

Comparison of Subsets, Immunophenotype and Function Between PBMC Isolation Methods



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Introduction

The quality of peripheral blood mononuclear cells (PBMC) isolated from whole blood has a significant impact on subsequent analysis. Maximizing the recovery, viability and functionality of isolated PBMCs are essential for reliable assessment of a variety of downstream applications, particularly cell-mediated immune responses.

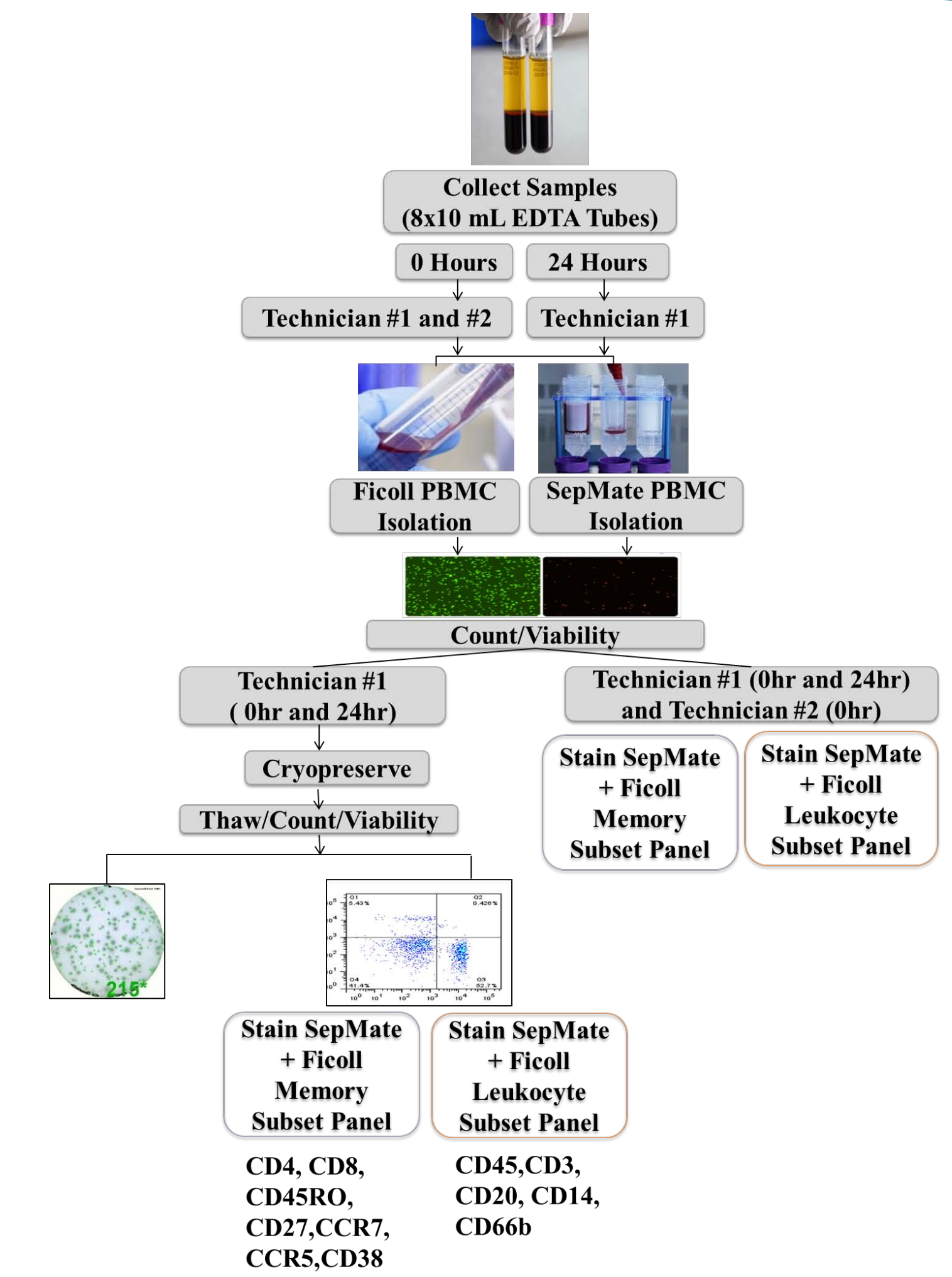
The standard method for purification of PBMCs is the use of density gradient centrifugation. This method requires precise layering of whole blood over density medium, careful pipetting of enriched cells after centrifugation, and meticulous care throughout the labor intensive isolation procedure to avoid mixing of layers before and after centrifugation steps.

