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

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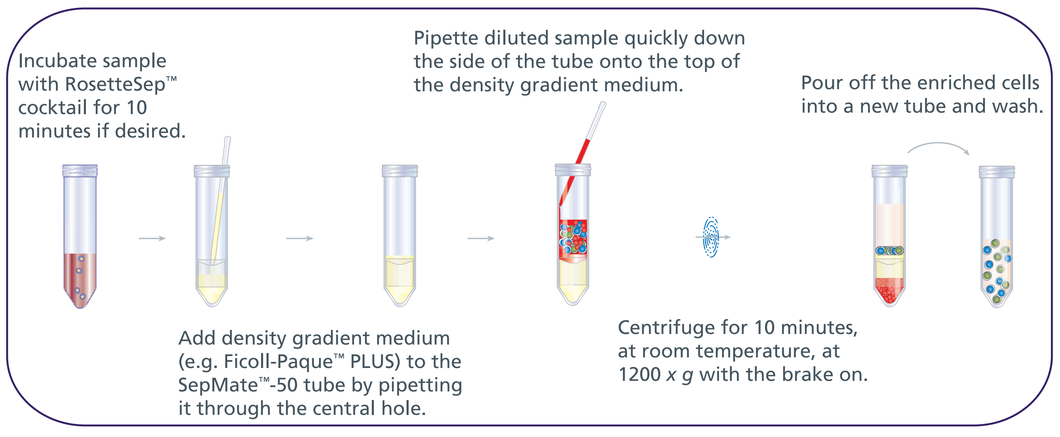
Introduction

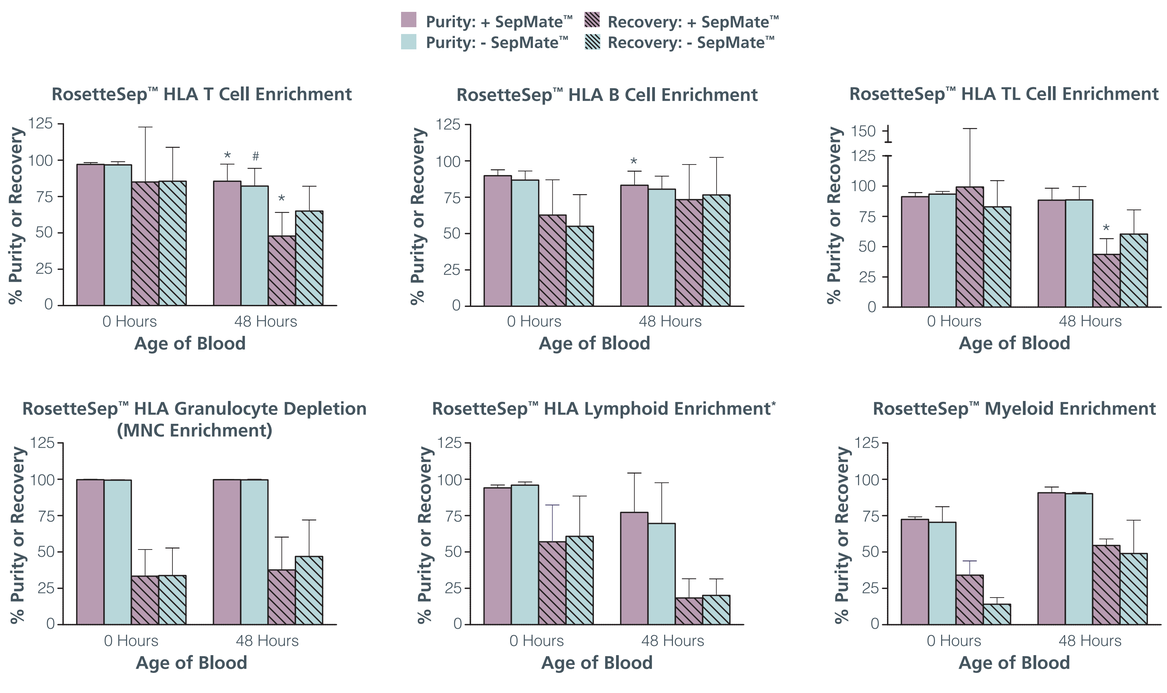
Sample analysis in HLA laboratories frequently requires enrichment of specific cell types, or at least preparation of mononuclear cells (MNCs). RosetteSep™ cell enrichment and standard MNC preparation both involve buoyant density centrifugation (density separation), which entails careful layering of the sample over the buoyant density 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, adding to the time required for the process. SepMate™-50, a centrifugation tube with a specialized insert, was developed to minimize mixing of the sample and the buoyant density medium. Buoyant density medium is pipetted through a central hole in the insert, partially filling the tube. The sample is incubated with RosetteSep™, diluted, and 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. The desired cells are washed and then are ready for use. We compared this procedure to RosetteSep™ cell enrichment using the "standard protocol" (standard centrifuge tubes, 20 minutes spin, brake off), and tested reducing the cocktail incubation time from 20 to 10 minutes. The purities and recoveries of cells enriched using various RosetteSep™ HLA Enrichment cocktails either with or without SepMate™-50 were evaluated, using blood that was fresh (0 hours) or 48 hours old. The effects of collecting blood in different anti-coagulants were also evaluated. Finally, the recovery of mononuclear cells (MNC) from whole blood centrifuged over a buoyant density medium with or without SepMate™-50 was compared.

