Extension of whole blood sample stability for flow cytometry analysis through the use of specialized blood collection tubes

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Introduction

Flow cytometry (FCM) is an information-rich methodology most commonly used to enumerate cells and monitor antigen expression. The use of FCM in multicenter clinical trials often requires sample shipment to specialized laboratories and hence requires prolonged sample stability.

One of the most standardized FCM enumeration assays is the TBNK assay. This is an *in vitro* diagnostic procedure used to determine absolute counts of T, B and NK cells from whole blood samples ≤24h post-venipuncture. This small sample stability window presents a challenge for clinical trials.

Objective and Methodology

Objective:

To identify vacutainer blood collection tubes and storage conditions that can extend sample stability.

Methodology Overview:

To assess the stability of cell populations and their frequencies, blood was collected from different healthy donors using EDTA tubes (K2EDTA), CYTO-CHEX® BCT or TransFix® vacutainers, and stored at different temperatures for up to 14 days prior to analysis using a validated TBNK assay.

To assess marker stability, samples were also collected from healthy donors using CYTO-CHEX® BCT tubes and stored at different temperatures for ≤28 days prior to analysis. Samples were analyzed using a FCM antibody panel which includes the following types of markers: (1) gating/lineage (2) activation/naïve/memory (3) functional/monitoring. The performance of the panel was established previously in whole blood spiked with a cell line or with *in-vitro* stimulated PBMC, through the following steps:

1 – Identification of optimal antibody titers.
2 - FMO controls and isotype control testing for fluorescence spill over and specificity
3 – Full panel testing for Intra-assay precision on a minimum of 3 donors