SepMate[®] tubes are a new commercially available product that greatly reduces the time for optimal PBMC isolation from whole blood, but requires validation to ensure that they perform equivalently to traditional Ficoll methods. Here we compare the two methods for PBMC yield, viability, subset frequency and functionality.

Objective

1. To compare subset frequencies, immunophenotype and T-cell function between standard Ficoll density gradient PBMC cell separation and a novel method using STEMCELL Technologies SepMate tubes.
2. To compare both methods of PBMC isolations from whole blood processed immediately after phlebotomy, 24 hours after phlebotomy as well as pre- and post cryopreservation.
3. To determine if there were differences in inter-technician variability between the Ficoll and SepMate methods of PBMC isolations.

Methods



Memory Subset Frequencies

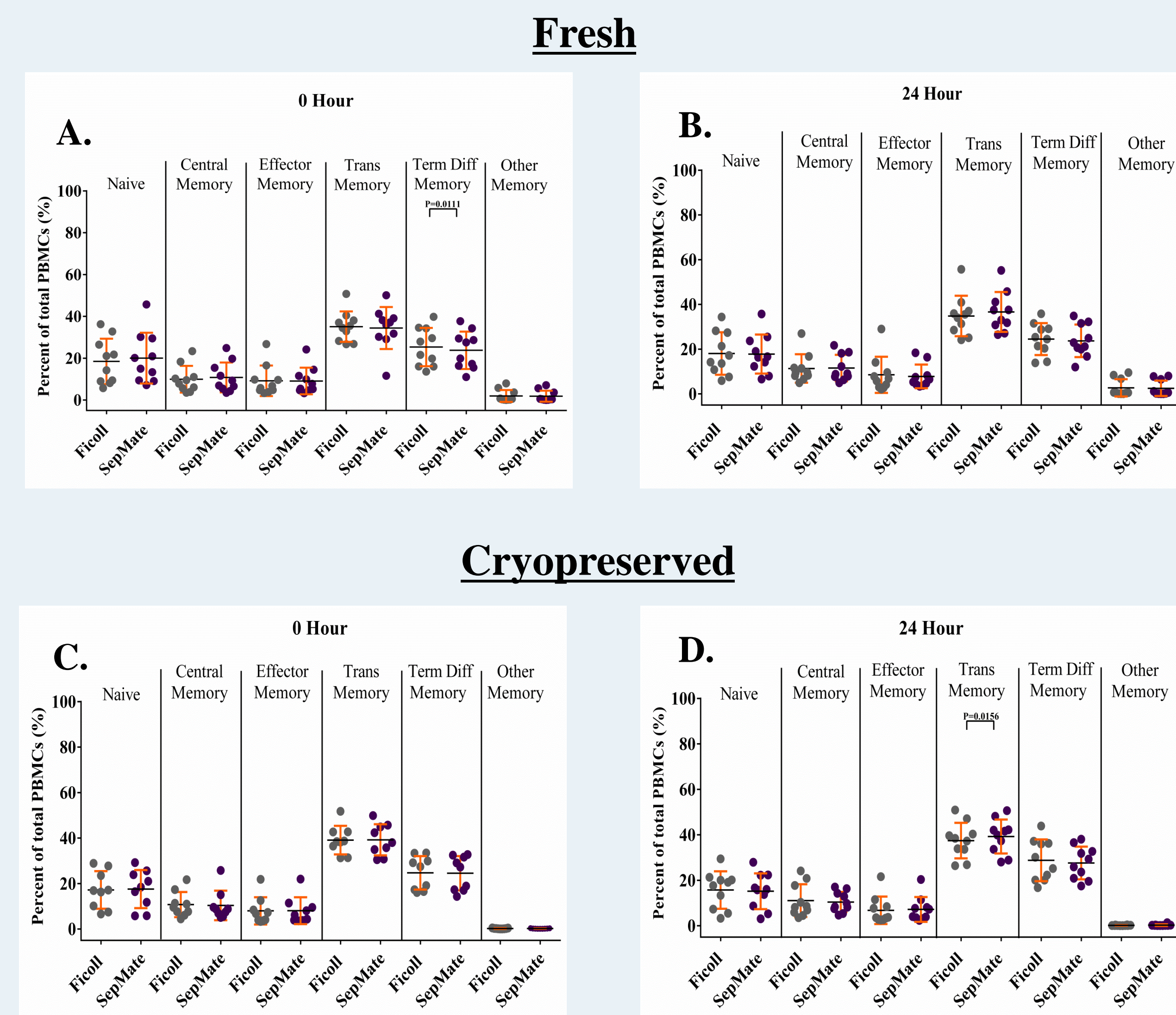


Figure 1. Paired comparison of CD4⁺ Memory T-cell subset frequencies in both freshly and cryopreserved PBMCs isolated by Ficoll and SepMate methods measured by flow cytometry. Overall, there was no significant difference in subset frequencies in PBMCs isolated using both methods in fresh or cryopreserved cells ($p > 0.05$ for nearly all comparisons).

ELISPOT

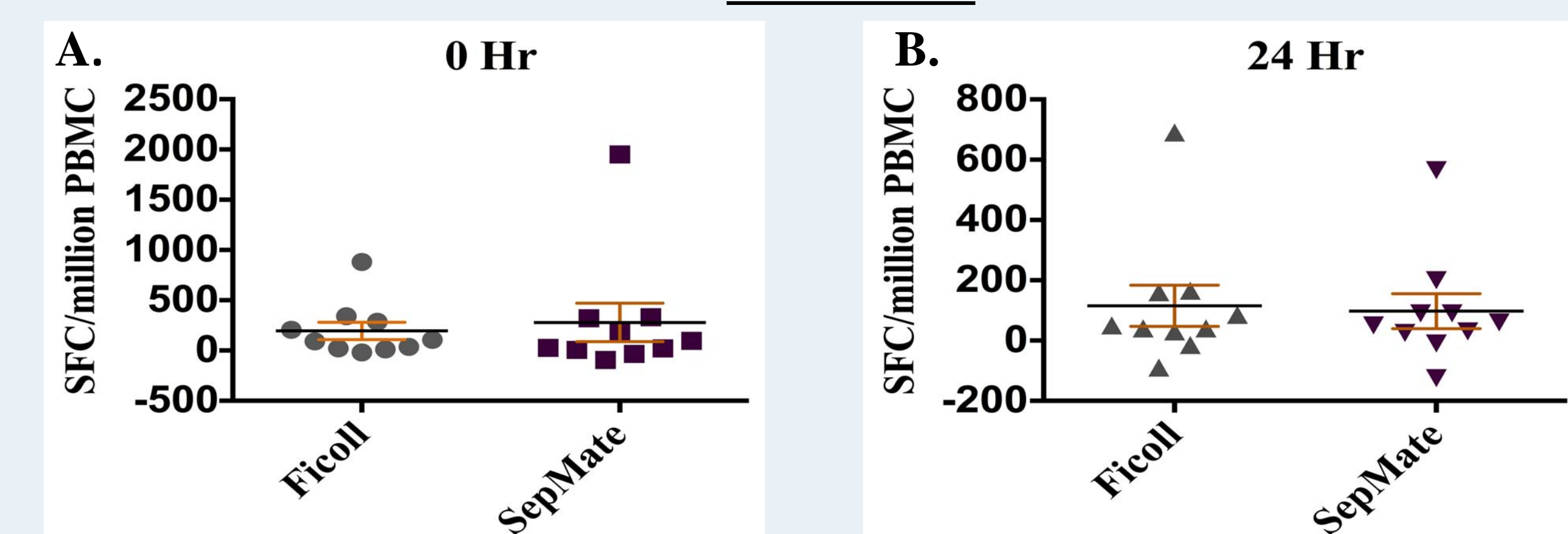


Figure 2. Comparison of IFN- γ SFC in cryopreserved PBMCs isolated by Ficoll and SepMate methods. T-cell function was measured by IFN- γ response to FEC antigen pool using ELISPOT. No differences were observed in T-cell responses of PBMCs isolated immediately and 24 hours after draw ($p > 0.05$).

