Peripheral Blood Mononuclear Cells for High Throughput Screening and Cell Based Assays

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INTRODUCTION

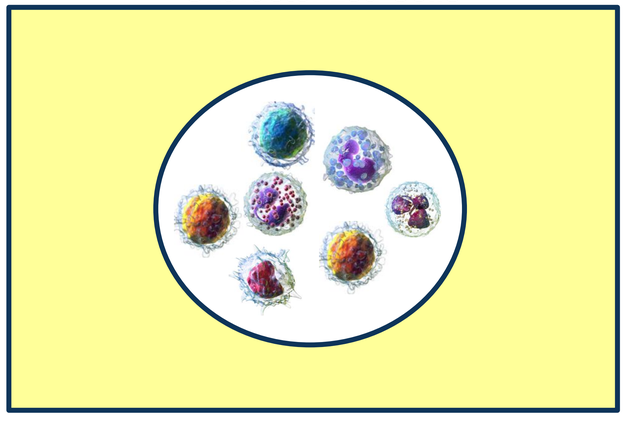
Peripheral blood mononuclear cells (PBMCs) are extremely useful for understanding in vivo physiological and metabolic activity. PBMCs have broad ranging applications from basic discovery, preclinical and clinical studies, including direct monitoring of immune responses to therapeutic and vaccine development. The availability of sufficient cells, especially in the case of assay development for large screening campaigns, and ensuring lot to lot reproducibility, have been limiting constraints.

Precision's AccuCell® cryopreserved PBMCs solve these challenges by providing large quantities of consistent and reproducible cells from a single donation. Here we demonstrate our PBMCs are available with diverse donor demographics and superior viability at 92.2% ± 0.03% and lots with up to 10 billion cells ensures high throughput screening (HTS) assays have matched cellular standards. Here we highlight some of our routine phenotypic characterization which includes viability, proliferation, apoptosis, HLA Class I & Class II typing, Antibody Dependent Cellular Cytotoxicity (ADCC), and IFNγ ELISpot T memory cell cytokine responses to PHA, CMV, and CEF.

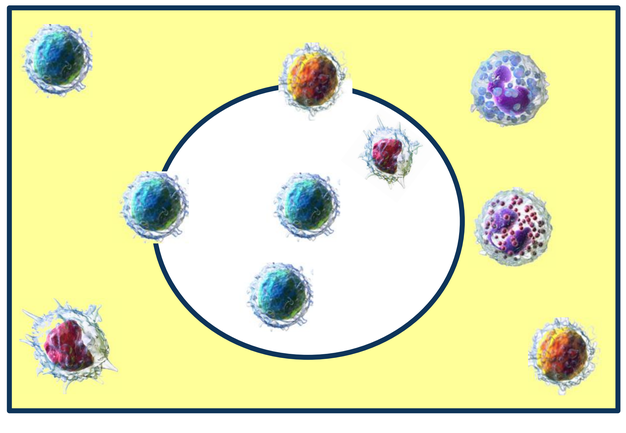
Primary human cells are more representative of an in vivo response than cells lines for the development of therapeutics. Due to the diversity and heterogeneity of humans and therefore human primary cells we provide a panel of validation assays on our AccuCell® PBMCs. Here we establish the importance of donor characterization and mechanism based validation of these cells, performed for all lots of Accucell® prior to QC release. The integration of this data empowers consistent and reproducible results for research and high throughput screening.

PBMC FUNCTION IN CELL BASED ASSAYS

AccuCell® PBMC Characterized Cells



Generic PBMCs Non-Characterized Cells



White Blood Cell Image credits:
http://commons.wikimedia.org/wiki/Category:White_blood_cells#mediaviewer/File:Blausen_0909_WhiteBloodCells.png

