

A specialized tube to make RosetteSep™ enrichment of specific cell subsets faster and easier

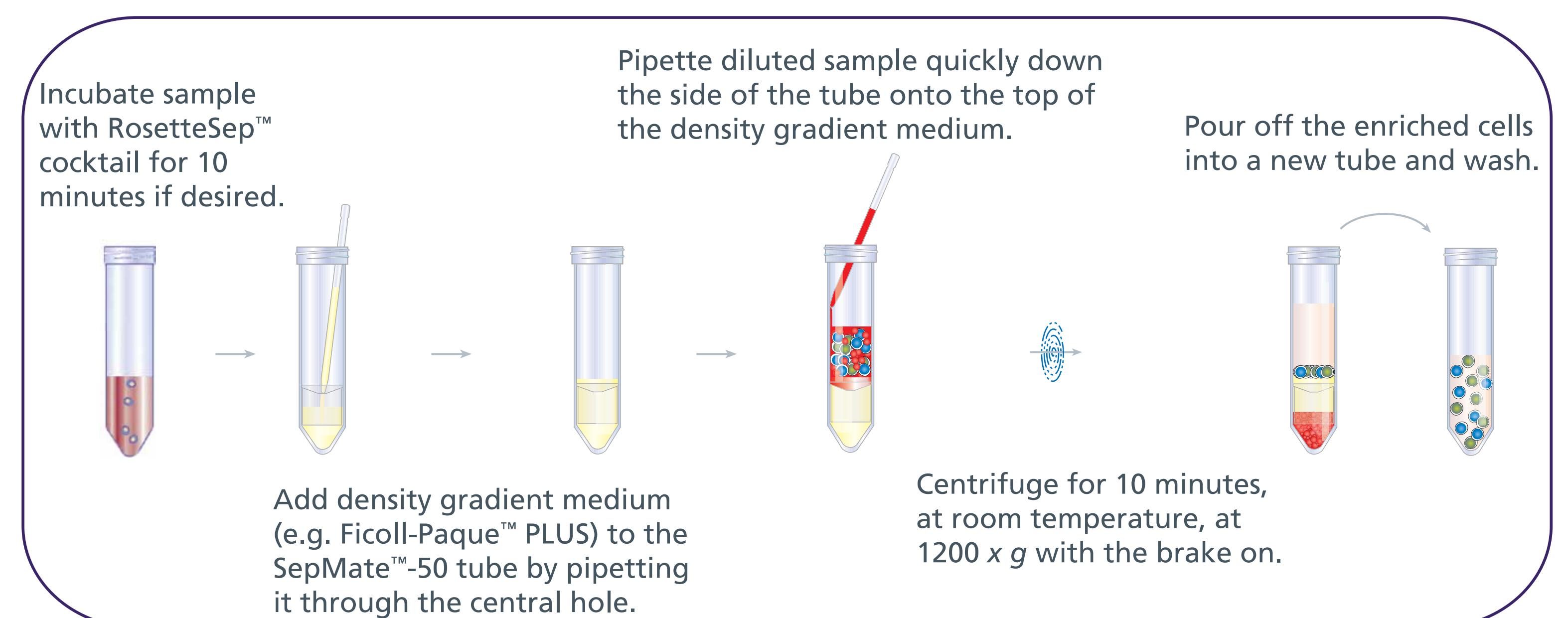
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Abstract

Many experimental protocols require the enrichment of specific cell subsets from peripheral blood. RosetteSep™ cell enrichment and standard MNC preparation both involve density gradient centrifugation, which entails slowly layering the sample over the density gradient medium to avoid mixing, and careful pipetting to remove the enriched cells after centrifugation. Centrifugation must be performed with the brake off to avoid disturbing the enriched cell layer, further lengthening the process. SepMate™, a centrifugation tube with a specialized insert, was developed to allow rapid layering of the sample on the density gradient medium and pouring off of the enriched cells after centrifugation, thus simplifying the entire process and making RosetteSep™ cell enrichment faster and easier. RosetteSep™ enrichments of mononuclear cell subsets using the SepMate™ tubes and protocol gave equivalent purity and recovery of desired cells compared to using the standard RosetteSep™ protocol, and desired cells could be enriched from whole blood in ~36 min. The protocol is easily scalable to process multiple samples simultaneously, and the SepMate™ tube can also be used to prepare MNCs.