PBMC Viability

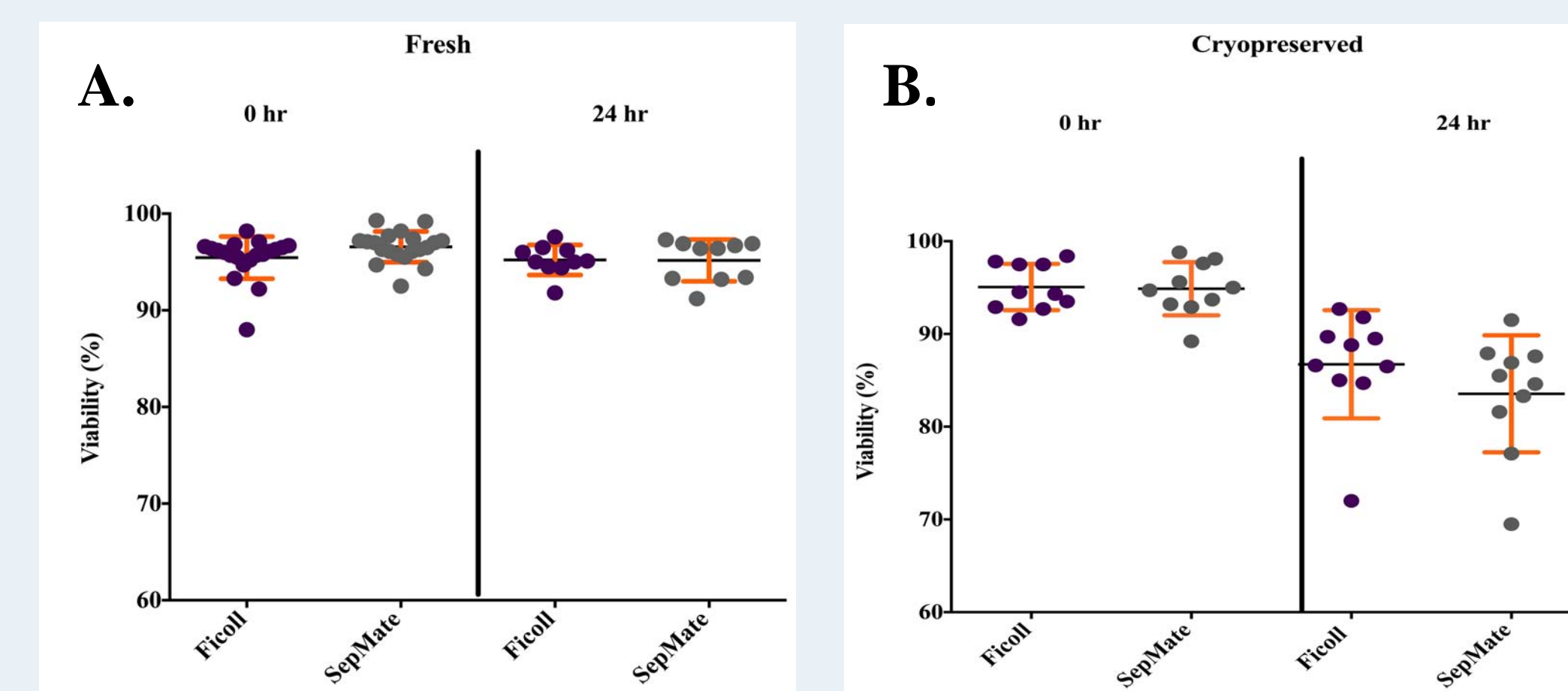


Figure 3. Mean viability of paired PBMCs isolated immediately after phlebotomy and after 24 hours using traditional Ficoll and SepMate methods (A). Mean viability post cryopreservation of paired PBMCs isolated immediately after phlebotomy and after 24 hours (B).

