Advancing Peripheral Blood Mononuclear Cell Isolation (PBMC) for ELISPOT Analysis – time is critical

Johannes Stanta, Teresa Urlacher, Tyler Moore

Celerion Inc. Lincoln, NE

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PURPOSE

The isolation of PBMCs from whole blood is a well-established approach in bioanalysis, with downstream applications functional assays, such as intracellular cytokine staining (ICCS) flow cytometry and ELISpot analysis following *ex-vivo* stimulation. Although PBMC analysis has grown in popularity, the lack of PBMC processing capabilities at clinical sites often leads to shipments of whole blood prior to PBMC isolation. Delays in PBMC processing, variation in protocols between labs, and non-optimized procedures can impede the successful utilization of PBMCs. Our work quantifies the impact of pre-isolation storage time on key health indicators in freshly isolated and cryopreserved PBMCs. In addition, we correlate storage time, cell viability, and cellular apoptosis in the presence or absence of cell health mitigators, which are key indicators for successful functional assays.

METHOD(S)

1. PBMC Isolation and Cell Health Assessment

- PBMCs were isolated via density gradient centrifugation
- Post-thaw, cell count, viability, and apoptosis were measured using Annexin V/PI staining and the Muse Cell Analyzer
  - Healthy cells: Annexin V (-) and 7-AAD (-)
  - Early apoptotic cells: Annexin V (+) and 7-AAD (-)
  - Late stage apoptotic/Dead: Annexin V (+) and 7-AAD (+)

2. IFN-γ ELISPOT Assay Workflow

- Cryopreserved PBMCs thawed at 37°C, washed, and assessed for initial viability/apoptosis
- Cells rested for 4 hours at 37°C in assay medium, then reassessed
- Pre-coated ELISpot plates were blocked; peptide pools, controls (PHA, CEFX), and DMSO added
- PBMCs plated at 2 × 10^6 cells/mL and incubated for 18–24 hours at 37°C with 5% CO_2
- Cytokine detection via biotinylated anti–IFN-γ and streptavidin-ALP.

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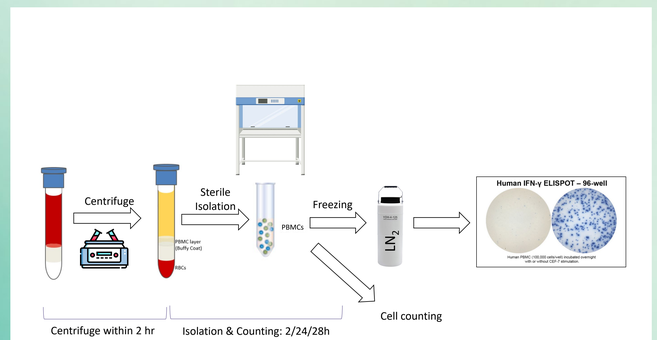
PBMC integrity declines rapidly

Processing within 24 hours is essential for reliable ELISpot results



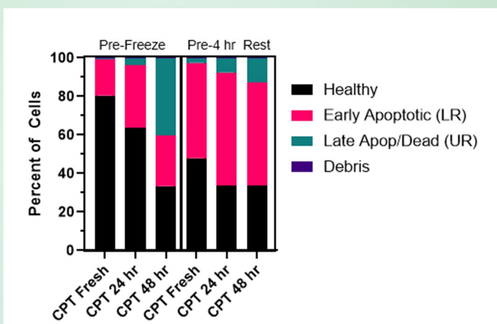


Figure 1: PBMC health by storage time and resting. Prolonged storage prior to isolation results in a shift from healthy cells to early and late apoptotic populations. A 4-hour rest post-thaw does not restore cell health.

Presenter: Johannes Stanta
johannes.stanta@celerion.com

RESULTS

Viability assessment

- Pre-freeze: Fresh and 24h-stored samples retained >99% viability; slight decline at 48h
- Post-thaw: Only fresh samples retained viability >70%. Significant loss observed in 24h and 48h samples
- Post-rest: A 4h resting period post-thaw did not improve viability in 48h samples

Apoptotic Indicator (Annexin V)

- Apoptosis increased with storage time: from ~20% in fresh to ~80% in 48h-stored samples (pre-freeze)
- High viability at 24h masks early apoptosis stages
- No significant improvement in apoptosis levels after resting post-thaw

ELISpot responses

- IFN-γ ELISpot responses correlate with PBMC viability; samples with <50% viable cells show minimal spot formation.

CONCLUSIONS

- Fresh PBMCs show optimal health pre-freeze and post-thaw, with minimal apoptosis and debris
- Cell health deteriorates over time: 24h and 48h samples shift toward early/late apoptosis, especially after freezing/thawing
- Post-thaw resting is not beneficial for recovering viability or reducing apoptosis in 48h samples
- Processing within 24 hours post-draw in CPT tubes is critical for ELISpot
- High post-thaw viability is critical for robust ELISpot performance. Cell health directly impacts assay sensitivity.
- Processing within ~48 hours may suffice for phenotyping and diagnostic, but not suitable for most functional assays

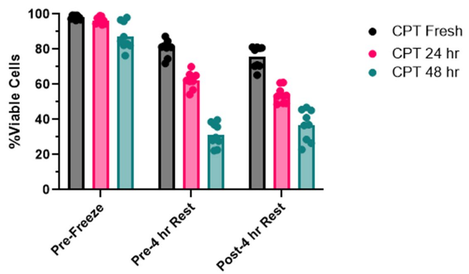


Figure 2: Viability Assessment of PBMCs Isolated at fresh, 24, and 48 hours after blood draw. PBMCs isolated after 24–48h storage show reduced viability after thawing, with minimal recovery following a 4-hour rest.

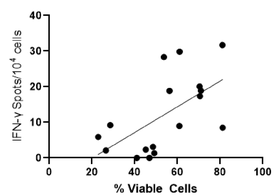


Figure 3: Correlation Between PBMC Viability and IFN-γ ELISpot Response. A positive correlation was observed between % viable PBMCs and IFN-γ spot counts, confirming reduced assay performance in samples with low viability.