A Specialized Tube to Make Enrichment of Hematopoietic Progenitors Faster and Easier

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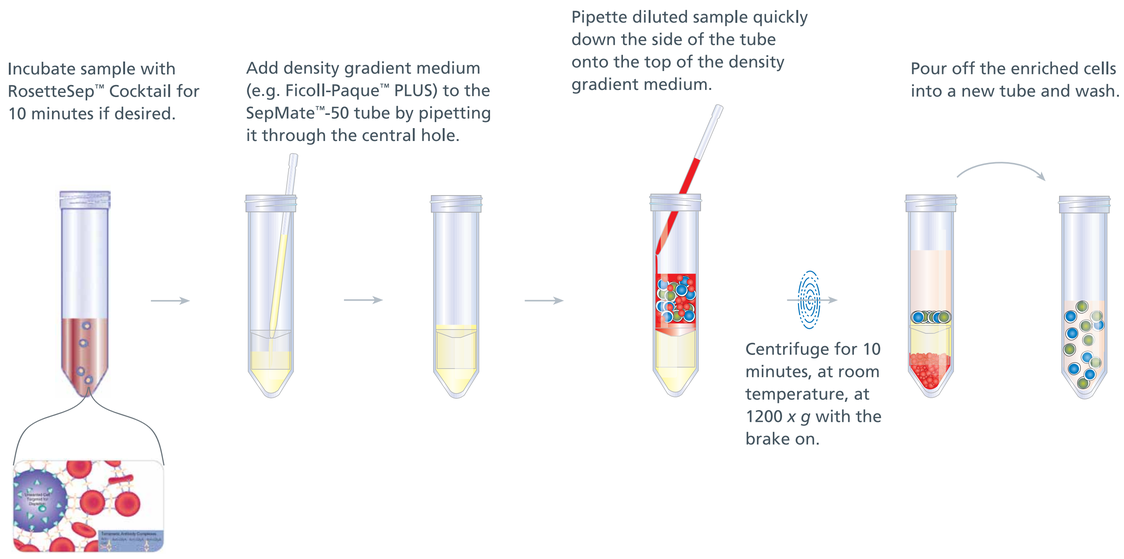
Abstract

Many experimental protocols begin with the enrichment of specific cell subsets from whole blood or cord blood. RosetteSep™ is an immunodensity-based method of cell enrichment, in which unwanted cells are directly cross-linked to red blood cells (RBCs) present in the sample. The sample is then centrifuged over a density gradient medium, and the RBCs and the unwanted cells linked to them pellet, while the desired cells are collected at the plasma : density gradient medium interface. However, density gradient centrifugation entails slowly layering the sample over the density gradient medium to avoid mixing, and carefully pipetting to remove the enriched cells after centrifugation. Centrifugation must be performed with the brake off to avoid disturbing the enriched cell layer. The process, including washes, requires 65 minutes. SepMate™, a centrifugation tube with a specialized insert, was developed to allow rapid layering of the sample onto the density gradient medium, and pouring off of the enriched cells after centrifugation, thus simplifying the density gradient centrifugation step. Total mononuclear cell recovery from whole blood (cells/mL) is similar with ($7.7 \pm 2.7 \times 10^5$) and without ($8.0 \pm 3.4 \times 10^5$) the SepMate™ tube (n=7, p = 0.7). We tested the use of the SepMate™ tube for isolation of hematopoietic progenitors from cord blood using the RosetteSep™ Human Cord Blood Progenitor Cell Enrichment Cocktail, which depletes cells expressing CD2, CD3, CD14, CD16, CD19, CD24, CD56, CD66b, or glycophorin A. To further shorten the process, we also tested decreasing the RosetteSep™ cocktail incubation time from 20 to 10 minutes. The enriched cells were evaluated for $CD34^+$ and colony-forming unit (CFU) cell enrichment and recovery (n=5). There was no significant difference in any of the parameters among the three groups: RosetteSep™ (20 min.) alone, RosetteSep™ (20 min.) + SepMate™, and RosetteSep™ (10 min) + SepMate™ (n=5, p>0.05) although there was a trend towards higher $CD34^+$ cell and CFU recovery when the sample was incubated with the cocktail for 20 minutes prior to centrifugation within the SepMate™ tube. The use of the SepMate™ tube simplifies the RosetteSep™ procedure and reduces the time needed for the whole procedure to only 35-45 minutes, depending on the cocktail incubation time used. The protocol is easily scalable to process multiple samples simultaneously and can also be used with RosetteSep™ cocktails for isolating other cell types from whole blood.

