Analysis of the Effects of Processing Method on Peripheral Blood Mononuclear Cell Isolation and Plasma Total Protein Levels

Muise, A., White, L., Lensch, M., Badowski, M., and Harris, D.T.
University of Arizona, AHSC Biorepository and Laboratory Exchange, Tucson, AZ

Background

Various processing methods are available for enrichment of peripheral blood mononuclear cells (PBMCs) from whole blood. The effects of these different processing methods on plasma and PBMC recovery before and after cryopreservation is largely unknown. Additionally, verification of best methods for thawing of PBMC included testing of thaw temperatures of media.

Methods

- 50-60 ccs of whole blood was collected in 10ml heparin vacutainers from each donor.
- The blood from each individual donor was pooled and then divided into three portions for processing by three methods: a CPT vacutainer (BD), a SepMate-50™ (StemCell Technologies), device and a Leucosep-50 (Grainier) device.
- Before and after each processing method, the cells were analyzed for:
 - Cell viability
 - Total cell numbers
 - Cell Differentials
- Plasma from each processing method was analyzed for total protein content using the Nanodrop-2000 analyzer.
- Cryopreserved PBMC were thawed using two different thaw methods, cold-thaw versus warm-thaw.
 - The Cold Thaw Method involved placing thawed cells in cold (4°C) media.
 - The Warm Thaw Method involved placing thawed cells in warm (32°C) media.
- Thawed PBMC were analyzed based on processing method before freezing, and thawing method for:
 - Cell viability
 - Total cell numbers
 - Cell Differentials

Results

While the viability and total cell recovery was not significantly affected by the different processing methods ($p>0.05$), the percentages of cell types from the CPT vacutainer method varied, but not significantly ($p>0.05$), when compared to the other two methods.

Cells recovered from processing by CPT vacutainer contained a much higher percentage of neutrophils than those processed by the SepMate or Leucosep methods; an average of 15.6% neutrophils in the final cell suspension as compared to the 4.4% from the SepMate method, and the 5.6% of neutrophils from the Leucosep method (N=8), with a p -value of 0.0048. The percentage of lymphocytes and monocytes recovered for the CPT vacutainers at 83.1% was less compared to 95.3% and 93.8% for the SepMate and Leucosep methods, respectively. This difference was also deemed significant by ANOVA analysis ($p=0.0042$).

It was also noted in the analysis of plasma from four independent donors (N=4) that the total protein content from the CPT processing method was much lower than the other two processing methods at 56.4 mg/ml, and below the normal range (60-80 mg/ml) for plasma (1). The SepMate processing method showed a plasma total protein level nearly double the normal range (135mg/ml), but the Leucosep processing had an average plasma total protein content of 77.8 mg/ml, within the expected normal range. Additional donors are in progress for continued data analysis.

Thawed PBMC were analyzed (N=6) for viability after thawing, and it was found that the warm thaw method yielded a significantly higher viability ($p=0.0028$) than the cold thaw method. The number of cells recovered before was significantly related to the thaw method. Preliminary data for cell differentials after thawing showed a lack of significant difference in percentage of neutrophils between processing methods, and the same was seen for lymphocytes and monocytes.

Results

FIGURE 1a. Viability of PBMC after processing by the three different methods were comparable.

FIGURE 1b. Average total number of PBMC recovered after processing by the three different methods were comparable.

FIGURE 2. Percentage of PBMC that were Lymphocytes and Monocytes after processing by the three different methods showed a significant difference for the CPT processing method.

FIGURE 3. Percentage of PBMC that were neutrophils after processing by three different methods showed a significant increase of neutrophils were retained by the CPT processing method.

FIGURE 4. Total protein found in plasma from the three different processing methods showed that only the total protein found in the plasma from the Leucosep method was within the expected range.

Results

FIGURE 5. PBMC viability after thawing paired samples with both the warm and cold thaw methods showed that the warm thaw method had consistently higher viability.

Conclusions

Our initial analysis shows that all three blood processing methods are comparable, although the CPT vacutainer processing method may retain more granulocytes. The SepMate and Leucosep processing methods seem to isolate primarily lymphocytes and monocytes. This finding may be an important consideration when the number of cells aliquoted for cryopreservation is critical for downstream assays, as freezing of neutrophils may reduce the viability of the sample and usable cells recovered after thawing.

Additionally, for the purposes of plasma isolation and preservation, CPT and Leucosep should have been comparable in terms of total protein content, yet the CPT had consistently lower total protein compared to the Leucosep.

The variation in viability after thawing at the various temperatures indicates that a warm thaw approach is beneficial to a higher viability after cryopreservation.

Bibliography

- "BD Vacutainer® CPT™ Mononuclear Cell Preparation Tube - Sodium Heparin (16x125 Mm / 8mL)." *BD FACSAria III - Features - Optics / BD Biosciences-US*. www.bdbiosciences.com/us/applications/blood-collection/cell-biomarker-preservation/bd-vacutainerreg-cpttrade-mononuclear-cell-preparation-tube---sodium-heparin/p/362753.
- SepMate™ Stemcell Technologies. www.stemcell.com/products/brands/sepmate-pbmc-isolation.html
- "GREINER BIO-ONE." *227290 - LEUCOSEP TUBE, 50 ML, PP, 30/115 MM, CONICAL BOTTOM, POROUS BARRIER, NATURAL*. shop.gbo.com/en/row/products/bioscience/separation/leucosep/50ml-leucosep-tubes/227290.html. Kennelly, Peter J., et al.. "Plasma Proteins & Immunoglobulins." *Harper's Illustrated Biochemistry*, 30e Eds. Victor W. Rodwell, et al. New York, NY: McGraw-Hill, . <http://accessmedicine.mhmedical.com/content.aspx?bookid=1366§ionid=73247095>.