Program #240

A specialized tube for rapid PBMC preparation, pre-enrichment, or isolation of specific cell populations

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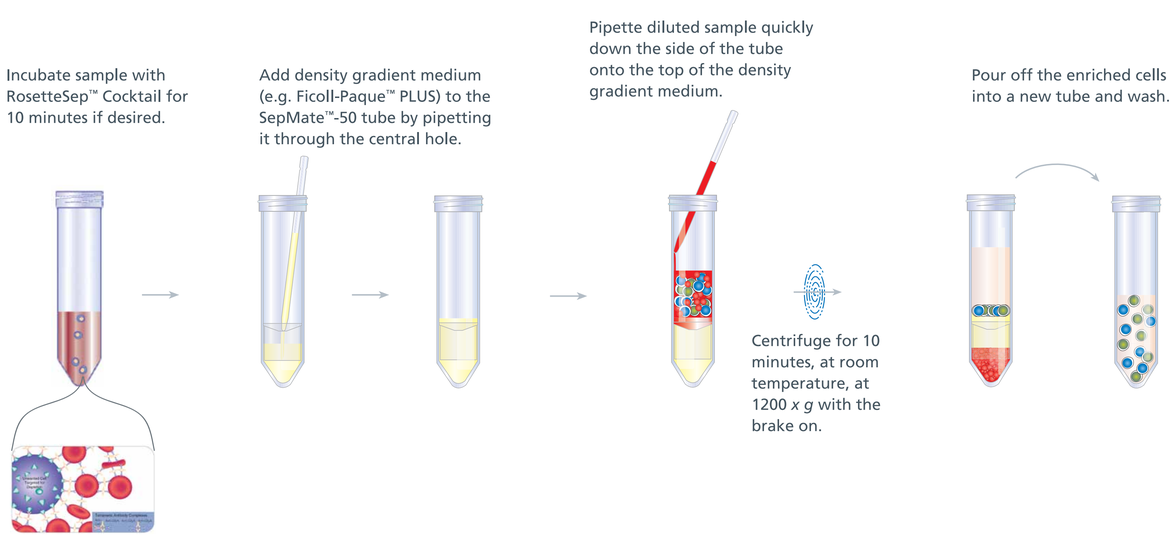
Introduction

Complex flow cytometric analysis of rare immune cells frequently requires peripheral blood mononuclear cell (PBMC) preparation or pre-enrichment of specific cell subsets (e.g. T cells, B cells, monocytes). PBMC preparation is accomplished using buoyant density separation, which is time-consuming and which entails careful layering of the sample over the buoyant density medium to avoid mixing, centrifugation with the brake off to avoid disturbing the density medium : plasma interface, and careful removal of the cell layer. If specific cell subsets are to be enriched, PBMC preparation is followed by magnetic cell selection. Alternatively, specific cell enrichment can be accomplished in a single step using RosetteSep™, which cross-links unwanted cells in whole blood directly to red blood cells (RBCs) present in the sample. The sample is then layered over a buoyant density medium and spun; the cells cross-linked to RBCs pellet along with free RBCs and granulocytes, while the unlabeled, desired cells are collected at the density medium : plasma interface. Although this process is faster than the two-step approach of isolating PBMCs and then the desired cell type, it does not obviate the time nor care required for buoyant density separation. SepMate™, a centrifugation tube with a specialized insert, was developed to minimize mixing of the sample and the buoyant density medium, thereby avoiding the need for careful layering and careful cell removal from the interface. Buoyant density medium is pipetted through a central hole in the insert, partially filling the tube. Either a whole blood sample, or a sample pre-incubated with RosetteSep™ for 10 minutes, is rapidly pipetted down the side of the tube to rest upon the buoyant density medium. After centrifugation for 10 minutes with the brake on, the enriched cell layer is simply poured off into a new tube, while the buoyant density medium, erythrocytes, granulocytes, and any cells targeted for depletion by RosetteSep™ are retained below the insert. The PBMCs or specific cell subsets are washed once and are then ready for use. We compared the isolation of specific cell subsets using this approach to RosetteSep™ cell enrichment using the "standard protocol" (incubation with RosetteSep™ for 20 minutes, standard centrifuge tubes, 20 minute spin with the brake off). The purities and recoveries obtained using SepMate™ were equivalent to those obtained using the standard protocol, and required 25 minutes less. SepMate™ can be used to prepare PBMCs or specific cell subsets rapidly and easily directly from whole blood. The protocol is easily scalable to process multiple samples simultaneously.

