Abstract #2375

Rapid unbiased enrichment (negative selection) of circulating non-hematopoietic tumor cells directly from whole blood

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Abstract

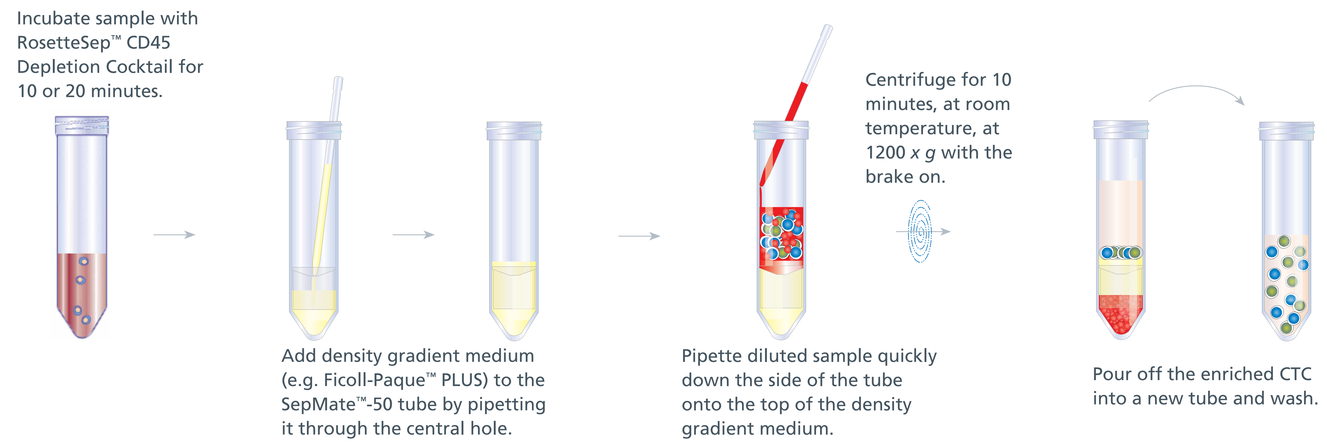
There is increasing interest in analyzing circulating non-hematopoietic tumor cells (CTC) in peripheral blood to evaluate disease progression or response to treatment; however, CTC enrichment is required prior to most analytic procedures. The ideal enrichment method would be rapid, permit a high recovery of viable CTC, and would be independent of the expression of specific epithelial cell surface markers, since CTCs in the peripheral blood may be undergoing epithelial mesenchymal transition (EMT) and may not express epithelial markers. RosetteSep™ CD45 depletion of hematopoietic cells directly from whole blood meets these criteria. However, RosetteSep™ enrichment of CTC involves density gradient centrifugation, which entails careful layering of the sample over the density gradient medium 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 minimize mixing of the sample with the density gradient medium, thus allowing rapid layering of the sample on the density gradient medium and easy pouring off of the enriched cells after centrifugation. We compared CTC enrichment using RosetteSep™ and the standard tubes and protocol with RosetteSep™ using SepMate™ tubes and reduced cocktail incubation and spin times on 5 donor whole blood samples seeded with ~1% CAMA cells.

Purity of viable (PI negative) CTC obtained with SepMate™ with RosetteSep™ was 85 ± 7%; purity of CTC obtained with RosetteSep™ alone was 91 ± 5% (no significant difference, paired t test, p=0.050). Purity of CTC obtained with density gradient centrifugation only (no RosetteSep™), either with or without SepMate™, was 4 ± 2%. There was no significant difference in the recovery of enriched CTC under any of the conditions tested (RosetteSep™ ± SepMate™, SepMate™ alone, and density gradient separation alone; Tukey-Kramer test, p>0.05). CTC enrichment was accomplished in <40 min using SepMate™ with RosetteSep™. Simplifying the layering and layer removal step makes the entire process easily scalable to processing multiple samples simultaneously. Utilizing this method, CTCs can be enriched directly from whole blood without bias regarding their surface antigen expression. Large samples can be rapidly volume-reduced in preparation for detailed examination in a microfluidic device. Finally, enriched CTC are not labeled with antibodies or beads; there is nothing to interfere with subsequent further enrichment, culture, or evaluation.