BILLIONS OF PBMC PER LOT & SUPERIOR VIABILITY

Figure 1. AccuCell® PBMCs Lot Cell Yields and Corresponding Cell Viability

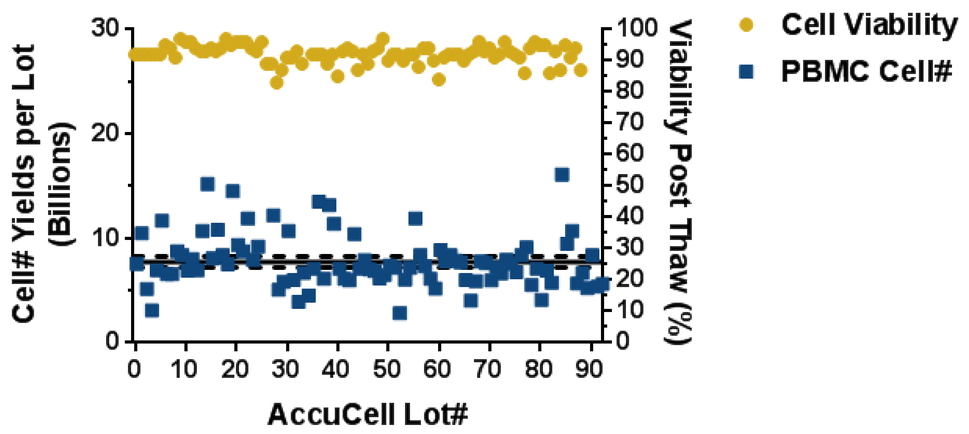


Figure 1: Data representative of PBMCs in Precision. Manufacturing of cryopreserved PBMCs yields cell lots with 7.5±2.5 billion cells. Upper 95% confidence limit (CL) is 8.26, Lower 95% CL is 7.22. In our optimized animal-free media and standardized controlled-rate freezing program, post thaw recovery of PBMCs on 10 million lot vials were 10 million viable cells or greater. Average viability in lots depicted above was 92.2%±0.03%.

DIVERSE DONOR DEMOGRAPHICS

Figure 2.

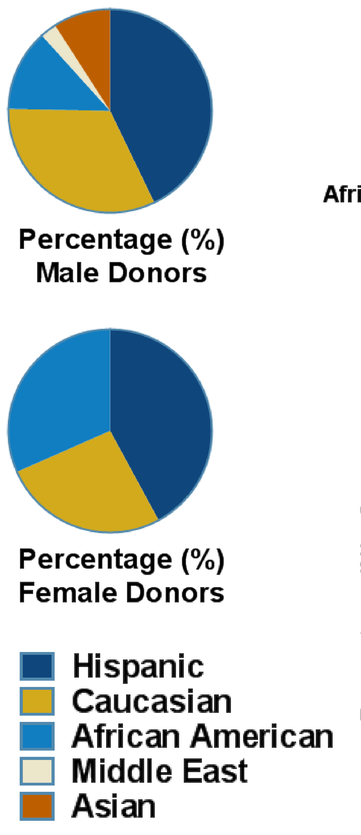


Figure 3.

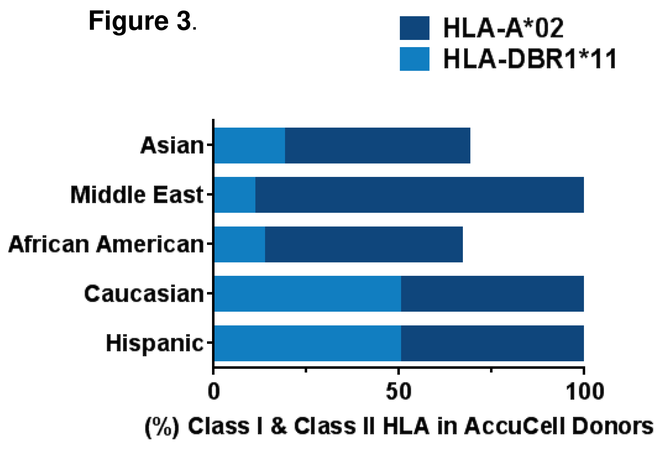


Figure 4.