Cellular Enumeration

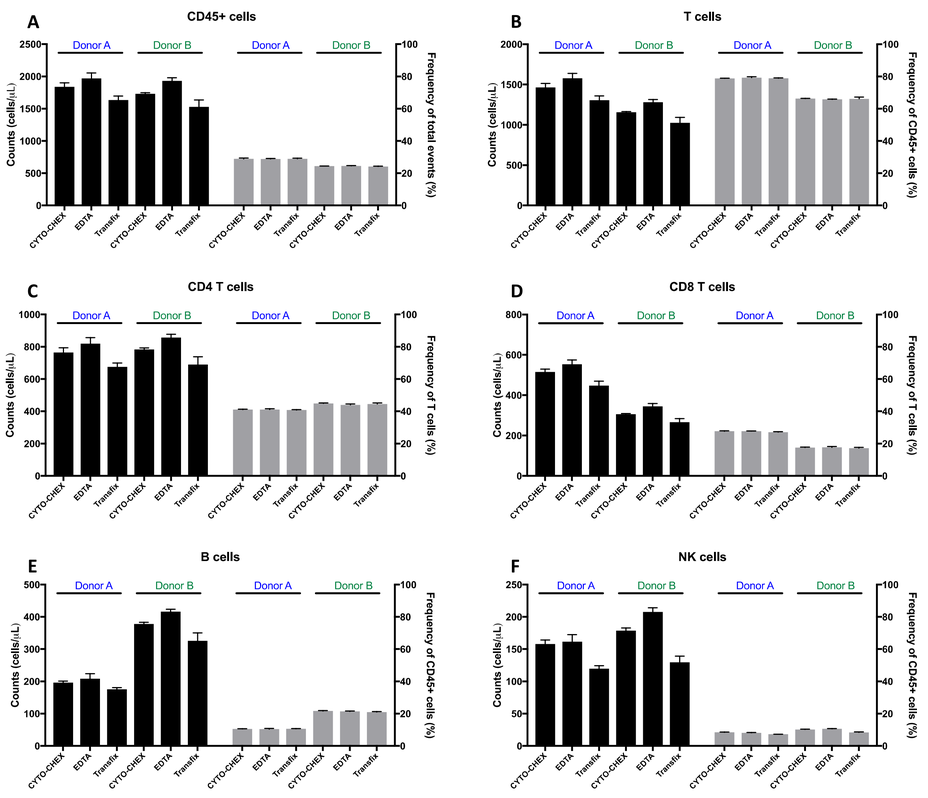


Figure 1: Cellular enumeration in various types of vacutainer tubes at Day 0
Whole blood was collected from 2 healthy donors into EDTA, CYTO-CHEX® or Transfix® vacutainers. A TBNK assay was performed immediately to enumerate cells at Day 0. Shown: Absolute cellular count (black columns) and cellular frequencies (grey columns) for the various cell types investigated for each donor.

➢ The 3 types of vacutainer tubes performed similarly at Day 0 and provided comparable results in terms of absolute counts and cellular frequencies.

Sample Stability in Various Tube Types

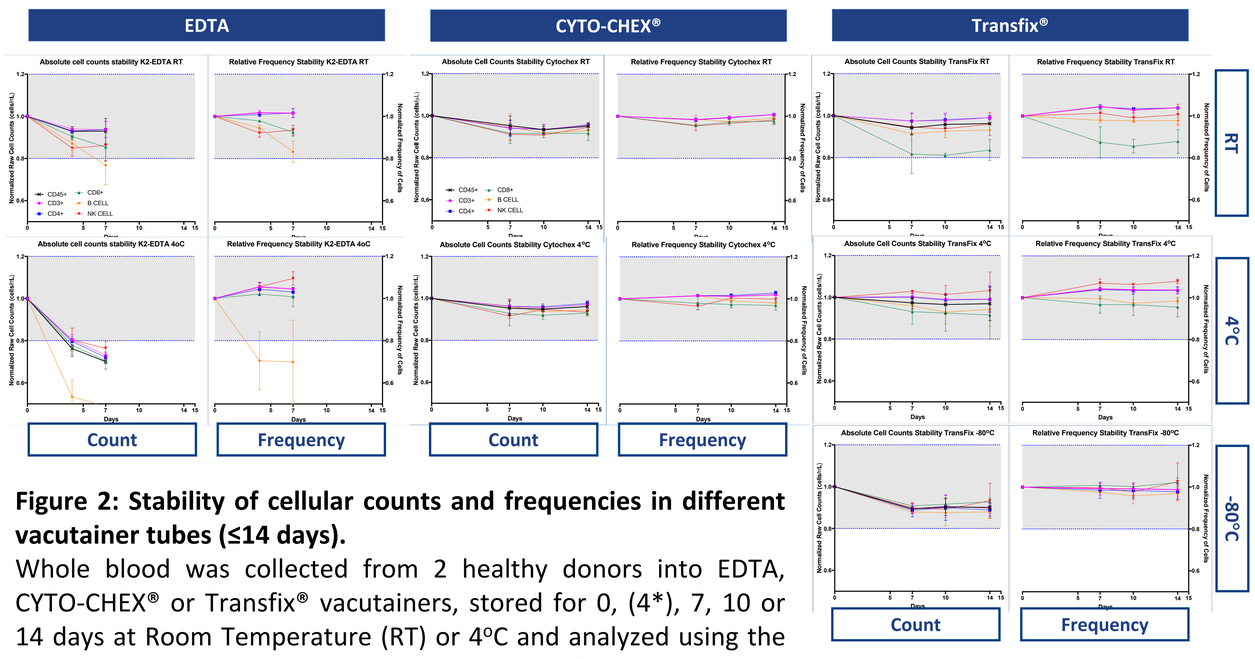


Figure 2: Stability of cellular counts and frequencies in different vacutainer tubes (≤14 days).
Whole blood was collected from 2 healthy donors into EDTA, CYTO-CHEX® or Transfix® vacutainers, stored for 0, (4*), 7, 10 or 14 days at Room Temperature (RT) or 4°C and analyzed using the TBNK assay. Shown: Stability time course data for cellular counts and frequency for each tube type tested.

