

A rapid automated method for the sequential isolation of CD19, CD3 and Myeloid cells from one tube of whole blood

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Summary

Decisions regarding immunomodulation treatment post hematopoietic stem cell (HSC) transplant are often made on the basis of chimeric analysis. Chimerism analysis is typically performed on small blood samples. Since lymphoid and myeloid engraftment is asynchronous, lineage-specific chimerism is needed. Analysis of purified cell subsets requires techniques which can isolate >1 cell type from an entire starting sample, rather than a divided sample. Performing flow cytometry as well as isolation of DNA from the purified subsets means that high cell recovery is essential. Ficoll steps often result in cell loss of 50% while certain lysis and wash steps can affect granulocyte content.

We describe a method of sequential selections to isolate cells of lymphoid (T and B cells) and myeloid lineages starting with 0.5 to 4.25 mL of human whole blood and using a fully automated pipetting robot (RoboSep®).

CD19, CD3 and myeloid cell fractions were isolated using immunomagnetic, column-free positive or optionally negative selection (EasySep®) from an

undivided sample of whole blood. Briefly, cells were first labeled with antibody targeting CD19 positive cells. These were then coupled to magnetic nanoparticles and the sample was placed in a magnet. The supernatant with unlabeled cells was removed to a new tube, leaving isolated CD19 positive cells in the magnet.

The supernatant was next labeled with anti-CD3 antibody, magnetic nanoparticles, placed in a magnet and the supernatant was removed to a new tube leaving isolated CD3 positive cells.

Finally, myeloid cells were enriched either by positive selection with a combination of anti-CD33 and CD66b antibodies or by negative selection with a cocktail of antibodies directed against the remaining unwanted cells (CD3, CD19, CD56, RBC) in the supernatant. Either method yielded enriched myeloid cells however the results reported here are based only on positive selection.

Methods

Preparation of sample: Whole blood was collected in a blood collection tube containing heparin. Blood was used directly or a buffy coat suspension was prepared. For buffy coat preparation, buffer (PBS with 2%FBS and 1mMEDTA) was added to the blood at a 5:1 ratio. The tube was then centrifuged at 1200rpm (300 x g) for 10 minutes, with no brake. The buffy coat layer (roughly 10% of total starting volume) was collected.

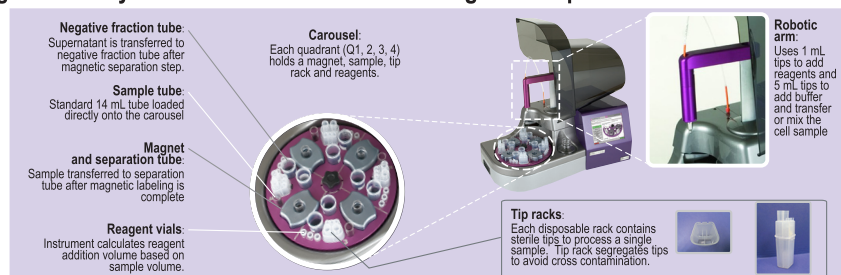
EasySep® lysis buffer (Cat# 20110) was added to the blood or buffy coat sample in a 14mL round-bottom tube at a ratio of 1:1. Separation was performed manually or with RoboSep®. The starting cell number per experiment ranged from 2.0×10^6 – 3.0×10^7 nucleated cells, from a starting blood volume of 0.5 - 4.25mL.

Whole blood selection kits from StemCell Technologies: CD19 Whole Blood Kit (Cat#18084); CD3 Whole Blood Kit (Cat# 18081); Myeloid Whole Blood Kit (Cat#18683).

Purity of selected cell populations was determined by flow cytometry. Alternate markers were used for assessment to avoid any receptor blocking due to the positive selection antibodies. CD19+ B cells were defined as CD19/20/5+ and CD19/20+. CD3+ T cells were defined as CD5+CD19/20-. Myeloid cells were defined as CD14/15+.

DNA was isolated using a QIAampDNA Blood Mini Kit (Qiagen) following manufacturer's instructions with one exception. The spin columns were spun at maximum speed at all steps.

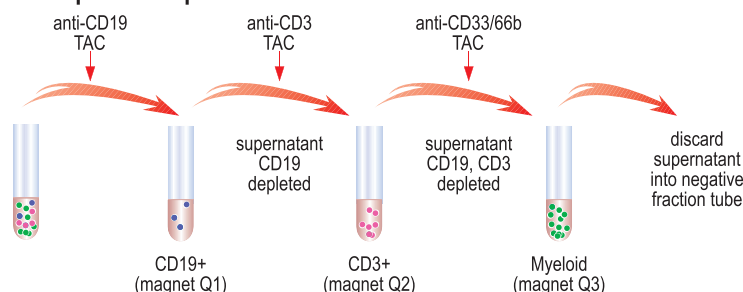
Figure 1. Fully automated cell enrichment using RoboSep®.