PBMC Recovery

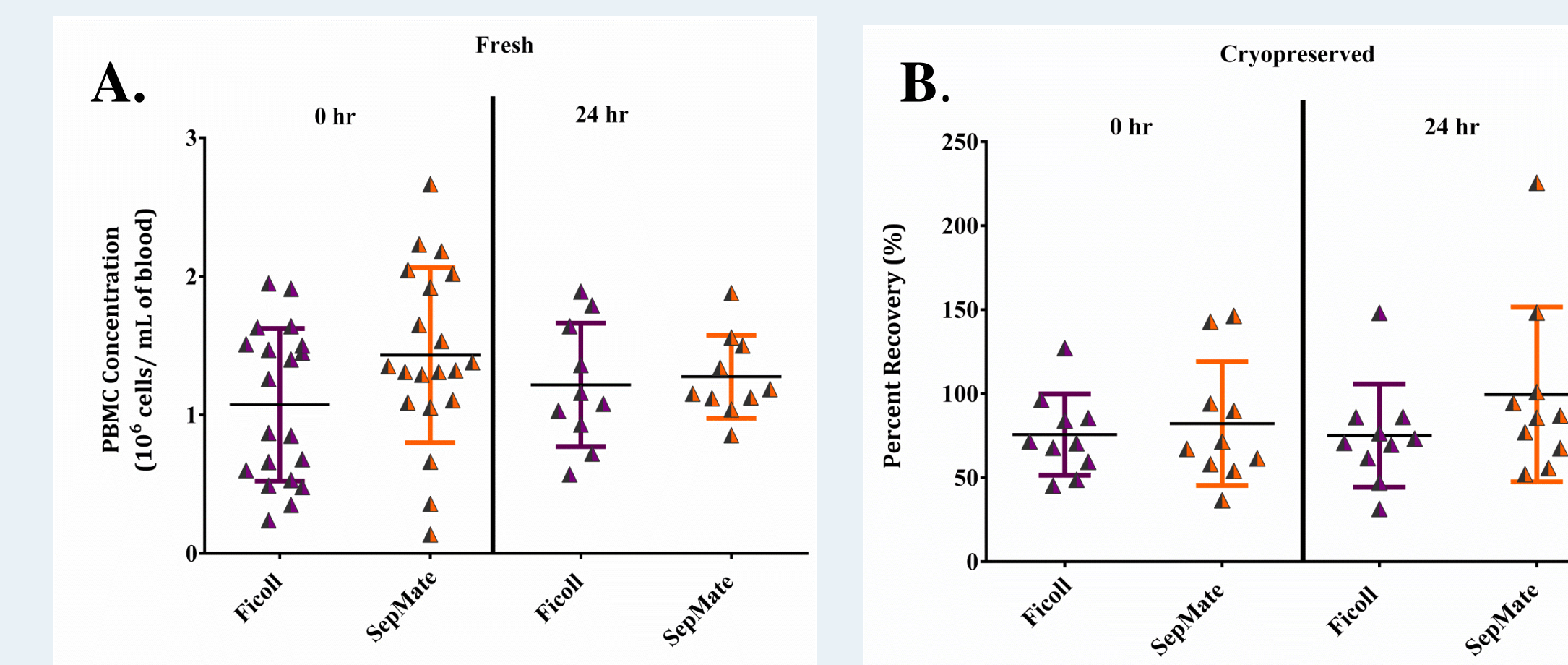


Figure 4. Mean recovery of paired PBMCs isolated immediately after phlebotomy and after 24 hours using traditional Ficoll and SepMate methods (A). Mean recovery post cryopreservation of paired PBMCs isolated immediately after phlebotomy and after 24 hours (B).

