Introduction

Immune monitoring is an essential method for quantifying changes in immune cell populations in chronic inflammation, infectious disease, autoimmune disease, and cancer studies. The extreme heterogeneity of immune cells demands a high-parameter approach to more fully and efficiently quantify the immune response in health and disease. CyTOF® technology, using mass cytometry by time-of-flight, provides an ideal solution, enabling simultaneous detection of more than 40 phenotypic and functional markers in a single tube of sample. We report development of a 29-marker panel for mass cytometry based on the Human ImmunoPhenotyping Consortium (HIPC) consensus panel (Maecker et al. Nature Reviews Immunology (2012)), expanded to allow identification of additional leukocyte subsets, particularly T cells.

Repeatability

Method

1. Single PBMC donor
2. Two lots of 2 donors each
3. Groups A and B on Helios
4. Group B on Helios and Helios
5. Group A on Helios and Helios

Results

Repeatability, a measure of precision over replicate staining and acquisition of a single sample, was measured as the coefficient of variation (CV) of the immune cell population frequencies. All populations measured had standard deviations (SD) of less than 2% and CVs of less than 20% for both instruments.

Reproducibility

Method

1. PMBC donors 1-4
2. Each biosource starts four tubes.
3. PBMC donor: 1, 2, 3, 4.
4. Each PMBC donor stained with four different panels.

Results

Reproducibility of cell frequency measurements was determined by the CV of the mean population frequency for each PMBC donor and instrument. Populations with frequencies greater than 5% had CVs of less than 10% for both instruments except for central memory (CM) CD4 T cells and monocytes (total and classical) which still had CVs under 20%.

Conclusions

- The Maxpar Human Immune Monitoring Panel Kit shows a high level of repeatability in identifying immune cell populations and measuring population frequencies.
- This panel kit also shows a high degree of reproducibility across multiple PBMC donors.
- The high correlation of the full panel compared to subpanel population frequencies demonstrates that neither isotope reagent effects nor steric hinderance impacts immune cell population identification with the kit.
- We conclude that the Maxpar Human Immune Monitoring Panel Kit can provide consistent immune cell population identification and enumeration for any given lot of PBMC.

Automated Versus Manual Data Analysis

Method

FCS files were normalized using CyTOF software and analyzed using probability state modeling (PSM) and GemStone software and by manual gating with Cytobank or WinList™.

Results

- GemStone automated data analysis process:
  - Data cleanup model eliminated dead cells, debris, and normalization beads.
  - Immune profiling model performed deep immunophenotyping analysis via PSM.
  - Immunophenotyping results are displayed in Cen-ware® dimensionality reduction plots.
- Printable report summarizes population statistics:
  - Number of cells
  - Percentage of total intact, live cells
  - Percentage of parent population

Automated data analysis required only ~5 minutes for each FCS file and needed no initial setup of gating strategy or adjustment of gates between samples.