Method



Results

FIGURE 1: Cell enrichment using RosetteSep™ alone or RosetteSep™ and SepMate™

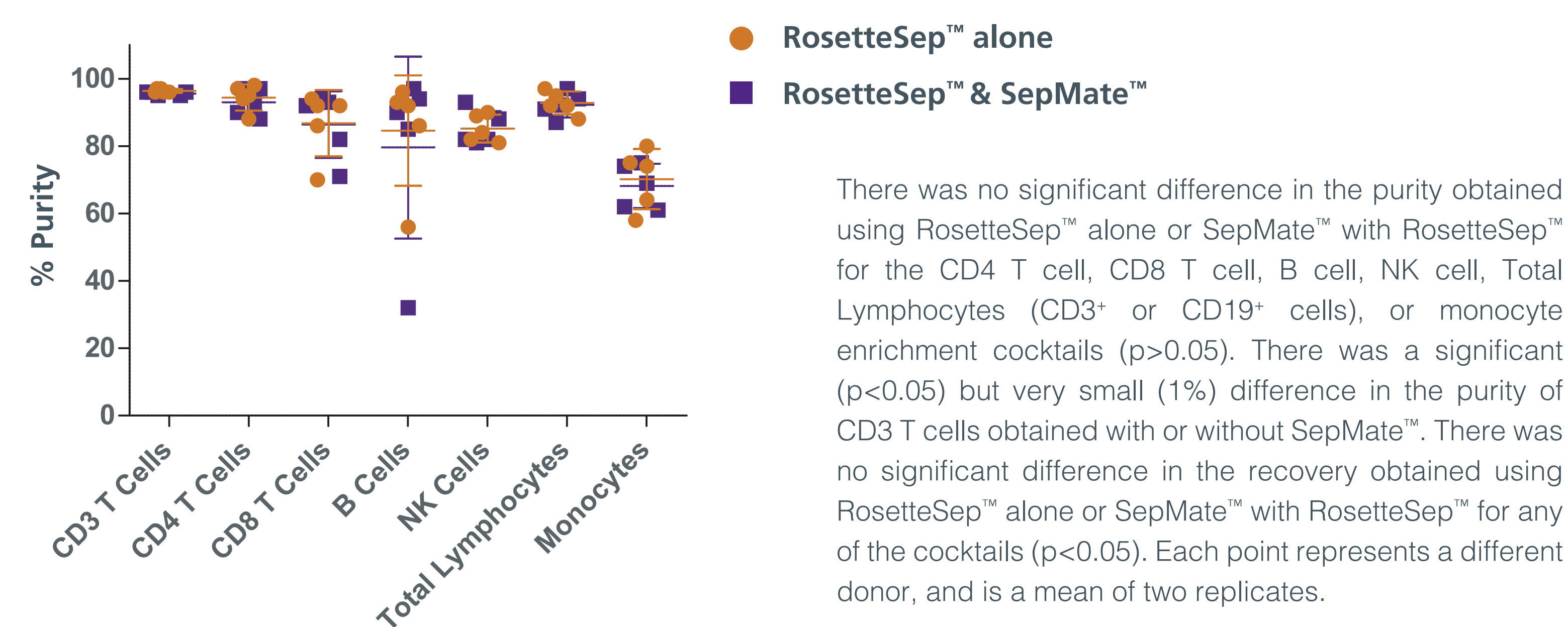
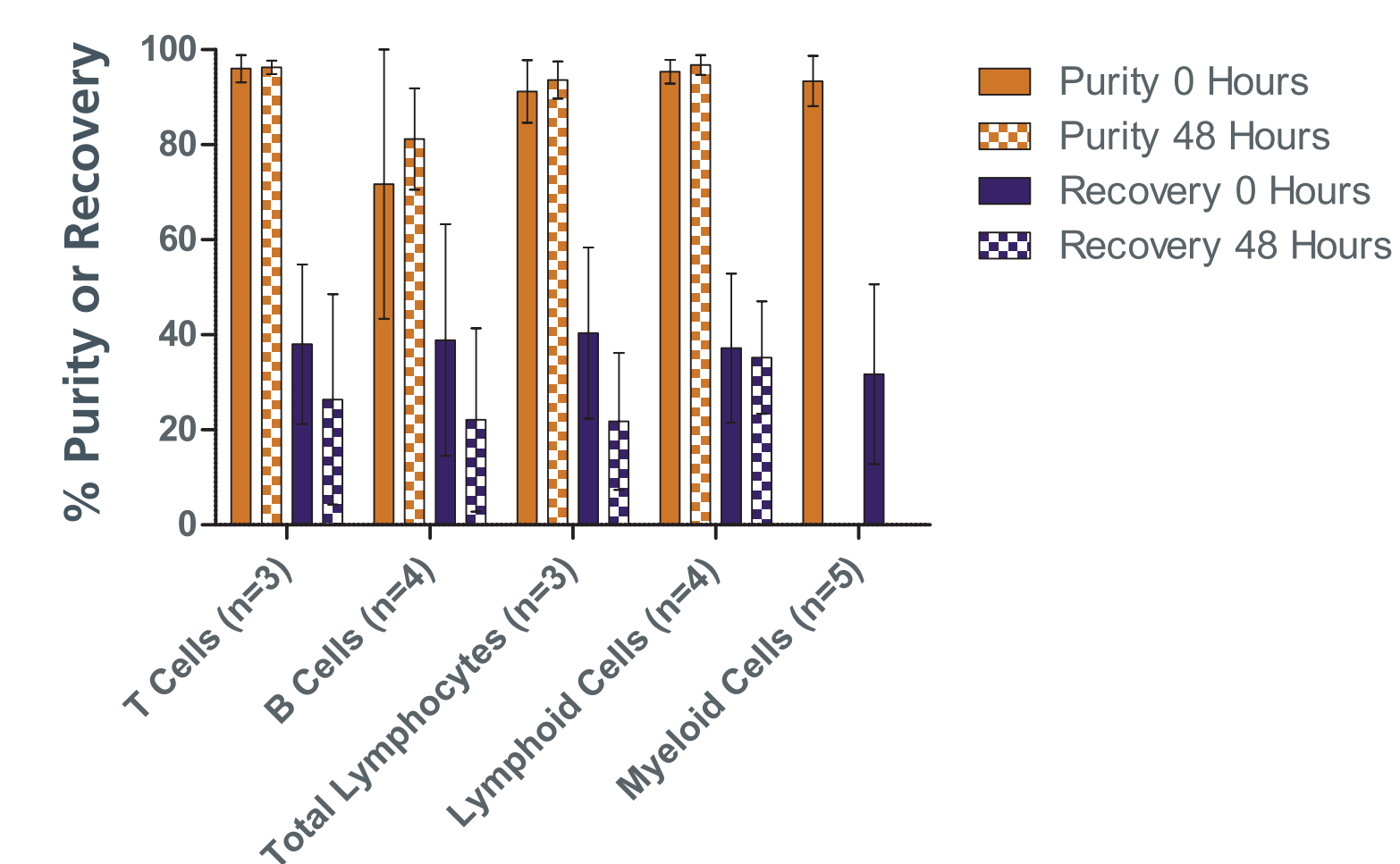