Leukocyte Subset Frequencies

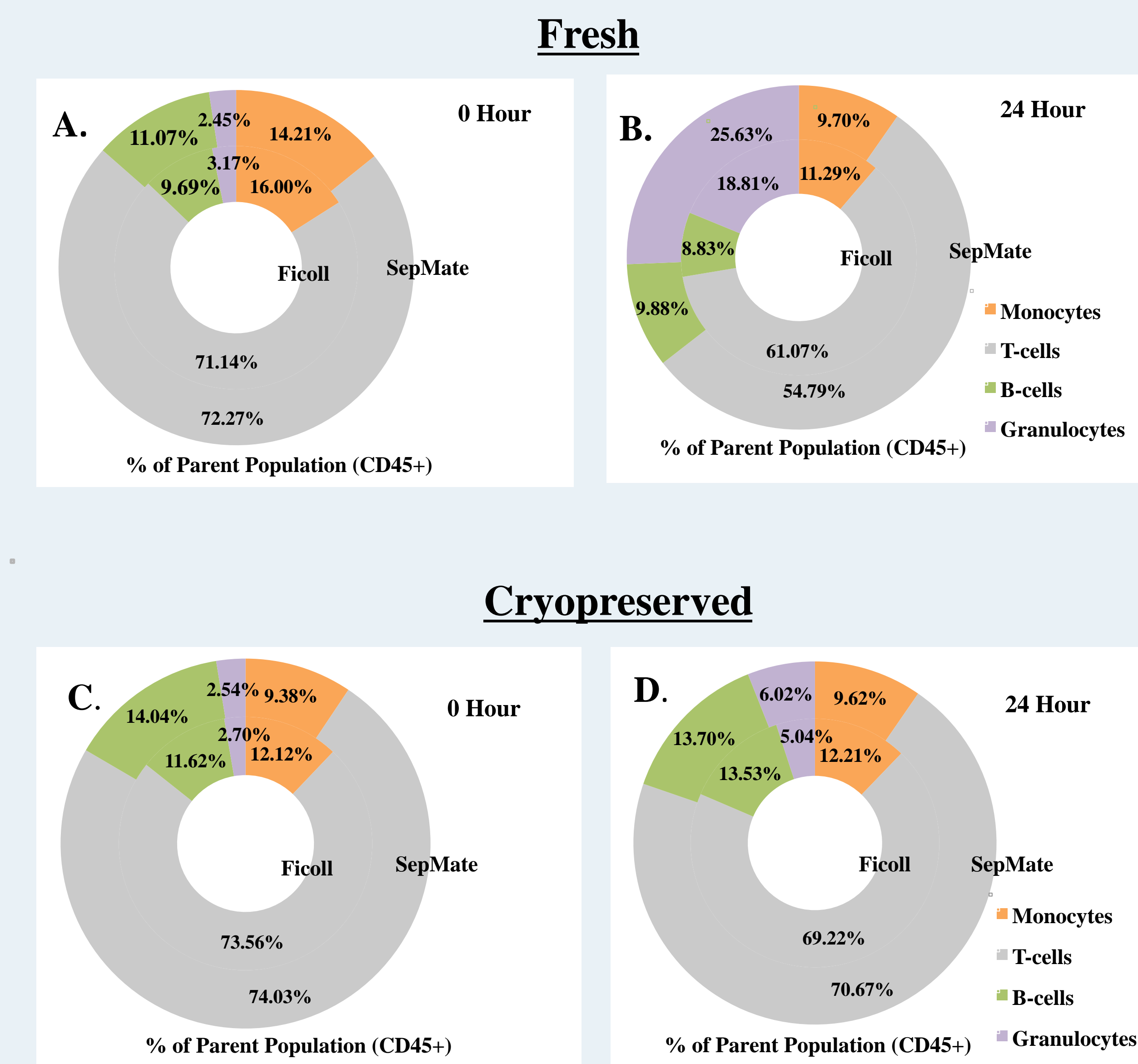


Figure 5. Comparison of Leukocyte subset frequencies of PBMCs isolated by Ficoll and SepMate methods measured by flow cytometry. There were no significant difference between subset frequencies when comparing both methods ($p > 0.05$).