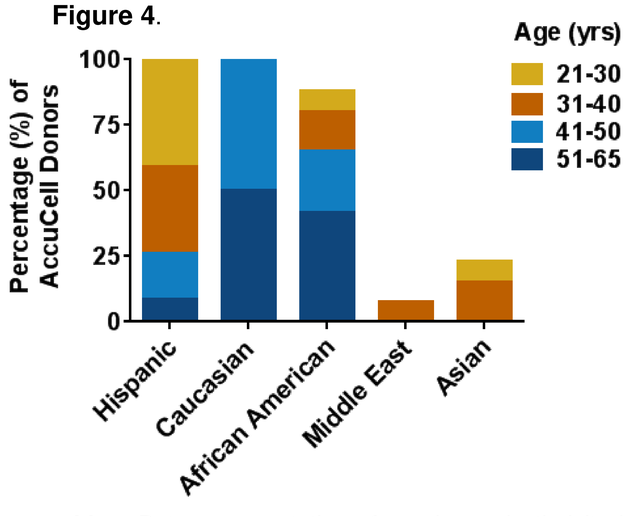


Figure 2: Gender & Ethnic Demographics. Data representative of gender and ethnicity in PBMC lots. Male donors 42.9% are Hispanic, 32.5% Caucasian and 13% African American. Female donors 42.1% are Hispanic, 26.3% Caucasian and 31.6% African American. **Figure 3: HLA Class I & Class II Demographics**. Data representative of Precision donors. In the HLA-A locus, the HLA-A*02 allele is present in 57% of Caucasians, and >50% in Hispanics and African Americans. Class II HLA-DBR1 locus, HLA-DRB1*11 prevalent in 50% Hispanics and Caucasians and 20% or less in the other ethnicities. **Figure 4. Gender and Age Matched Demographics on Diverse Ethnic Populations** Dynamic age ranges in both genders enables in vitro pharmacokinetic/pharmacodynamics (PK/PD) profiling of therapeutics.

PBMC PROLIFERATION PROFILING

Figure 5.

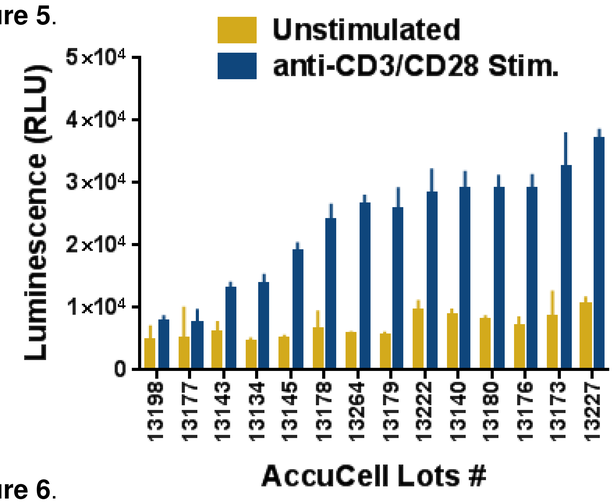
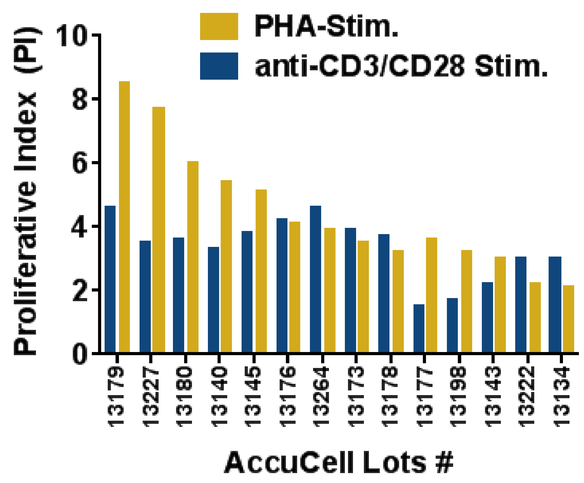


Figure 6.



Mechanism induced proliferation through the T cell receptor elicits a Proliferative Index (PI) ≥ 2 in the majority of donors. Due to the heterogeneity of Human Primary Cells more or less induction is expected with specific mechanisms, unlike Phytohemagglutinin(PHA which has a number of physiological effects.

Figure 5: T-Cell Receptor (TCR) Induced Proliferation with anti-CD3/CD28 Donors were thawed, cells were plated at 50K-200K per well, rested overnight at 37ºC and stimulated with pre-coated anti-CD3/CD28 beads at an optimum bead:cell ratio, proliferation was measured at 96hrs with Cell-Titer Glo™. **Figure 6: Comparison of PHA vs TCR Induced Proliferation**. Similarly, the same donors were stimulated with PHA and proliferation was measured at 96hrs with the MTT(reduction of tetrazolium salt) Assay. Proliferative Index is the ratio of stimulated versus unstimulated growth.

CONCLUSION

AccuCell® PBMCs are well characterized through multiple approaches including diverse donor demographics, mechanistic cell based assays for T cell proliferation, pan T cell and monocyte proliferation stimulation with PHA, culminated with ADCC Cytotoxicity(data not shown) for innate memory anti-tumor activity, and IFNγ ELISpot for T-memory cells cytokine responses.

Integrating these data, which are available for each lot of AccuCell®, empowers "fit for purpose" research, consistency, and reproducibility in HTS screens. Providing cellular standards for high-throughput screening and research applications is our goal.

ACKNOWLEDGMENTS

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