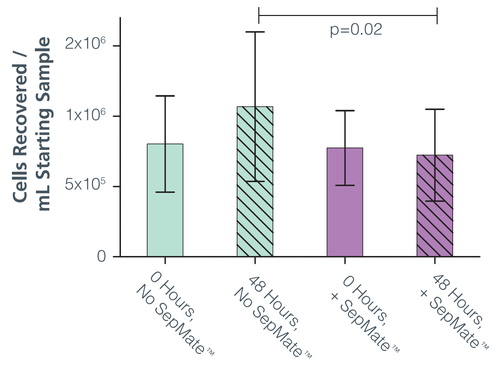
Method

FIGURE 1: Specific cell enrichment using RosetteSep™ and SepMate™



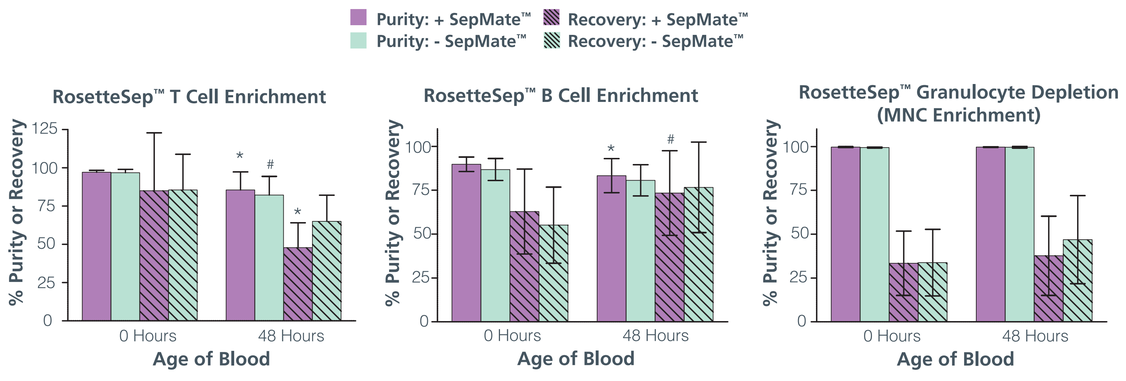
Results

FIGURE 2: Mononuclear cell recovery after buoyant density centrifugation with and without SepMate™ at 0 and 48 hours after blood draw



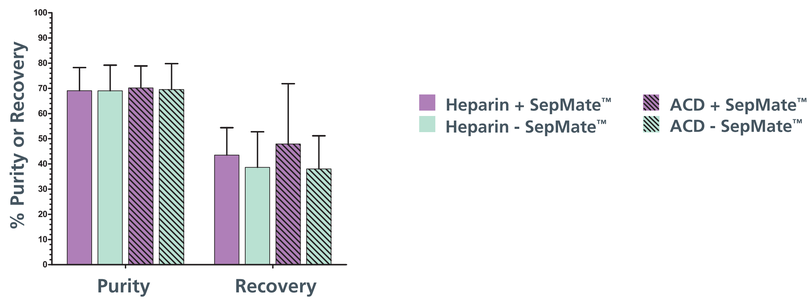
Blood samples were centrifuged over Ficoll immediately after blood draw or 48 hours later, either in a standard centrifuge tube or in a SepMate™ tube. The number of mononuclear cells (defined as $CD66b^-CD45^+$) recovered per mL of starting whole blood was calculated. Each sample was tested in duplicate under each condition and at each time point (n=7 different samples; paired t-test p=0.02).