PBMC Viability

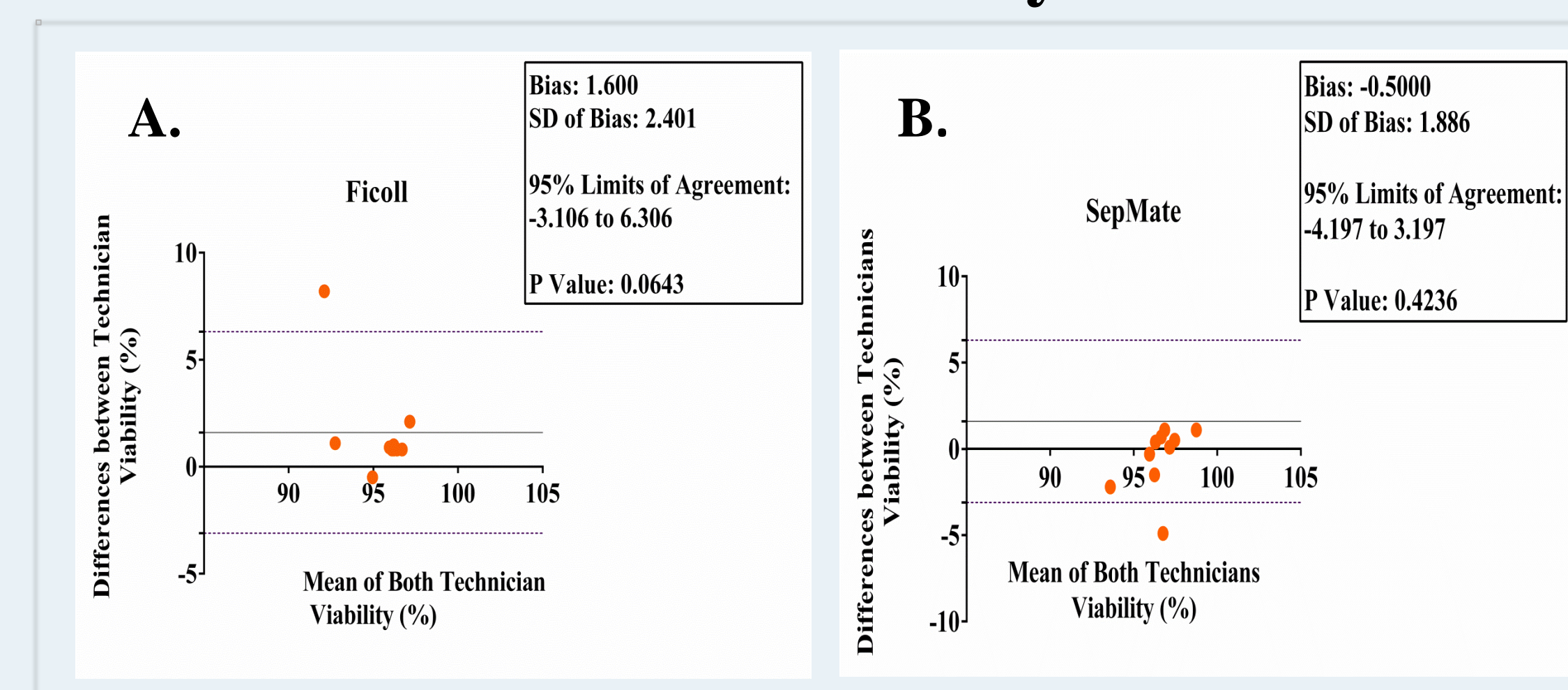


Figure 6. Bland-Altman Plot of paired Ficoll (A) and SepMate (B) PBMC isolation viability agreement. The mean viability of both technicians was plotted against the viability difference. Both methods showed insignificant variability between technicians ($p > 0.05$).

