Enhanced Preservation of Real-World Diseased Immune Signatures in Stabilized Whole Blood Enables Longitudinal Studies of Multiparameter Immune Monitoring

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Background

More than 1.2 million cytometry specimens are processed annually across clinical trials. Of those, more than 20% are rendered unusable because they fall outside the 24- to 72-hour live processing window. Fresh samples processed at 72 hours show a 48% drop in total cell count (Fig. 1), an 88% decrease in monocyte frequency, and a 48% decrease in neutrophil frequency compared to same-day processing within hours of the blood draw.

These limitations worsen in diseased specimens, where altered phenotypes and increased cellular fragility impact sample quality. Using TokuKit, a three-step whole blood stabilization workflow, we previously demonstrated 2-year stability in healthy donors, but whether diseased phenotypes are similarly preserved remains unknown. Here, we evaluated TokuKit's ability to preserve phenotypic and functional immune profiles from diseased donors for up to 6 months at -80°C.

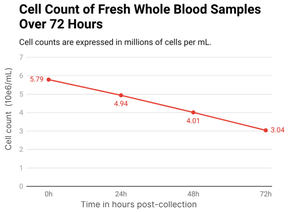
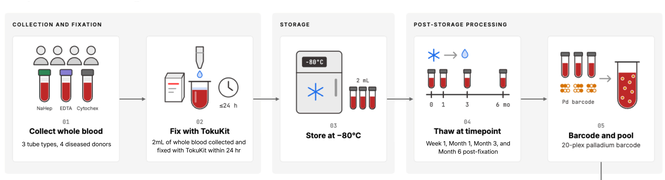


Figure 1. Line graph of cell counts (10e6) per milliliter(mL) of blood live samples over time.

Methods

A. Experimental Design



B. TokuKit Workflow

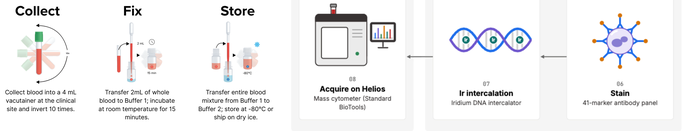
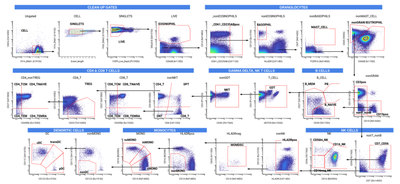


Figure 2. (A) Whole blood was collected into sodium heparin, K2EDTA, and Cyto-Chex tubes from four diseased donors at the Albert Einstein College of Medicine (IRB #: 2021-13730), stabilized using TokuKit (a PFA-based fixation) within 24 hours of collection, shipped on dry ice, and subsequently stored at −80 °C in multiple 2 mL aliquots. Samples were evaluated at Week 1 (baseline) and at 1, 3, and 6 months post-fixation. Upon thawing, cells were labeled with a 20-plex palladium barcode, pooled, stained with a 41-marker antibody cocktail, labeled with an iridium intercalator, and acquired on a Helios® mass cytometer (Standard BioTools). No viability dye was included. FCS files were normalized using EQ bead-based batch normalization and debarcoded before analysis in CellEngine (CellCarta), and cell populations were identified by manual gating. Population frequencies were calculated as a percentage of the top-level gate (non-granulocytes, or total leukocytes for granulocyte lineages), and functional marker frequencies as a percentage of the parent population. Parent–marker pairs with fewer than 100 parent events in either compared sample were excluded. Agreement between Week 1 and each later timepoint was assessed within each collection tube using Pearson correlation (r).

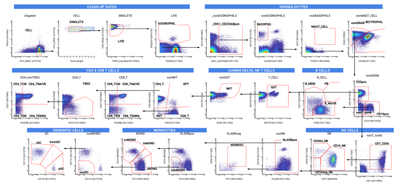
(B) TokuKit is a three-step blood preservation kit used to collect, fix, and store whole blood at the clinical site, allowing samples to be shipped and batch-processed centrally.

Fresh v. Fixed Comparison

A. Live Blood Gating Scheme



B. TokuKit-Fixed Blood Gating Scheme



C. Correlation of Population Frequencies, TokuKit-fixed vs. Fresh

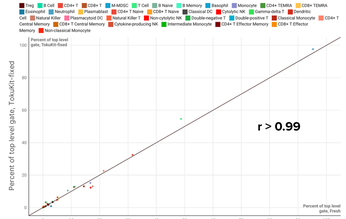
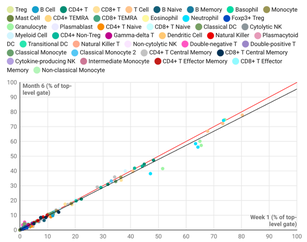


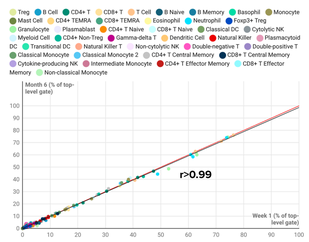
Figure 3. (A) Gating strategy for live whole blood samples processed within 24 hours of collection. (B) Gating strategy for TokuKit-fixed whole blood samples processed on the day of staining following storage at -80°C. (C) Correlation of immune cell population frequencies between fresh samples stored for 24 hours at room temperature and TokuKit-fixed samples stored for 9 days at -80°C across 3 donors (r > 0.99, n=3, 33-marker mass cytometry panel).

