A Comparison of PBMC Separation Methods – Under-layering, LeucoSep™ Separation, and CPT™ Cell Preparation Tube

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BACKGROUND: Immune monitoring utilizing PBMC collected from study subjects is a critical component of immunotherapeutic research. An important service of a successful biobank is its ability to provide high quality PBMC in support of both clinical and pre-clinical trials. Different PBMC separation techniques are currently utilized throughout the scientific community, bringing to question whether or not these methods are equivalent. Three common PBMC separation methods were examined for their effects on frequency of PBMC subsets by flow cytometry.

METHODS: Whole blood (WB) from healthy adult volunteers was collected in tubes containing acid-citrate-dextrose anticoagulant (ACD) or sodium citrate CPT™. PBMC were isolated using density-gradient separation methods; under-layering, LeucoSep™ tubes, or CPT™. Freshly isolated and cryopreserved/thawed PBMC were assessed for recovery, viability, and analyzed by flow cytometry for frequency of lymphocyte populations of T cells, helper T cells, cytotoxic T cells, NK and B cells, TREGs and monocytes.

RESULTS – Processing: Recovery of PBMC per mL of whole blood processed using the three different separation methods ranged from 0.5×10^6 cells per mL to 2.51×10^6 cells per mL, depending on the healthy control sample, with a p-value of 0.4105. Viability for all samples tested fell within normal ranges (>90% viable, fresh or thawed). Percent recovery of viable PBMC after cryopreservation and thaw yielded recoveries of 60% – 93%, p-value of 0.4925.

RESULTS – Flow Cytometry: Analysis of flow cytometric results by one-way ANOVA on the frequency of PBMC subsets T, helper T, cytotoxic T, and TREG cells yielded p values of 0.7295, 0.9991, 0.9999, and 0.9553, respectively, (fresh) and p values of 0.9739, 0.9891, 0.9929, 0.6959 (thawed). Frequency of B cells, NK cells and monocytes p values were 0.1740, 0.2470, and 0.4778 (fresh) and 0.9386, 0.9263, and 0.5153 (thawed). Analysis of viable populations (as measured by flow) across separation methods resulted in p values of 0.0034 (fresh cells) and 0.2143 (thawed).

Conclusion: Analysis of flow cytometric results by one-way ANOVA of PBMC isolated using three different separation methods showed there was no significant difference in the frequency of the PBMC subsets examined. Differences in the percent of viable cells across separation methods was considered significant, with the viability of PBMC from CPT™ being lower than viabilities for both LeucoSep™ and under-layering methods; however, the % of viable cells still fell within acceptable ranges of 86.7% - 95.9 % in fresh cells and 93.9% - 97.9% for the cryopreserved/thawed cells. These results support the use of all three PBMC isolation methods for use in both immediate (fresh cells) or future experiments (cryopreserved cells).