Inter-Technician Comparison

PBMC Yield

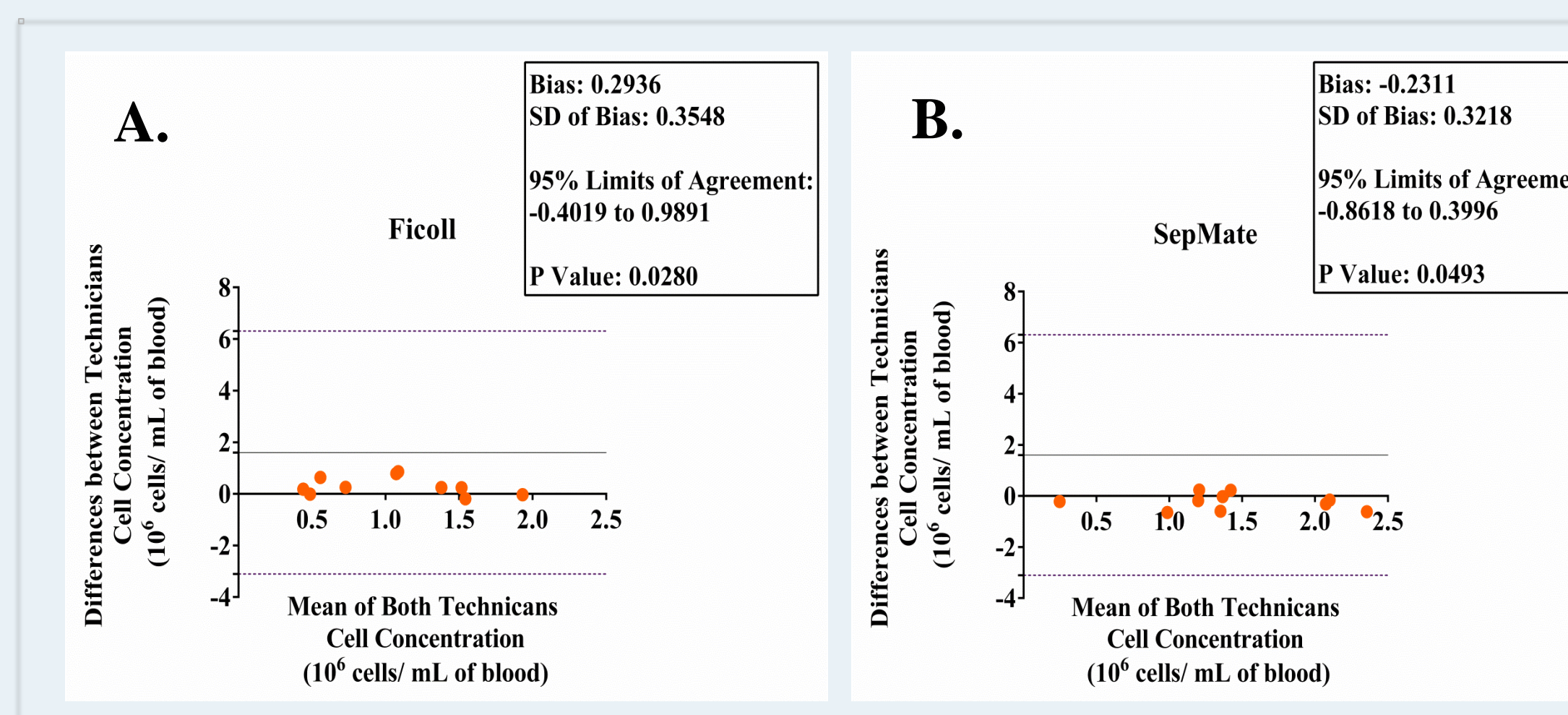


Figure 8. Bland-Altman Plot of paired Ficoll (A) and SepMate (B) PBMC yield agreement. The mean PBMC yield of both technicians was plotted against the viability difference. The SepMate method showed insignificant variability between technicians ($p > 0.05$).

Results

- Viability in both standard Ficoll vs. SepMate method were similar ($p > 0.05$).
- Yield of PBMCs using both methods was comparable ($p > 0.05$).
- Recovery of PBMCs post-cryopreservation from samples processed immediately after phlebotomy and after 24 hours was equivalent, with slightly higher yields in freshly processed blood using SepMate tubes.
- Memory CD4⁺ subset frequencies were the same using both methods (nearly all parameters measured $p > 0.05$).
- Leukocyte subset frequencies were comparable using both methods in all parameters measured ($p > 0.05$ for all comparisons).
- Overall there was no difference in the inter-technician variability of yield or viability.
- No differences were observed in T-cell responses of PBMCs isolated in both methods ($p > 0.05$).

Conclusions

- Isolation methods using SepMate tubes performed comparably to traditional Ficoll methods in all parameters measured.
- Use of SepMate tubes reduce the time and care needed for optimal PBMC recovery.

Acknowledgments

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