TokuKit preserves diseased patient samples across all standard tube collections

A. Population frequencies: T = 6 M vs. T = 0 M (EDTA)



B. Population frequencies: T = 6 M vs. T = 0 M (Cyto-Chex)



C. Functional marker frequencies: T = 6 M, T = 3 M, T = 1 M vs. T = 0 M

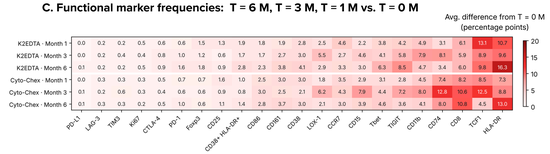
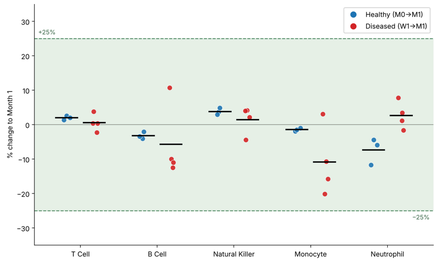


Figure 4. Immune cell population and functional marker frequencies remained stable across collection tubes. Correlation of immune cell population frequencies (% of top-level gate) between T = 0 M (Week 1 baseline) and T = 6 M in (A) K2EDTA (r > 0.99) and (B) Cyto-Chex (r>0.99) tubes (n = 4 donors × 41 immune cell populations). Across all collection tubes, including sodium heparin, population frequencies correlated with baseline at r ≥ 0.98 through Month 6. (C) Average difference from T = 0 M in functional marker frequencies (% marker-positive cells, percentage points) at Months 1, 3 and 6 in K2EDTA and Cyto-Chex tubes. Each cell averages all four donors and all parent populations for that marker; parent–marker pairs with <100 events were excluded. Together, these results indicate that TokuKit is compatible with standard collection tubes, allowing sites to use the tube their protocol already specifies.

Diseased and healthy samples show comparable changes from baseline

A. Per-donor % change from baseline to Month 1
Healthy vs. Diseased Samples in major immune cell compartments



B. Per-donor % change from baseline to Month 3
Healthy vs. Diseased Samples in major immune cell compartments

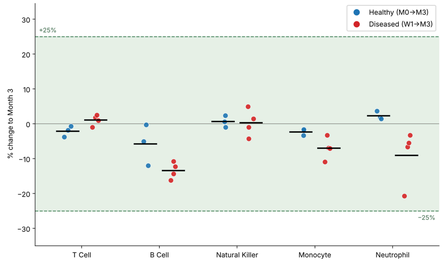
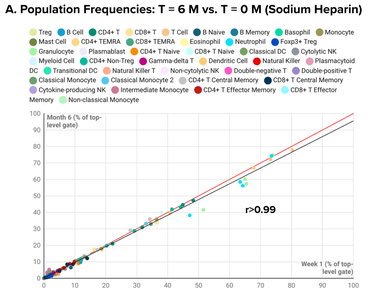


Figure 5. Relative % change in frequency from baseline in major immune compartments for diseased samples (red; sodium heparin, n = 4; baseline T = 0 M, Week 1) and healthy samples (blue, n = 3; baseline Month 0) at (A) Month 1 and (B) Month 3. Black bars show group means. Changes were small and similar in direction in T and NK cells (≤5%) in both groups, and all per-donor changes fell within ±25% of baseline, our standard acceptance criterion.

Diseased whole blood samples remained stable for 6 months

A. Population Frequencies: T = 6 M vs. T = 0 M (Sodium Heparin)



B. Functional Marker Frequencies: T = 6 M vs. T = 0 M (Sodium Heparin)

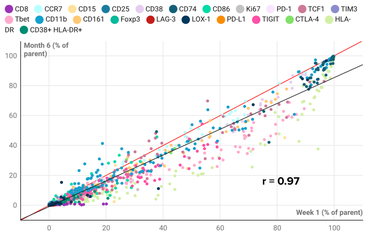


Figure 6. (A) Correlation of immune cell population frequencies between TokuKit-fixed diseased specimens processed within one week (T=0) and 6 months (T=6) post-fixation. Each point represents one immune cell population per donor, as a percentage of the top-level gate (r > 0.99). (B) Correlation of functional marker frequencies of TokuKit-fixed diseased whole blood samples processed within one week (T=0) and 6 months (T=6) post-fixation. Each point represents one immune cell population per donor, as a percentage of the parent gate (r = 0.97).

Key Findings and Conclusion

TokuKit preserves immune cell population frequencies and functional marker expression in diseased whole blood stored at −80 °C for up to 6 months. Sponsors running global trials across multiple sites can collect, fix and store whole blood at the point of collection and batch-process samples at a central laboratory, removing the 24–72-hour processing window that renders at least 20% of clinical samples unusable.

- Stability: immune cell frequencies r ≥ 0.99 and functional marker frequencies r ≥ 0.97 vs Week 1 through Month 6 (sodium heparin)
- Patient vs healthy donor: changes from baseline were comparable between patient and healthy donor samples; T and NK cells changed by ≤5% in both groups at every timepoint
- Collection tube: immune cell frequencies were preserved in K2EDTA, sodium heparin, and Cyto-Chex tubes (r ≥ 0.98 vs Week 1 through Month 6)