Method



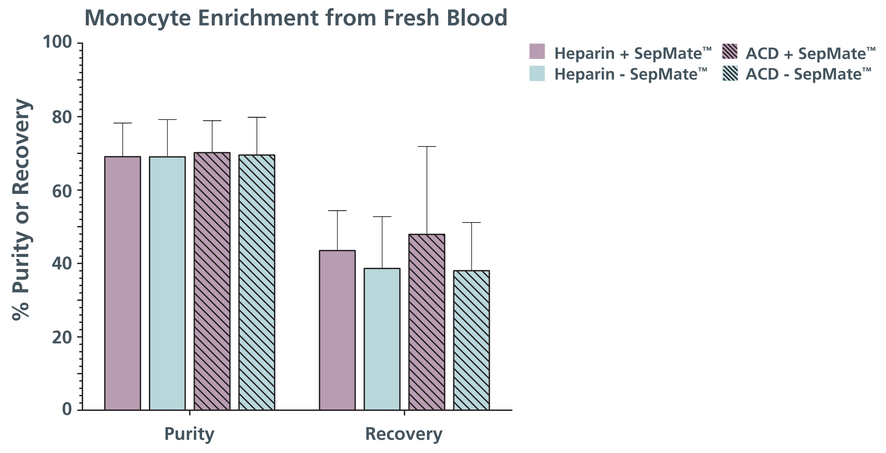
Results

FIGURE 1: Purity and recovery of cells enriched with RosetteSep™ with and without SepMate™-50 at 0 and 48 hours after blood draw



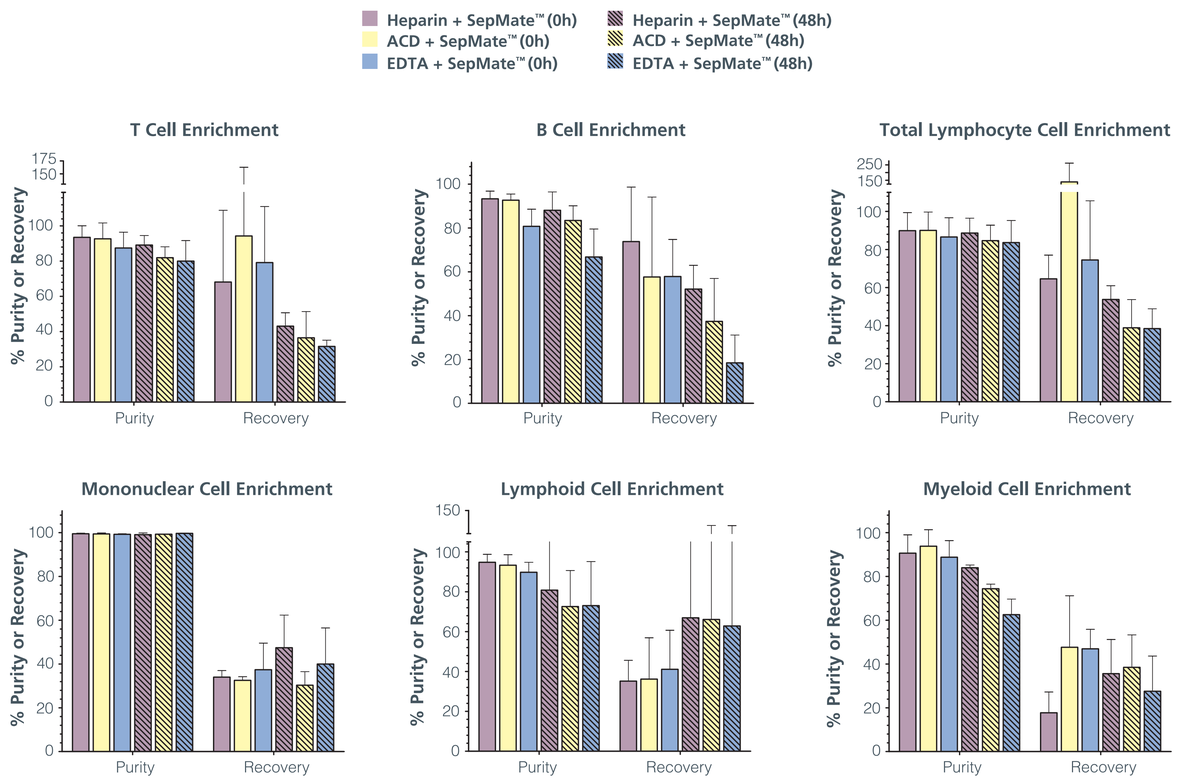
Blood was collected in heparin, incubated with RosetteSep™ cocktail for 10 min. (with SepMate™-50) or 20 min. (no SepMate™-50), and centrifuged over buoyant density medium either in a SepMate™-50 tube or a standard centrifuge tube. The purity and recovery of T cells ($CD3^+$ $CD45^+$), B cells ($CD19^+$ $CD45^+$), Total Lymphocytes (TL; $CD3^+$ or $CD19^+$, $CD45^+$), mononuclear cells ($CD66b^-$ $CD45^+$], Lymphoid Cells ($CD3^+$ $CD45^+$), or Myeloid Cells ($CD66b^+$ or $CD14^+$, $CD45^+$) was determined. Each condition was tested in duplicate; n=6 different samples for all cocktails except the myeloid cell enrichment (n=2).
*p<0.05 in comparison to without SepMate™-50 for the same time point; #p<0.05 compared to 0 hours.