FIGURE 2: Effect of sample age on cell enrichment using RosetteSep™ and SepMate™



Blood samples were collected in heparin and specific cell subsets were enriched immediately ("0 Hours") or after storage at room temperature for 48 hours ("48 Hours"). The purity and recovery of T cells, B cells, Total Lymphocytes (CD3+ + CD19+ cells), and Lymphoid Cells (CD3+ cells, enriched using RosetteSep™ DM-L instead of Ficoll-Paque PLUS) did not differ significantly at 0 vs. 48 hours ($p < 0.05$). Myeloid cells (CD33+, enriched using RosetteSep™ DM-M) were enriched at 0 hours but could not be successfully enriched in 3/5 samples at 48 hours.

FIGURE 3: Effect of sample age on recovery of peripheral blood mononuclear cells (PBMCs) using SepMate™

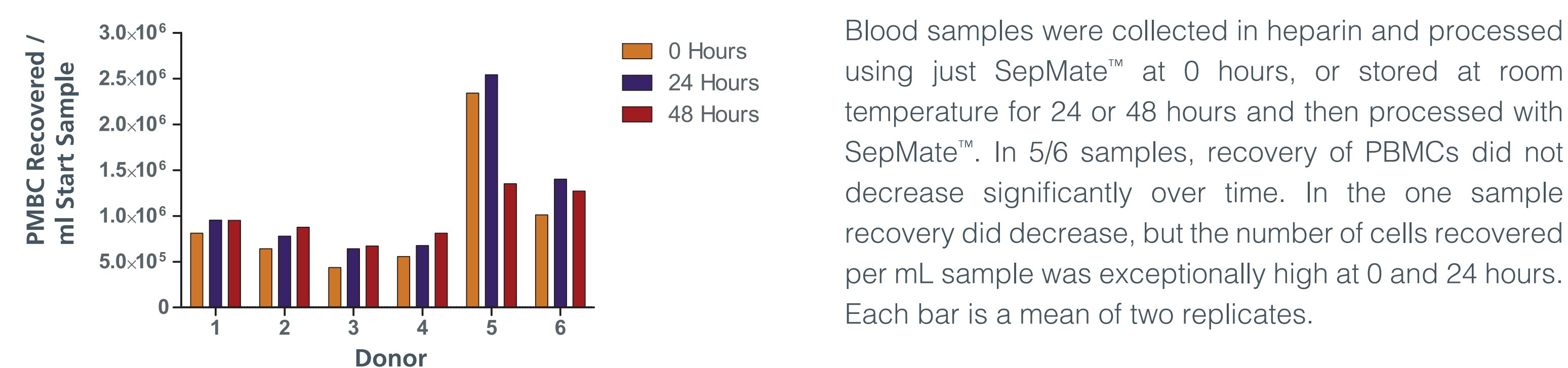
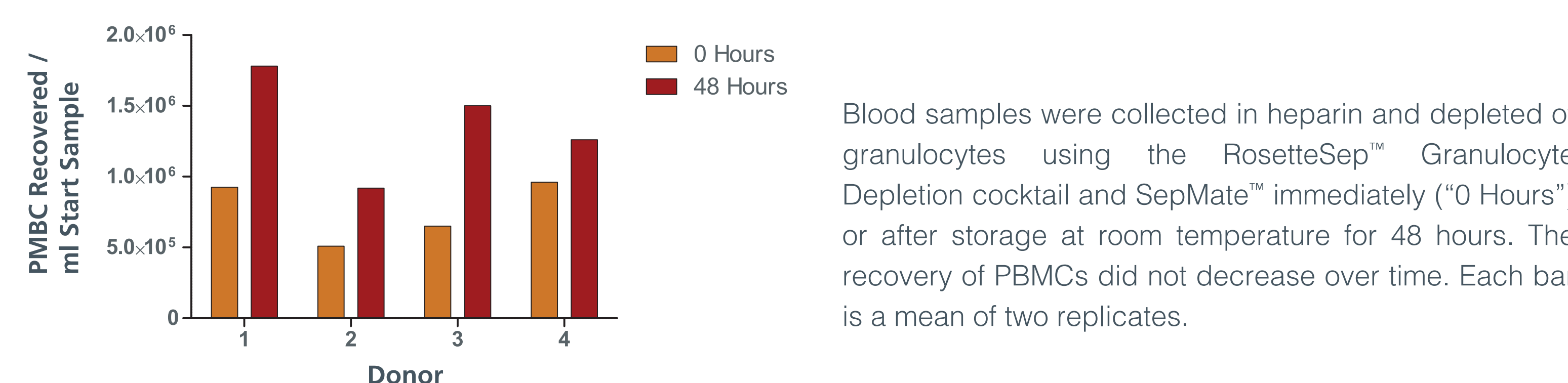


FIGURE 4: Effect of sample age on recovery of peripheral blood mononuclear cells (PBMCs) using RosetteSep™ Granulocyte Depletion and SepMate™



Conclusions

- **SepMate™ can be used with RosetteSep™ to isolate specific cell subsets directly from whole blood using in ~36 minutes.**
- **Purity and recovery of specific cell subsets isolated directly from whole blood using SepMate™ and RosetteSep™ are similar to those obtained with RosetteSep™ alone.**
- **Lymphocytes subsets (T Cells, B Cells, Total Lymphocytes, Lymphoid Cells) can be enriched from samples as old as 48 hours without a significant decrease in purity or recovery.**
- **Myeloid (CD33+) cells can be enriched from fresh samples, but cannot be consistently enriched from 48-hour old samples, under the collection and storage conditions used.**
- **SepMate™ can be used to isolate PBMCs from whole blood in < 30 minutes, including washes.**
- **The recovery of PBMCs does not significantly decrease if the sample is 24 or 48 hours old compared to the same fresh sample, in 5/6 samples.**
- **Granulocytes can be depleted using RosetteSep™ and SepMate™ on samples up to 48 hours old.**

*Ficoll-Paque™ PLUS is a trademark of GE Healthcare.