Is Fresh *Really* That Fresh? Time-Dependent Quality Loss in Live Specimens

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Background & Results

An estimated ~80% of specimens used for cytometry analysis are processed live. The use of live specimens spans from 0 to 72 hours post collection and has remained largely unquestioned due to concerns of antigen masking.

To quantify the impact of processing delays on sample quality, we analyzed blood samples from three healthy donors using mass cytometry (CyTOF) and spectral flow cytometry, comparing population frequencies at various time points from blood draw. We applied a 25% threshold for frequency changes to identify unstable populations.

Mass cytometry analysis showed that all cell populations were stable up to 5 hours post-collection, but by 7 hours, monocyte populations showed statistically significant changes, which worsened with longer delays. Spectral flow cytometry analysis confirmed these findings, demonstrating that monocyte and neutrophil frequencies were significantly affected by processing delays.

Results are consistent across two cytometry platforms, highlighting the significant changes in aged samples and challenges the traditional use of live specimens, emphasizing the need for fixation to preserve samples in high-parameter cytometry analysis.

Methods

Whole blood from three healthy donors was collected in 10 mL sodium heparin vacutainers. Each specimen was split into two aliquots, one for live processing and staining, and the other one for fixation. Analysis was performed on a Helios mass cytometer or a 5 laser Cytek® Aurora spectral flow cytometer.

Live samples

Samples designated for live processing were diluted in ACK lysis buffer to lyse red blood cell (RBC). Samples were subsequently counted, and stained with a 41-marker (mass cytometry) or a 25-marker antibody cocktail (spectral flow cytometry). After staining, samples were fixed with 1.5% PFA and analyzed on a Helios® mass cytometer or a 5 Laser Cytek® Aurora Spectral Flow Cytometer.

Fixed samples

Whole blood was fixed immediately after collection using Teiko's TokuKit. This kit contains buffers "Stable Lyse" and "Stable Store," manufactured by Smart Tube®. Following the manufacturer's instructions, whole blood was incubated in buffer "Stable Lyse" at a ratio of 1:1.4 (blood:buffer) for 15 min at room temperature (RT). After incubation, buffer "Stable Store" was added at a ratio of 1:1.6. Samples were then frozen at -80C before processing. Upon thawing, buffers were washed out and cells were permeabilized, followed by staining with a 41-marker (mass cytometry) or 25-marker panel (spectral flow cytometry) and analyzed on the same instruments mentioned above.

Panel

Mass Cytometry panel design was performed in-house. The final panel consists of 41-markers, each conjugated to a unique heavy metal. Spectral flow cytometry panel design was adapted from Cytek® 25-color Immunoprofiling Assay. Some antibody clones and fluorophores were exchanged after in-house research indicated that they are incompatible with fixation. The final panel consists of 25 markers with unique spectral signatures. The panel does not contain a viability stain.

Conclusions

Do live samples remain stable over time? ***Not really.***

- Live processing of samples results in 49% loss in cell number within 72 hours.
- Cell loss is not uniformly distributed across cell populations.
  - Monocytes show a 87% reduction when processed live at 72 hours.
  - Neutrophils show a 48% reduction when processed live at 72 hours.
  - Rare lymphocyte subsets show a >25% change in frequency when processed live within 51 hours.
- Fixation can preserve cell count and population frequencies over time.

Live processing of blood samples results in loss of monocyte and rare lymphocyte subsets

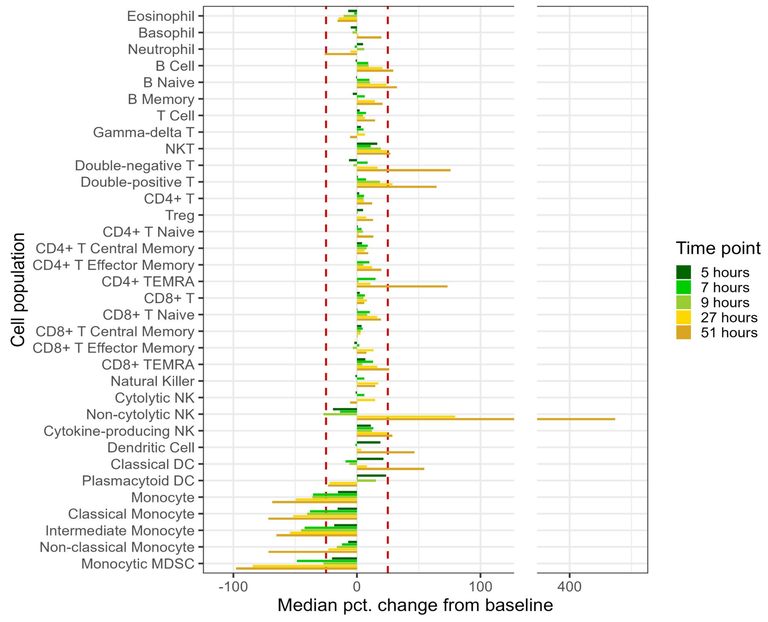

Figure 1: Bar chart of median percent change of all analyzed cell populations. Red line indicates +/- 25% change threshold. Analyses are compared to baseline (T=0 hours). Percent change calculated as (100*(current value minus baseline value)/baseline value)

