Column-Free Enrichment of CD34+CD117+ Cells Directly from Bone Marrow and Peripheral Blood

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INTRODUCTION

Blast cells in patients with acute myeloid leukemia (AML) often express markers associated with hematopoietic stem and progenitor cells, including CD34 and CD117. In a diagnostic setting, these markers are used to identify leukemic cells and monitor measurable residual disease. Current methods for enriching these cells include fluorescence-activated cell sorting or column-based isolation using magnetic particles directly conjugated to anti-CD34 or CD117 antibodies. Both methods require lysis of red blood cells (RBCs) prior to cell isolation, which can lead to loss of these precious, low-frequency cells. To address this limitation, we have developed a rapid, column-free method (EasySep™) to enrich CD34+CD117+ cells directly from bone marrow (BM) or peripheral blood. This method also incorporates RBC lysis into the cell isolation workflow.

METHODS

Contrived Human Specimens: As whole blood (WB) and/or BM from patients with AML were not available, two types of human contrived specimens were generated and used to evaluate performance of the CD34+CD117+ isolation reagents:

- **Cell-line contrived samples** were generated by spiking the CD34/CD117-expressing cell line KG-1 into WB from healthy donors at varying CD34+CD117+ cell frequencies: low (< 3%), medium (3 - 15%), high (15 - 40%), and very high (> 40%).
- **Clinical contrived samples** were generated by spiking BM from a healthy donor with commercially available, previously frozen peripheral blood mononuclear cells (PBMCs) derived from patients with AML at various stages of disease. As the frozen AML-derived PBMC samples contained a relatively low percentage of CD34+CD117+ cells, the frequency of CD34+CD117+ cells in the starting clinical contrived samples was typically < 5%.

Cell Isolation: Cell line-contrived and clinical contrived samples were first diluted with an equal volume of EasySep™ Red Blood Cell Lysis Buffer. Cells were then labeled with CD34 and CD117 antibody complexes and magnetic particles, then separated either manually using a specialized magnet (the "Big Easy" EasySep™ Magnet), or automatically using the RoboSep™-S instrument.

Purity Assessment: The percentage of CD34+CD117+ cells before and after enrichment was determined by flow cytometry using fluorochrome-conjugated antibodies against CD34, CD117, CD45, and CD33 in the presence of a dye to detect dead cells (7-amino-actinomycin D). RBCs in the starting samples were lysed using ammonium chloride prior to flow cytometry analysis.

Cell Yield Assessment: The number of CD34+CD117+ cells recovered per mL of starting sample was determined using a combination of flow cytometry to determine the frequency of CD34+CD117+ cells and cell counting using a hemocytometer.

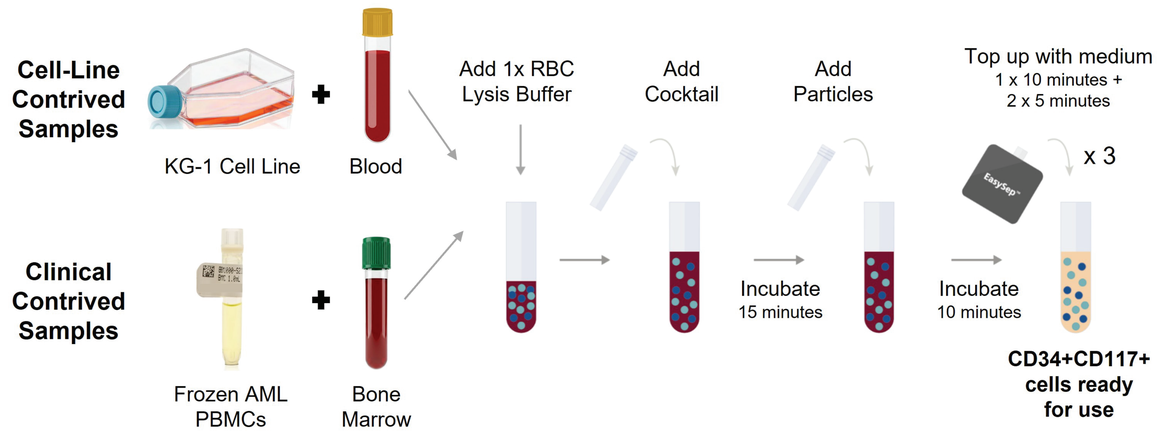


FIGURE 1. Workflow for Generating Contrived Specimens and Isolating CD34+CD117+ Cells.

