Advancing Whole Blood Stabilization Techniques for Immunological Analytical Outcomes

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Introduction

PBMCs are essential for many clinical trial endpoints such as ELISpot and ICCS flow cytometry, but clinical sites often lack processing capabilities, leading to shipment delays and suboptimal handling. These factors, along with protocol variability, can compromise cell viability and assay performance. While DMSO is the standard for cryopreservation, it is cytotoxic, with time-dependent cell loss and reported clinical adverse events. Its impact on cell health adds further variability to downstream assays. This study evaluates how pre-isolation storage time affects PBMC viability and apoptosis in both fresh and cryopreserved samples, and how these parameters, with or without mitigators, influence ELISpot and ICCS outcomes.

Methods

1. Whole blood collection in CPT and heparin tubes
2. CPT tubes centrifuged within 2h
3. Heparin tubes: addition of DMSO and frozen or stored at RT for 48h
4. Stimulation of anti-CD3/anti-CD28 with Con A + Brefeldin A
5. Readout with ELISpot and ICCS Flow Cytometry

Results

- Processing PBMCs within ~24hours post blood draw in CPT tubes is required for ELISpot.
- Processing PBMCs within ~48hours post blood draw in CPT tubes is sufficient for culturing and ex-vivo stimulation for intracellular cytokine assay by flow cytometry for Interferon-γ.
- DMSO treated whole blood negatively affects gradient formation during traditional PBMC isolation.
- DMSO preservation of whole blood for 48hrs prior to PBMC processing interferes with ELISpot and ex-vivo stimulation and intracellular cytokine assay by flow cytometry for Interferon-γ.

Conclusion

- Whole blood preservation for PBMC isolation only works for some applications.
  - Cell numbers were drastically reduced in DMSO treated whole blood.
  - DMSO treated cells (pre-frozen) showed higher apoptosis populations than freshly isolated; however, DMSO treated cells had reduced populations of cells in apoptosis upon thawed.
- Collection in CPT Tubes shows the most promising stability compared to freshly collected blood.
  - ELISpot analysis is best achieved with samples collected within 24 hours.
  - Ex-vivo stimulation and ICCS flow cytometry is best achieved with samples collected within 48 hours.

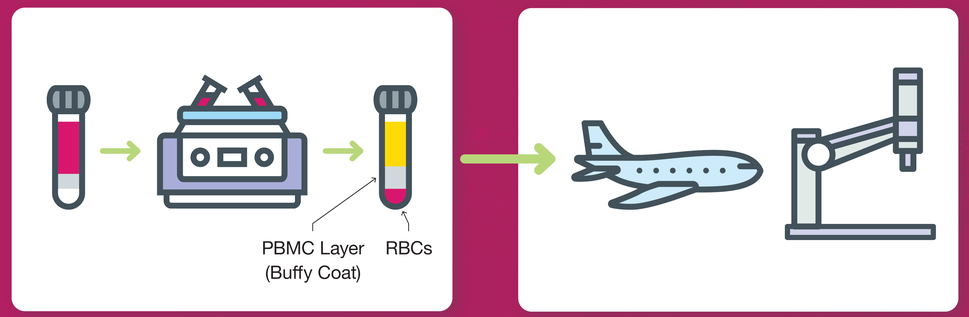
References

1, Braudeau, C., Salabert-Le Guen, N., Chevreuil J., Rimbert, M., Martin, J.C. & Josien, R. (2021) An easy and reliable whole blood freezing method for flow cytometry immuno-phenotyping and functional analyses. Clinical Cytometry, 100 (6) 652-665.