FIGURE 2: Purity and recovery of monocytes with and without SepMate™-50 from samples collected in different anti-coagulants at 0 hours after blood draw



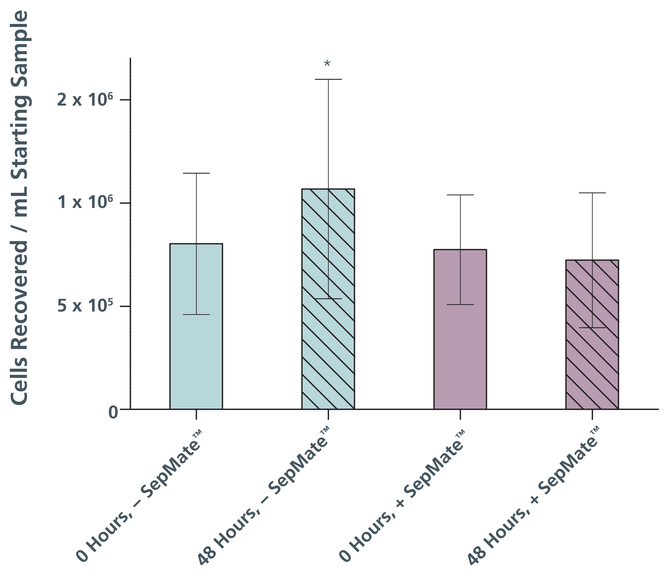
Blood was collected in heparin or ACD anti-coagulant, incubated with RosetteSep™ monocyte enrichment cocktail for 10 min. (with SepMate™-50) or 20 min. (no SepMate™-50), and centrifuged over Ficoll either in a standard centrifuge tube or in a SepMate™-50 tube. The purity and recovery of monocytes ($CD14^+$ $CD45^+$) was determined. Each condition was tested in duplicate (n=5 different samples). There was no significant difference between groups in purity or recovery.

FIGURE 3: Purity and recovery of T cells, B cells, Total Lymphocytes, Mononuclear Cells (MNC), Lymphoid Cells, and Myeloid Cells with SepMate™ from samples collected in different anti-coagulants 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 RosetteSep™ cocktail for 10 min. and centrifuged over the appropriate buoyant density medium in a SepMate™-50 tube for 10 min., with the brake on. The purity and recovery of T cells ($CD3^+$ $CD45^+$, n=3), B cells ($CD19^+$ $CD45^+$, n=3), Total Lymphocytes (TL; $CD3^+$ or $CD19^+$, $CD45^+$, n=3], mononuclear cells ($CD66b^-$ $CD45^+$, n=2), Lymphoid Cells ($CD3^+$ $CD45^+$, n=4), or Myeloid Cells ($CD33^+$ $CD45^+$ or ($CD66b^-$ or $CD14^+$, $CD45^+$), n=2) was determined. Each condition was tested in duplicate. There was a tendency for values to drop from 0 to 48 hours. There was a tendency for values to be best with blood collected in heparin and worse with blood collected in EDTA, particularly at 48 hours after blood draw.

FIGURE 4: Mononuclear cell recovery after buoyant density centrifugation with and without SepMate™-50 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™-50 tube. The number of mononuclear cells (defined as $CC66b^-$ $CD45^+$) 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.
* p=0.02 in paired t-test compared to 48 hours + SepMate™-50.

Conclusions

- **Use of the SepMate™-50 tube reduces the time and care needed for buoyant density centrifugation ("ficolling") of whole blood.**
- **The SepMate™-50 tube can be used with RosetteSep™ cell enrichment cocktails to isolate specific cells from whole blood in ~35 min., including washes, up to 48 hours post blood-draw.**
- **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.**
- **The SepMate™-50 tube can be used to enrich mononuclear cells (MNC) from whole blood in ~25 min., including washes, up to 48 hours post blood-draw.**

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