Methods

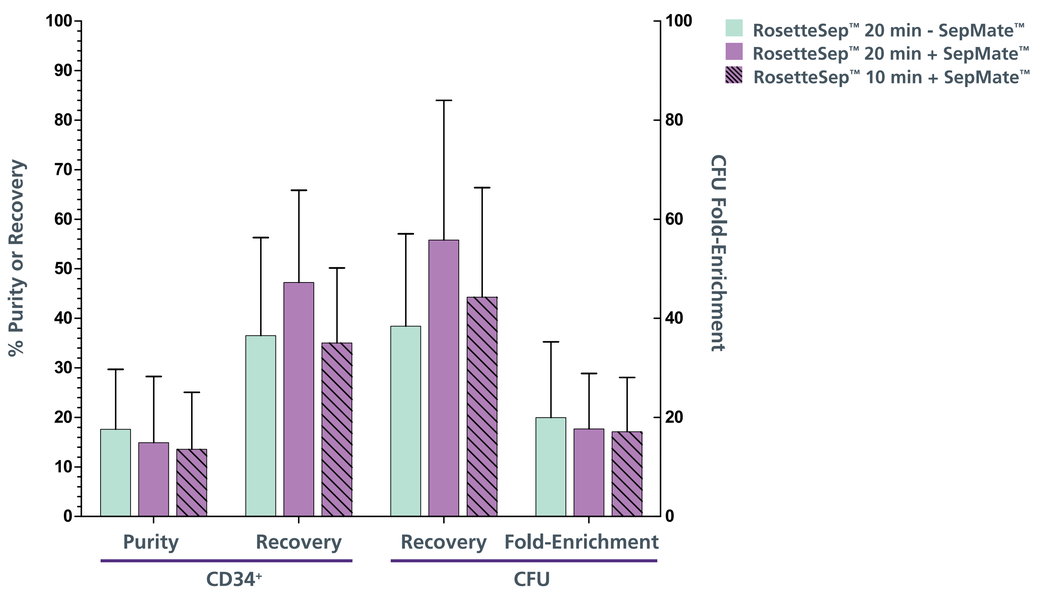
FIGURE 1: Method of enrichment of hematopoietic progenitor cells from cord blood using RosetteSep™ and SepMate™



Hematopoietic progenitor cells were enriched from cord blood by incubation with the RosetteSep™ Human Cord Blood Progenitor Cell Enrichment Cocktail for 10 or 20 minutes, and then by buoyant density centrifugation either in a standard tube or using SepMate™. Enriched cells were manually counted using a haemocytometer. Samples were evaluated for $CD34^+$ purity by flow cytometry, and for CFU content using the 14-day CFU assay and MethoCult™ H4034 (STEMCELL Technologies Inc.) using standard procedures.

Results

FIGURE 2: Enrichment of hematopoietic progenitor cells from cord blood using RosetteSep™ and SepMate™



Hematopoietic progenitor cells were enriched from cord blood by incubation with the RosetteSep™ Human Cord Blood Progenitor Cell Enrichment Cocktail for 10 or 20 minutes, and then by buoyant density centrifugation either in a standard tube or using SepMate™. Values are means ± 1 standard deviation; n=5. There are no significant differences between the groups.

Conclusions

- **Total mononuclear cell (MNC) recovery from cord blood by density gradient centrifugation is similar with ($7.7 \pm 2.7 \times 10^5$) and without ($8.0 \pm 3.4 \times 10^5$) the SepMate™ tube (n=7, p = 0.7; data not shown).**
- **SepMate™ can be used with the RosetteSep™ Human Cord Blood Progenitor Enrichment Cocktail to achieve 5 – 25 fold enrichment of $CD34^+$ cells and 5 – 34 fold enrichment of CFU from cord blood in 35 – 45 minutes.**
- **The recovery of $CD34^+$ cells and of CFU from cord blood using RosetteSep™ and SepMate™ was similar to that achieved with RosetteSep™ alone. When using SepMate™, there was a trend for higher recovery when the sample was incubated with RosetteSep™ for 20 minutes instead of 10 minutes, but the difference was not significant.**
- **The purity of $CD34^+$ cells and the fold-enrichment of CFU from cord blood using RosetteSep™ (incubated for 10 or 20 minutes) and SepMate™ were similar to those achieved with RosetteSep™ alone.**
- **Large samples can be rapidly and easily reduced in volume by density gradient centrifugation using SepMate™ tubes. SepMate™-50 can be used to process 5 – 17mL of whole cord blood or whole blood.**
- **Enriched cells have not been labeled with antibody or particles and are suitable for further stem and progenitor isolation, e.g., by cell sorting, and for use in many downstream applications.**

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