Whole Blood Stabilization for PBMC Isolation and Characterization Can Work for Some Applications

PBMC isolation requires specialized training and equipment at clinic sites

Shipping whole blood impacts PBMC quality (24–48 hr)



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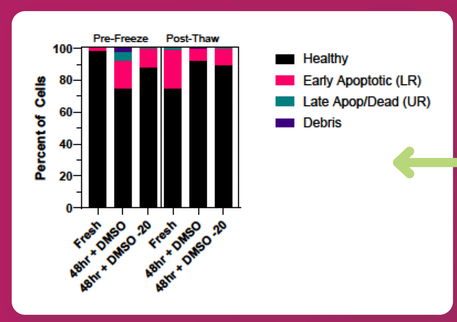
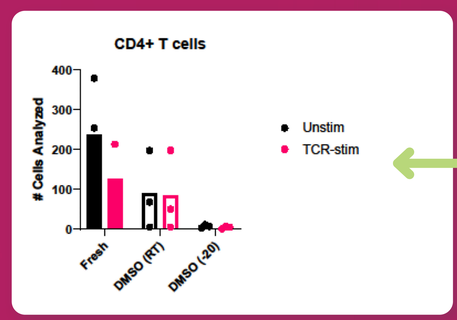
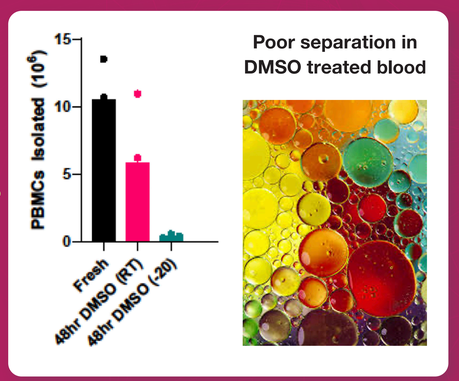
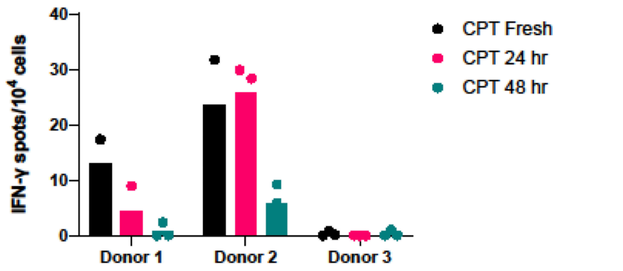
DMSO treated whole blood



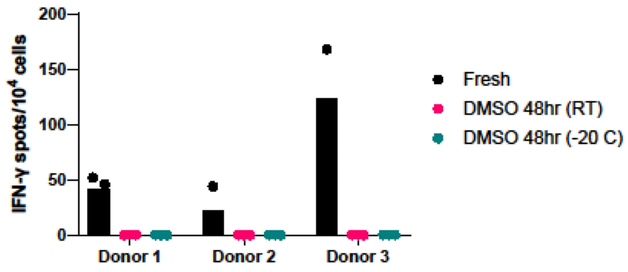




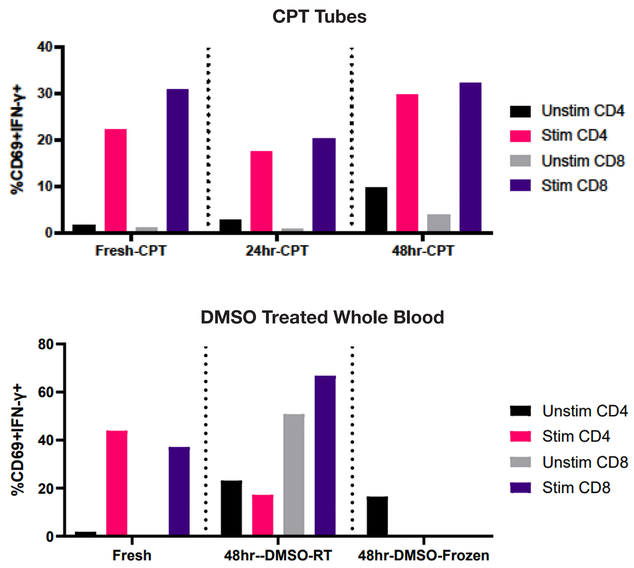
IFN-γ ELISPOT: PBMCs from CPT Tubes



IFN-γ ELISPOT: PBMCs from DMSO Treated Whole Blood



Intracellular Cytokine Flow Cytometry



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