Add EasySep® whole blood enrichment cocktail. Cells targeted for selection are labeled with tetrameric antibody complexes (TAC) recognizing either CD19 or CD3 or CD33/CD66b (myeloid) and dextran-coated nanoparticles.

When cell enrichment is automated using RoboSep®, the first wash from the CD19 positive selection (Q1) is removed to the sample tube for the CD3 positive selection (Q2). RoboSep® continues with the washing of the CD19 sample and begins to label the CD3 sample. As the labeling progresses, the first wash from the CD3 sample, now in the magnet, is removed to the sample tube in Q3 to begin the myeloid cell selection. After the three samples have been processed, the enriched cells can be found in the tube in the appropriate magnet on RoboSep®. Samples are ready for flow cytometric analysis or other downstream processing.

Figure 2. Sequential separation



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Results

Table 1. % Purity and % Recovery of CD19, CD3 and myeloid cells enriched from whole blood using RoboSep®

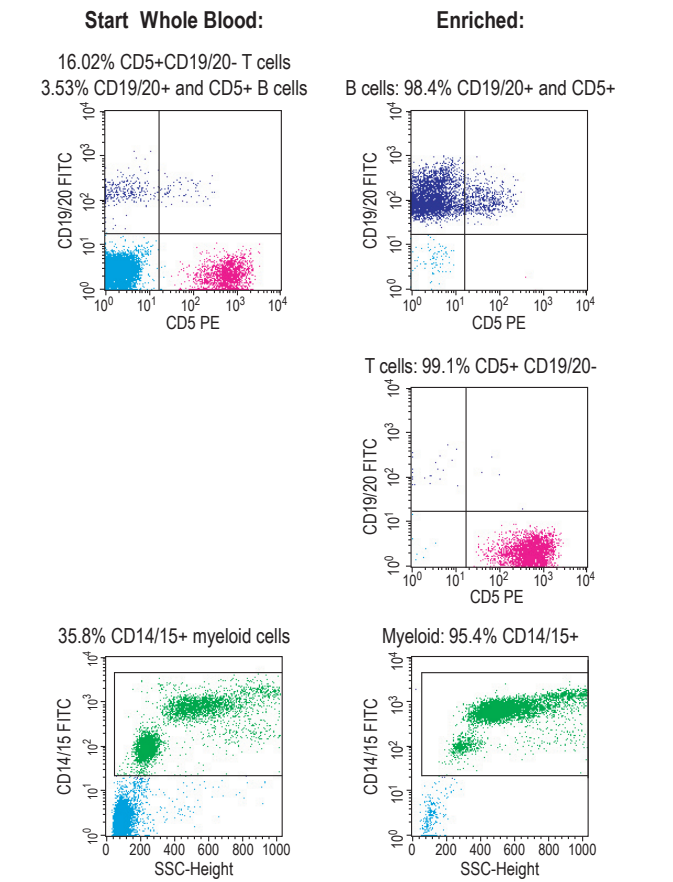
Cell Type	% start blood	% purity	% recovery from start
CD19+	1.9 ± 1.5	96.6 ± 2.0	26 ± 11
CD3+	17.0 ± 2.2	99.0 ± 2.2	49 ± 37
Myeloid	64.2 ± 21.4	97.8 ± 1.9	43 ± 21

Purities determined by flow cytometry. All samples gated on CD45+, viable (PI negative) cells. Values are expressed as means +/- 1 sd (n= 3)

Table 2. Amount of genomic DNA isolated from samples enriched from approximately 2.4 mL of whole blood. Results are the average of 3 donors.

Cell Type	average number of cells	DNA (ug)
CD19+	2.2 x 10 ⁵	4.8
CD3+	1.3 x 10 ⁶	1.4
Myeloid	1.4 x 10 ⁶	5.3

Figure 3. Typical FACS plots before and after enrichment of selected cells (plots show viable (PI-) cells gated on CD45)



Conclusions

- CD19, CD3 and myeloid cells can be enriched sequentially from the same sample of whole blood using RoboSep®. No columns are required. Entire procedure takes less than 2 hours.
- All three cell types can be collected after loading the RoboSep® machine one time with the whole blood sample and reagents.
- No cross contamination during enrichment as different tips (filtered) are used during processing for each cell sample.
- No layering over Ficoll™ or post-enrichment lysis step is required to achieve high cell purity and viability (96-99%) with good recovery of desired cells.
- In the few experiments performed, no advantage was seen by first preparing a buffy coat.
- Small volumes of blood (0.5 - 4.5mL containing 2 x 10⁶ - 3 x 10⁷ cells) yield sufficient cells to run flow cytometry and DNA analysis.

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