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White Paper

Unlocking Value of Flow Cytometry During Drug Development: Establishment Of Whole Blood Sample Stability For Extended Analysis

- **David Adle, PhD** Scientist
- **Jesse Thompson, PhD** Senior Analyst
- **Danielle Shea, BA** Senior Analyst
- **Curtis Sheldon, BS** Associate Director, Bioanalytical Principal Investigator
- **Rafiqul Islam, MS** Senior Director, Global Bioanalytical Services

Introduction

Current clinical protocols for enumeration of specific cell populations within whole blood stipulate that the sample should be stained with antibody and run via flow cytometry within 24-48hrs. Staining fresh whole blood is not practical in large clinical studies, and it is not possible to batch or re-run samples. Furthermore, it is very challenging if not impossible to ship samples from clinical study sites to a central analytical laboratory given these time constraints. Isolation and cryopreservation of PBMCs for downstream analysis by cell flow cytometry is an alternative, but is technically demanding and labor intensive. In addition, processing of PBMCs introduces variability and can deplete certain cell types, leading to inaccurate results.

Here, a simple staining method is described for multiple markers of clinical relevance by cell flow cytometry. Whole blood samples containing stabilizer are stable for at least 30 days post-freezing at -80°C and likely can be stored indefinitely for future analysis. Importantly, values closely match those of fresh samples from the same source run within 24hrs following current recommendations. This method is a very attractive solution for use in clinical studies which utilize cell flow cytometry during the drug development process.

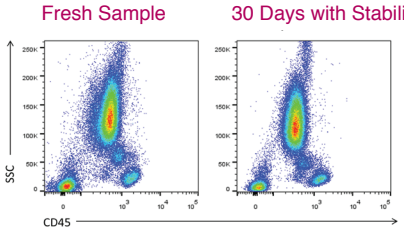
Methods

Human whole blood with or without stabilizer was processed and stained with FDA approved Trucount™ kits (BD Biosciences San Jose, CA) and acquired on a Cytek DxP6 FACSCalibur. G-CSF mobilized human blood was purchased from AllCells (Alameda, CA).

Results

Cell integrity and positioning of leukocytes analyzed within 24hrs after blood draw are nearly identical compared to the same sample containing stabilizer analyzed post-freezing 30 days later *(Figure 1)*.

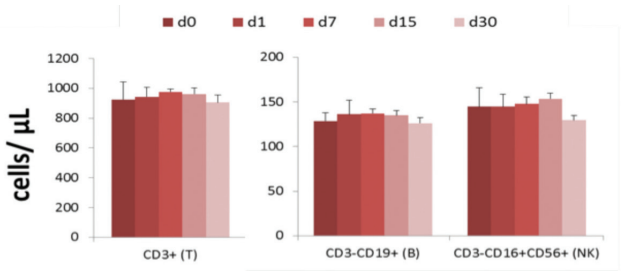
Figure 1. Stability Profiles



Stability profiles of fresh blood compared to whole blood with stabilizer stored for 30 days at -80°C, as represented by dot plots. SSC= side scatter, CD45= leukocytes.

The ability to quantify variations among lymphocyte subsets is important in clinical settings as well as in drug development. Therefore, the ability to enumerate T, B, and NK lymphocytes in stabilized blood was tested. Stability was demonstrated in multiple individual donors for up to day 30 post-freezing. Representative values from one individual are shown in *Figure 2*. Average values of stabilized blood never deviated more than 10% compared to the same fresh sample without stabilizer analyzed within 24hrs.

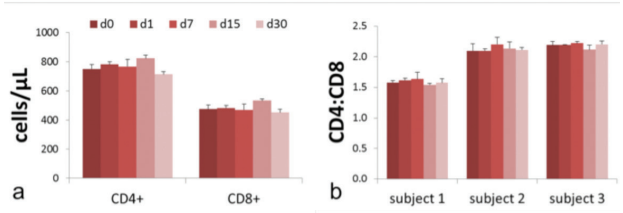
Figure 2. TBNK Stability in Human Whole Blood



Absolute $CD3^+$ T lymphocytes, $CD3^-CD19^+$ B lymphocytes, and $CD3^-CD16^+CD56^+$ NK lymphocytes per microliter from one individual donor. Day 0 (fresh sample without stabilizer) and days 1, 7, 15, and 30 (with stabilizer) samples were run via flow cytometry in triplicate. Error bars represent standard deviation.

The ability to enumerate CD4+ and CD8+ subsets of CD3+ T lymphocytes was then tested. Samples were stable for up to day 30 post-freezing in all three individual donors. Representative values from one individual are shown in *Figure 3a*. Average values of stabilized blood never deviated more than 12% compared to the same fresh sample without stabilizer analyzed within 24hrs. Additionally, CD4:CD8 ratios for all three individuals remained constant *(Figure 3b)*.

Figure 3. CD4 CD8 T-cell Stability in Human Whole Blood



Stability up to 30 days of CD3+ CD4+ and CD3+CD8+ T lymphocyte absolute cell counts per microliter in stabilized blood from one individual donor, and CD4:CD8 ratios in stabilized blood from three donors, all tested in triplicate. Error bars represent standard deviation.

Hematopoietic stem cells (HSC) are characterized by the expression of CD34, and are relevant in a number of clinical settings including the treatment of various leukemias.

A recombinant form of granulocyte colony stimulating factor (G-CSF) can be administered following chemotherapy to accelerate recovery by releasing and producing granulocytes and CD34 HSCs. Elevated circulating CD34 HSCs is an indicator of G-CSF efficacy. G-CSF mobilized blood without stabilizer was analyzed within 24hrs after blood collection. The same sample with addition of stabilizer was analyzed post-freezing for up to 30 days and yielded nearly identical results *(Table 1)*.

Table 1. Long-Term Stability of CD34 HSCs in Human Whole Blood

	Day 7	Day 14	Day 30
Mean CD34+ Day 0	**73.5 cells/µL**	**73.5 cells/µL**	**73.5 cells/µL**
	71.3	67.4	78.1
	75.3	71.9	70.6
	75.1	77.5	73.7
Mean	73.9	72.2	74.1
%CV	3.0	7.0	5.1
%Theoretical	100.6	98.4	100.9

Table 2. Cell Types and CD Markers Evaluated for Stability

Cell Type	Cell Surface Markers	Stability
T Cell	CD45, CD3, CD4, CD8	> 30 days
B Cell	CD45, CD19	> 30 days
NK Cell	CD45, CD16, CD56	> 30 days
Hematopoietic Stem Cell	CD45, CD34	> 30 days

Conclusions

A method has been developed for long-term stabilization of whole blood for the detection of several specific cell populations by flow cytometry without compromising sample integrity *(Table 2)*. This provides the foundation for analysis of stabilized whole blood for the determination of many more specific subsets which may have applications to drug development. The benefits of whole blood stability for flow cytometric analysis are:

- Reduced personnel/instrument cost by batching samples for analysis during regular working hours.
- Samples may be shipped from a clinical CRO to specialized flow cytometry labs for analysis without impacting quality.
- Assay reproducibility can be confirmed by performing incurred sample reanalysis as required by FDA bioanalytical guidance.
- New opportunities to analyze banked samples for additional phenotypic markers without need to repeat studies will significantly reduce client costs.