*EDTA was expected to be less stable, thus a Day 4 timepoint was included for this condition.

The EDTA vacutainers demonstrate a rapid reduction of the absolute count, for all cell types already by 4 days. In addition, storage at 4°C accelerates cell loss. Observation of the frequency shows that although all populations are affected, B cells are more dramatically reduced, an effect enhanced by storage at 4°C. The stability assessment was stopped past 7 days due to the low quality of the sample.

The CYTO-CHEX® BCT vacutainers demonstrate a stability for both cell count and frequency. This stability is observed until the last time point assayed, being 14 days. Both RT and 4°C storage provide similar performance.

The Transfix® vacutainers demonstrate a stability for both cell count and frequency. This stability is observed until the last time point assayed, being 14 days. Interestingly, the Transfix® vacutainers can be cryopreserved at -80°C, so this condition was also assayed. The results demonstrate that storage at 4°C or at -80°C both enhance the stability of both cell counts and cell frequencies.

➢ The EDTA tubes have a limited stability, while both CYTO-CHEX® BCT and Transfix® vacutainers can prolong sample stability to 14 days, with Transfix® tubes being compatible with -80°C storage.

Transfix® Effect on Cell Gating

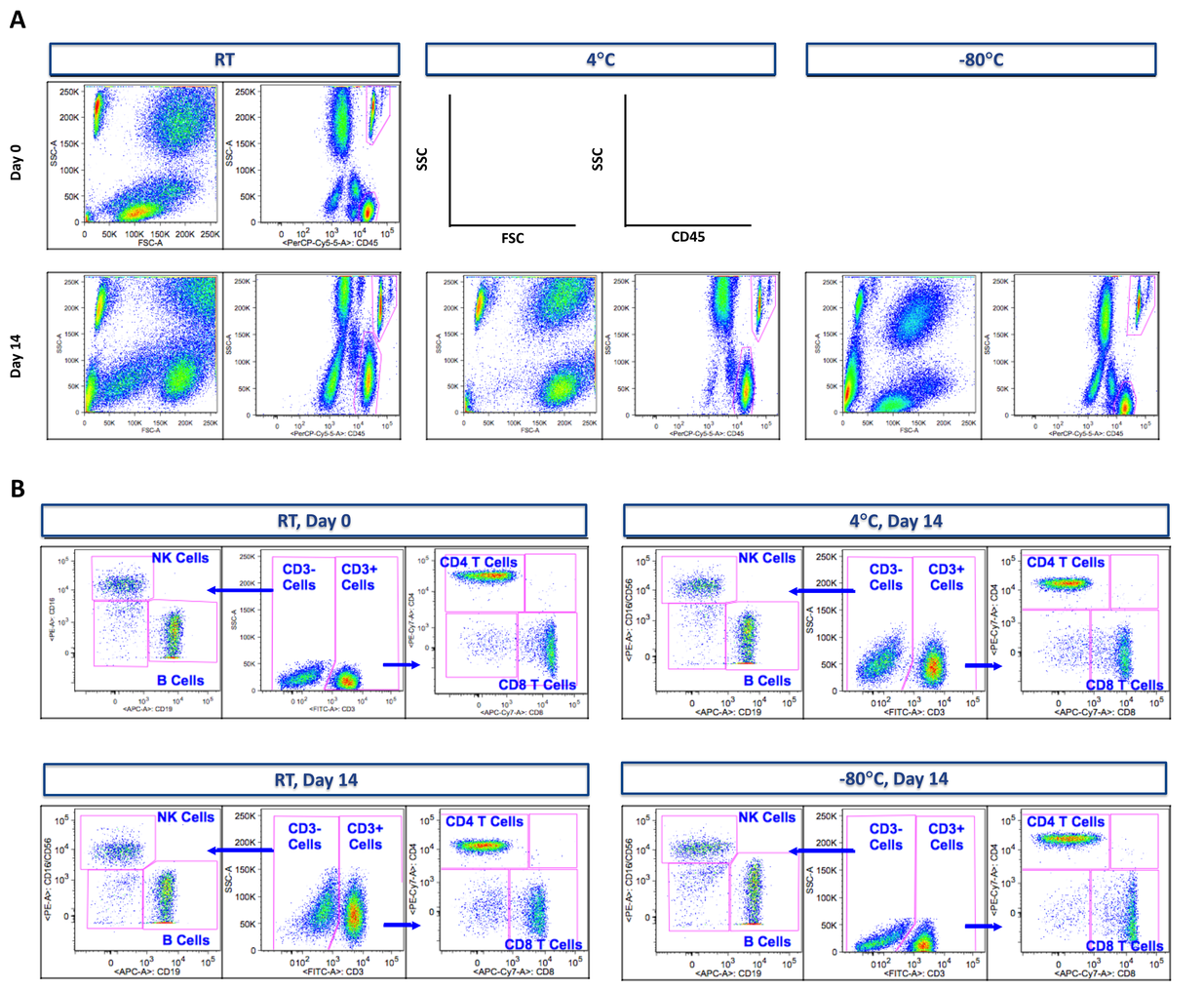
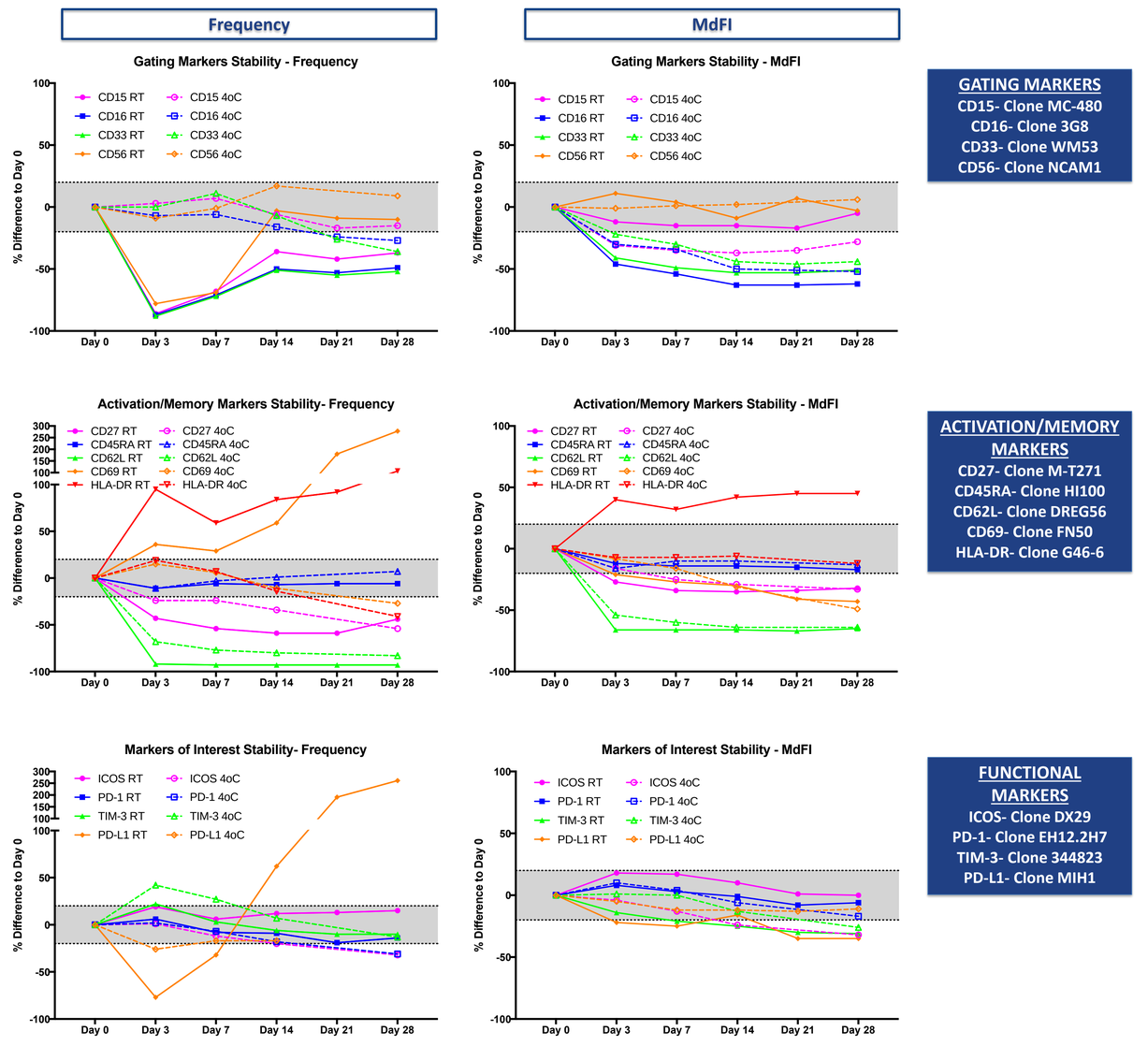