RESULTS

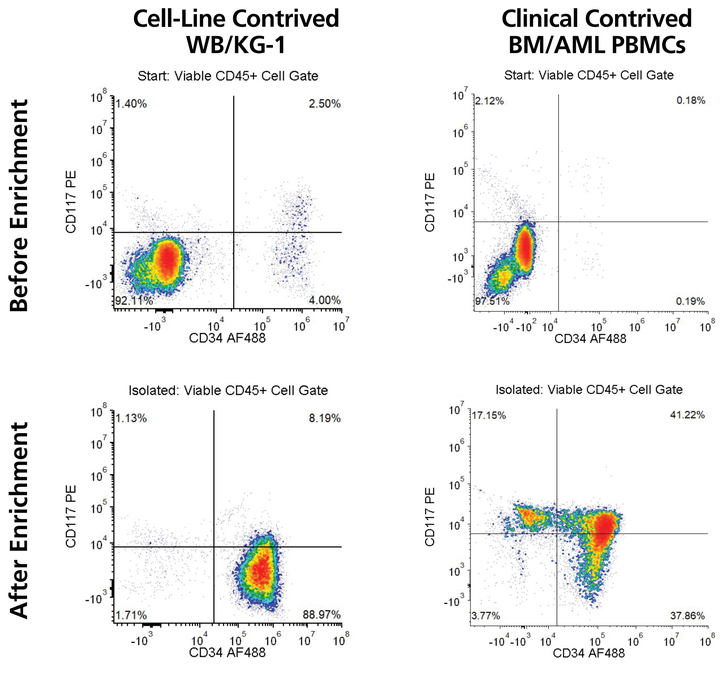


FIGURE 2. Representative Flow Cytometry Plots of Cell-Line Contrived and Clinical Contrived Specimens Before and After CD34+CD117+ Enrichment.

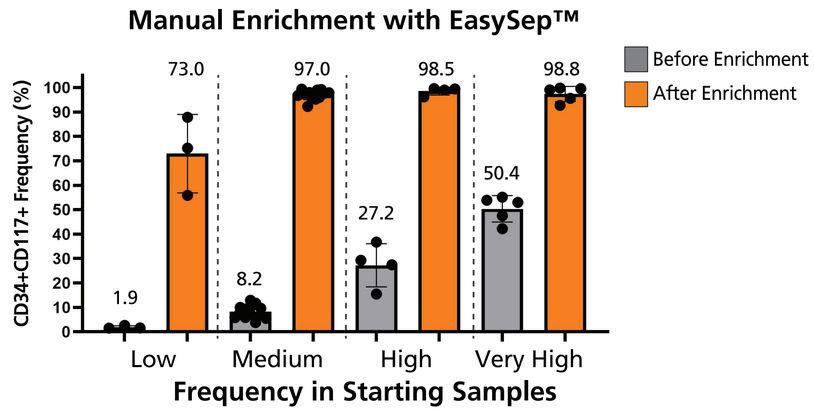


FIGURE 3. CD34+CD117+ Frequency in Cell-Line Contrived Specimens Before and After Manual Enrichment.