FIGURE 3: Purity and recovery of T, B, and mononuclear cells with and without SepMate™ at 0 and 48 hours after blood draw



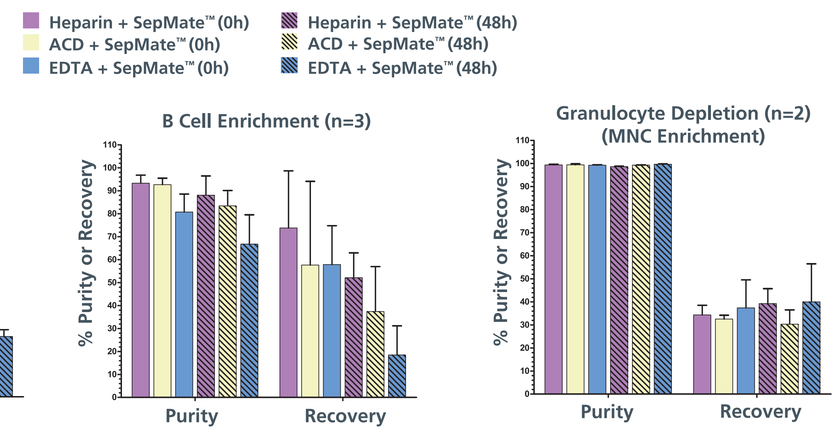
Blood was collected in heparin, incubated with the T cell, B cell, or granulocyte depletion RosetteSep™ cocktail for 10 min (with SepMate™) or 20 min (no SepMate™), and centrifuged over Ficoll either in a standard centrifuge tube or in a SepMate™ tube. The purity and recovery of T cells ($CD3^+CD45^+$), B cells ($CD19^+CD45^+$), or mononuclear cells ($CD66b^-CD45^+$) was determined. Each condition was tested in duplicate (n=6 different samples).
*p<0.05 in comparison to without SepMate™ for the same time point. #=p<0.05 compared to 0 hours.

FIGURE 4: Purity and recovery of monocytes with and without SepMate™ from samples collected in different anti-coagulants at 0 hours after blood draw



Blood was collected in heparin or ACD anti-coagulant, incubated with RosetteSep™ monocyte enrichment cocktail for 10 min (with SepMate™) or 20 min (no SepMate™), and centrifuged over Ficoll either in a standard centrifuge tube or in a SepMate™ tube. The purity and recovery of monocytes ($CD14^+CD45^+$) was determined. Each condition was tested in duplicate (n=5 different samples). There was no significant difference between groups in purity or recovery.

FIGURE 5: Purity and recovery of T, B, and mononuclear cells with SepMate™ from samples collected in different anti-coagulant at 0 and 48 hours after blood draw



Blood was collected in heparin, ACD, or EDTA anti-coagulant, and processed either immediately or after 48 hours storage at room temperature. Samples were incubated with the T cell, B cell, or granulocyte depletion RosetteSep™ cocktail for 10 min and centrifuged over Ficoll in a SepMate™ tube. The purity and recovery of T cells ($CD3^+CD45^+$, n=3), B cells ($CD19^+CD45^+$, n=3), or mononuclear cells ($CD66b^-CD45^+$, n=2) was determined. Each condition was tested in duplicate. There was a tendency for values to drop from 0 to 48 hours, although significance was not reached with such small samples. There was a tendency for values to be best with blood collected in heparin and worst with blood collected in EDTA, particularly at 48 hours after blood draw.

Conclusions

- **Use of the SepMate™ 50 tube reduces the time and care needed for buoyant density centrifugation ("ficolling") of whole blood.**
- **SepMate™ can be used to enrich mononuclear cells (MNC) from whole blood in ~25 min, including washes, up to 48 hours post blood-draw.**
- **SepMate™ can be used with RosetteSep™ cell enrichment cocktails to isolate T and B cells from whole blood, or to deplete granulocytes, in ~35 min including washes, up to 48 hours post blood-draw.**
- **SepMate™ can be used with RosetteSep™ to isolate monocytes from whole blood in ~35 min, including washes.**
- **T cells, B cells, and mononuclear cells can be isolated from blood collected in heparin, ACD or EDTA anti-coagulants up to 48 hours post blood-draw. There is a tendency for better purity and recovery from blood collected in heparin, particularly at 48 hours. There is a tendency for poorer purity and recovery from blood collected in EDTA.**

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