Figure 3: Effect of the sample stabilization in Transfix® vacutainers
The Transfix® vacutainers contain a proprietary fixative that stabilizes blood cells. In FCM analysis, the use of such reagents often results in a variation of physical properties (FSC/SSC plot) and may alter epitope recognition, preventing antibody binding. Shown: TBNK assay data from samples collected in Transfix® tubes **(A)** Physical properties (FSC for general cell size and SSC for cell granularity) as well as CD45 expression **(B)** Selection of the different cell populations: CD3+ Lymphocytes (T cells), CD4 T cells, CD8 T cells, B cells and NK cells.

The effect of the Transfix® fixative on SSC characteristics is visible in the form of a spreading of the SSC values. On the other hand, the fluorescence characteristics are mostly stable in both time and storage conditions.

➢ These results demonstrate that although stabilization affects the physical properties of the cells (FSC/SSC), the different cell populations can be adequately identified.

Stability of Multiple Markers in CYTO-CHEX® BCT



GATING MARKERS
CD15- Clone MC-480
CD16- Clone 3G8
CD33- Clone WM53
CD56- Clone NCAM1

ACTIVATION/MEMORY MARKERS
CD27- Clone M-T271
CD45RA- Clone HI100
CD62L- Clone DREG56
CD69- Clone FN50
HLA-DR- Clone G46-6

FUNCTIONAL MARKERS
ICOS- Clone DX29
PD-1- Clone EH12.2H7
TIM-3- Clone 344823
PD-L1- Clone MIH1

Figure 4: Stability of various cell markers in CYTO-CHEX® BCT (≤28 days)
Whole blood from 3 healthy donors was collected into CYTO-CHEX® BCT, and stimulated peripheral blood mononuclear cells (PBMC) were spiked into the blood samples to provide positive signal for weakly expressed markers. Samples were then stored for 0, 3, 7, 14, 21 or 28 days at RT or 4°C and assayed in triplicate using a FCM antibody panel which included various markers (gating/lineage, activation/naïve/memory, functional/monitoring). Shown: Stability time course data normalized to Day 0 and averaged across the 3 donors for each marker.

➢ The stability of the various markers vary greatly and should be investigated on an individual basis.

Conclusions

- **Whole blood sample stability can be significantly extended with the use of specialty blood collection tubes (CYTO-CHEX® and Transfix®), enabling batching of samples.**
- **The Transfix® can be cryopreserved, offering long-term storage possibilities.**
- **Stability of expression/detection must be confirmed for each markers, and can be influenced by different storage temperature. Some markers (ICOS, PD1 and CD45RA) show stability for ≤28 days when samples are stored at RT.**