CD34+CD117+ cells were enriched manually in cell-line contrived samples with low (< 3%; n = 3), medium (3 - 15%; n = 13), high (15 - 40%; n = 4), and very high (> 40%; n = 5) initial cell frequency. Samples were enriched from 1.9 ± 0.6% to 73.0 ± 16.1%, from 8.2 ± 2.7% to 97.0 ± 1.9%, from 27.2 ± 8.8% to 98.5 ± 1.6%, and from 50.4 ± 5.4% to 98.8 ± 3.1%. Data represent mean ± standard deviation.

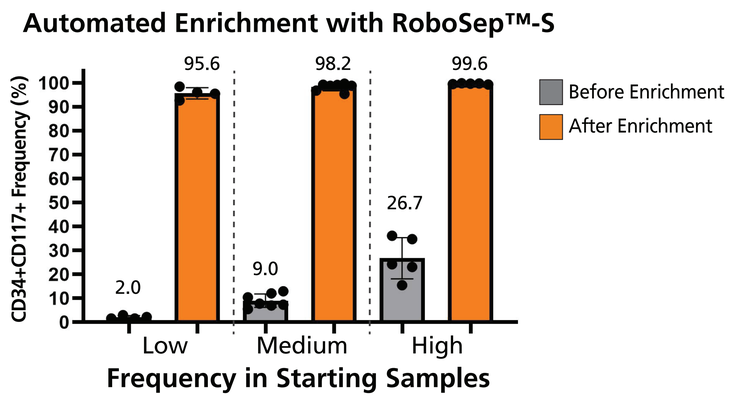


FIGURE 4. CD34+CD117+ Frequency in Cell-Line Contrived Specimens Before and After Automated Enrichment with RoboSep™-S.

CD34+CD117+ cells were enriched using RoboSep™-S in cell-line contrived samples with low (< 3%; n = 4), medium (3 - 15%; n = 7), and high (15 - 40%; n = 5), initial cell frequency. Samples were enriched from 2.0 ± 0.7% to 95.6 ± 2.3%, from 9.0 ± 2.8% to 98.2 ± 1.6%, and from 26.7 ± 8.6% to 99.6 ± 0.2%. Data represent mean ± standard deviation.

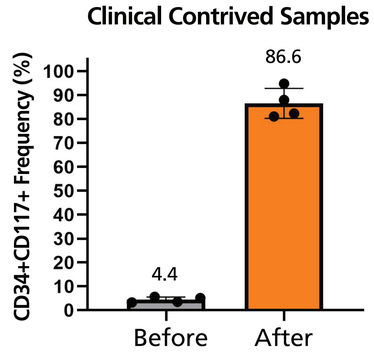


FIGURE 5. CD34+CD117+ Frequency in Clinical Contrived Specimens Before and After Manual Enrichment.

Clinical contrived specimens were enriched from 4.4 ± 1.2% to 86.6 ± 6.3% (n = 4). Data represent mean ± standard deviation.

TABLE 1. The Number of CD34+CD117+ Cells Recovered per mL of Cell-Line or Clinical Contrived Sample Based on Initial Starting CD34+CD117+ Cell Frequency.

Cell Frequency in Starting Sample	n	Number of Cells Recovered per mL Sample (Mean ± SD)
Cell-Line Contrived (WB)		
< 3%	3	5.2 ± 4.1 x10^4
3 - 15%	13	4.4 ± 1.5 x10^5
15 - 40%	4	8.7 ± 6.2 x10^5
> 40%	5	4.4 ± 1.7 x10^6
Clinical Contrived		
< 5%	4	1.7 ± 1.0 x 10^5

SUMMARY

- CD34+CD117+ cells can be isolated directly from WB or BM using EasySep™ methodology.
- CD34+CD117+ cell isolation can be performed manually using the "Big Easy" EasySep™ Magnet or automatically using RoboSep™-S.
- Cell enrichment is performed concurrently with RBC lysis, providing an efficient, streamlined workflow.
- High purity of CD34+CD117+ cells can be achieved (> 94%).
- High yields of CD34+CD117+ cells can be recovered even from samples with low frequency of cells.

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