 7 days – to simulate shipping time – with fresh blood in standard EDTA vacutainer tubes. Our results show that protein expression data and cell population annotations of blood in Cyto-Chex BCT are comparable to those of fresh blood. With this data, researchers can have confidence that there is flexibility when planning a large ChipCytometry project using blood samples collected in Cyto-Chex BCT.

In summary, ChipCytometry is a highly multiplexed solution for single cell profiling of protein targets, enabling unbiased characterization of cell types and cell states in blood samples. We demonstrate the advantage of Cyto-Chex BCT to circumvent cryopreservation and streamline large-scale analyses of clinical blood samples.

To learn more about ChipCytometry, contact us at hello.canopy@bruker.com or visit www.canopybiosciences.com/chipcytometry

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