Method

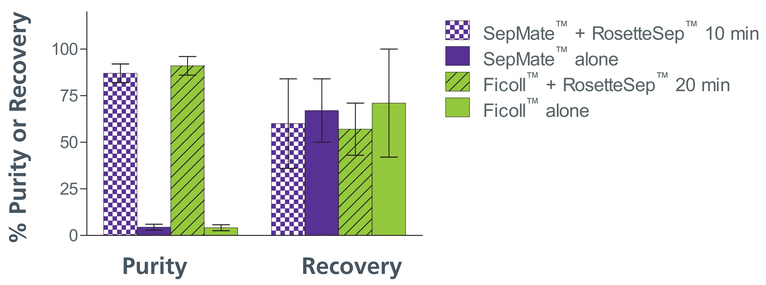
FIGURE 1: Enrichment of CTC using RosetteSep™ and SepMate™

The breast adenocarcinoma cell line CAMA was seeded into whole blood at ~1% or 0.1%. Samples were then incubated with RosetteSep™ CD45 Depletion Cocktail and then either carefully layered over a buoyant density medium in a standard centrifuge tube, spun for 20 minutes with the brake off, and carefully removed ("Standard RosetteSep™ Protocol"), or pipetted rapidly into a SepMate™ tube containing buoyant density medium, spun for 10 minutes with the brake on, and simply poured off. CTC purity was evaluated by flow cytometric analysis of nucleated cells expressing EpCAM, and recovery was determined using the purity and cell counts.



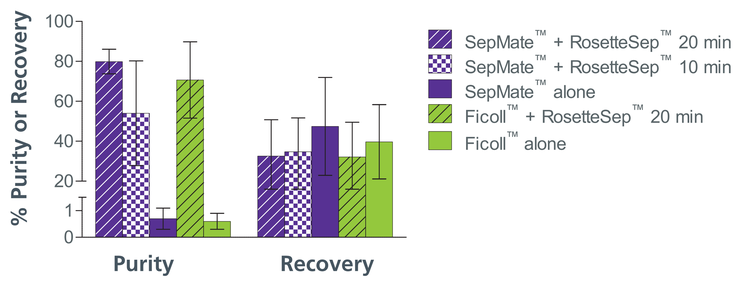
Results

FIGURE 2: Purity and recovery of CAMA cells seeded at 1% into whole blood



Means ± standard deviation; n= 5 donors, each in duplicate. Samples were incubated with RosetteSep™ CD45 Depletion Cocktail for either 10 or 20 minutes prior to centrifugation. There was no significant difference in recovery between the groups (ANOVA, p=0.97). Purity was slightly higher (paired t test, p=0.050) in the "Ficoll™ + RosetteSep™ 20 min" group versus the "SepMate™ + RosetteSep™ 10 min" group.

FIGURE 3: Purity and recovery of CAMA cells seeded at ~0.1% into whole blood



Means ± standard deviation; n= 6 donors, each in duplicate. Samples were incubated with RosetteSep™ CD45 Depletion Cocktail for either 10 or 20 minutes prior to centrifugation. There was no significant difference in recovery between the groups (ANOVA, p=0.28). There was no significant difference in purity between the three groups where the RosetteSep™ CD45 Depletion Cocktail was used (ANOVA, p=0.10), although there was a tendency for higher purity when the sample was incubated with the cocktail for 20 min rather than 10 min.

Conclusions

- SepMate™ can be used with the RosetteSep™ CD45 Depletion Cocktail to easily enrich epithelial tumour cells seeded into whole blood in 35 – 45 minutes.
- Recovery of epithelial tumour cells from whole blood using RosetteSep™ and SepMate™ is similar to that achieved by simply spinning the sample over a density gradient medium.
- Purity of epithelial tumour cells enriched from whole blood using SepMate™ and RosetteSep™ is similar to that obtained when using only RosetteSep™.
- Rare cells such as CTCs, including those that may be undergoing EMT, can be enriched directly from whole blood using the RosetteSep™ CD45 Depletion Cocktail without bias regarding their surface antigen expression.
- Large samples can be rapidly and easily volume-reduced using SepMate™.
- Enriched cells have not been labeled with antibody or particles and can be evaluated using many downstream technologies, such as microfluidic devices.