Live processing of blood samples causes neutrophil populations lose CD66b positivity over time

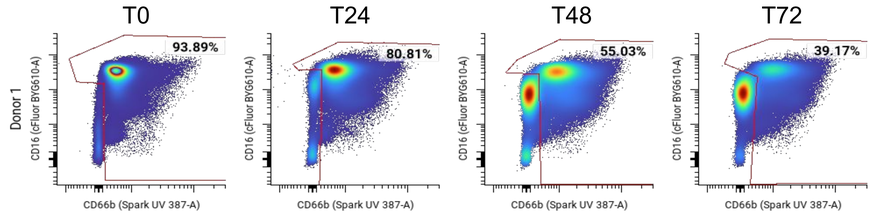

Figure 2: Density plots of events plotted against CD66b and CD16 to gate neutrophil populations of samples stored live and analyzed at T=0, 24, 48, and 72 hours post-blood draw.

Fixation preserves cell count and population frequencies over time

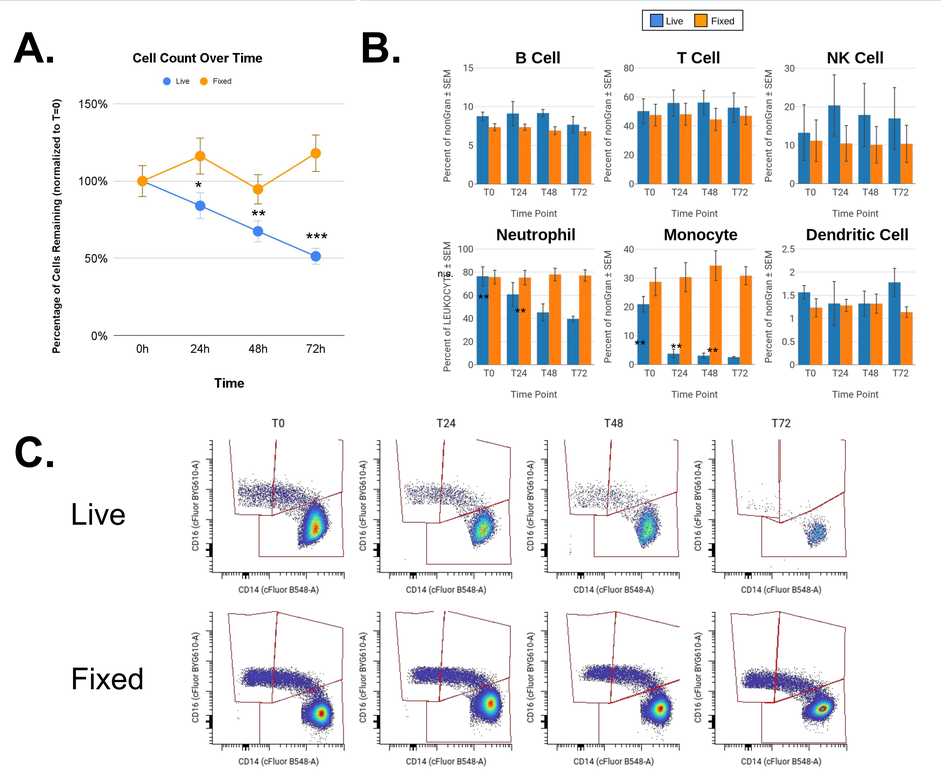

Figure 3: (A) Line graph of cell counts (10e6) per milliliter(mL) of blood for both live and fixed samples over time. **(B)** Immune cell frequencies calculated as percent of non-granulocytes (B cell, T cell, NK cell, Monocyte, and Dendritic cell populations) or leukocytes (Neutrophil) for three whole blood donors over time. **(C)** Density plot of Monocyte subpopulations for live and fixed samples over time. Significance values are indicated (*p<0.05, **p<0.01, ***p<0.001).

Live processing does not preserve immune cell frequencies over time

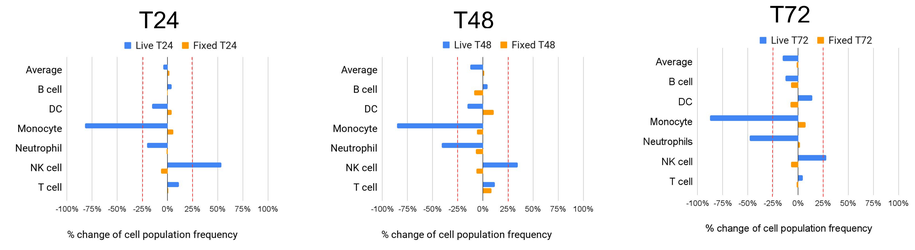

Figure 4: Bar charts of percent change in cell population frequencies calculated as percent of non-granulocytes (B cell, T cell, NK cell, Monocyte, and Dendritic cell populations) or leukocytes (Neutrophil) for three whole blood donors at T= 0, 24, 48, and 72 hours post-blood draw. Percent change calculated as (100*(current value minus baseline value)/baseline value)