Fully automated magnetic labeling and separation of hematopoietic cells from multiple samples

Steven M Woodside[1], Ron C Makowichuk[1], Jodie Fadum[1], Albertus W Wognum[1], Allen C Eaves[1,2] and Terry E Thomas[1].
[1]Research and Development, StemCell Technologies, Vancouver, BC, Canada, V5Z 1B3 and [2]Terry Fox Laboratory, BC Cancer Research Centre, Vancouver, BC, Canada, V5Z 1L3.

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Introduction

Laboratory process automation is an important requirement for streamlining and standardizing technical procedures. Despite extensive use of magnetic cell separation, these procedures have not been fully automated. Currently magnetic cell labeling is done manually followed by automated magnetic separation (e.g. AutoMACS and Isolex). Additionally, current technology only allows for processing of a single sample at a time. The objective of this study was to fully automate the purification of hematopoietic cells from multiple blood and bone marrow samples.

Methods

For manual separations, cells were labeled using EasySep® reagents and then magnetically separated as shown in Figure 1. Automated separations followed the same sequence of steps, with an automated pipettor being used to add reagents and buffer, mix, transfer the sample to the magnet and remove the supernatant.

The hematopoietic progenitor content of the original and enriched samples was assayed using a colony-forming-cell assay in semi-solid cell culture medium (MethoCult™) as shown in Figure 2.

Figure 1. Immunomagnetic Separation

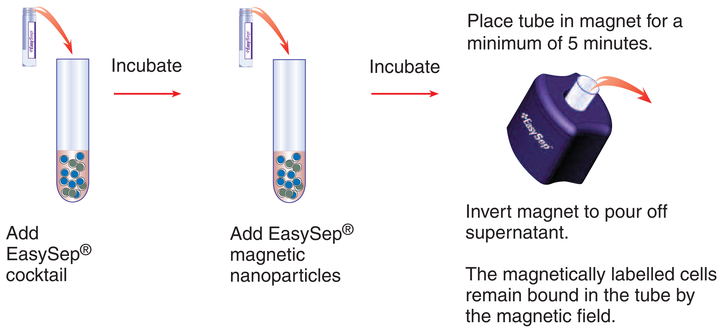
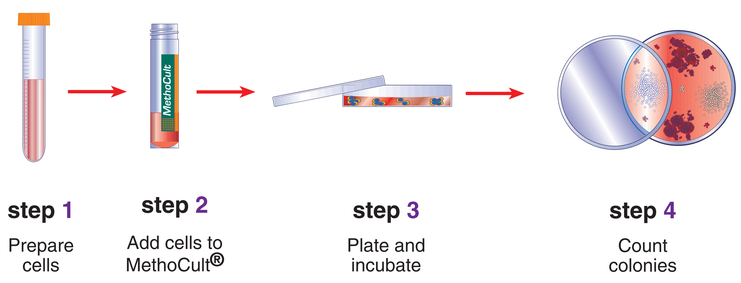


Figure 2. Hematopoietic CFC Assay

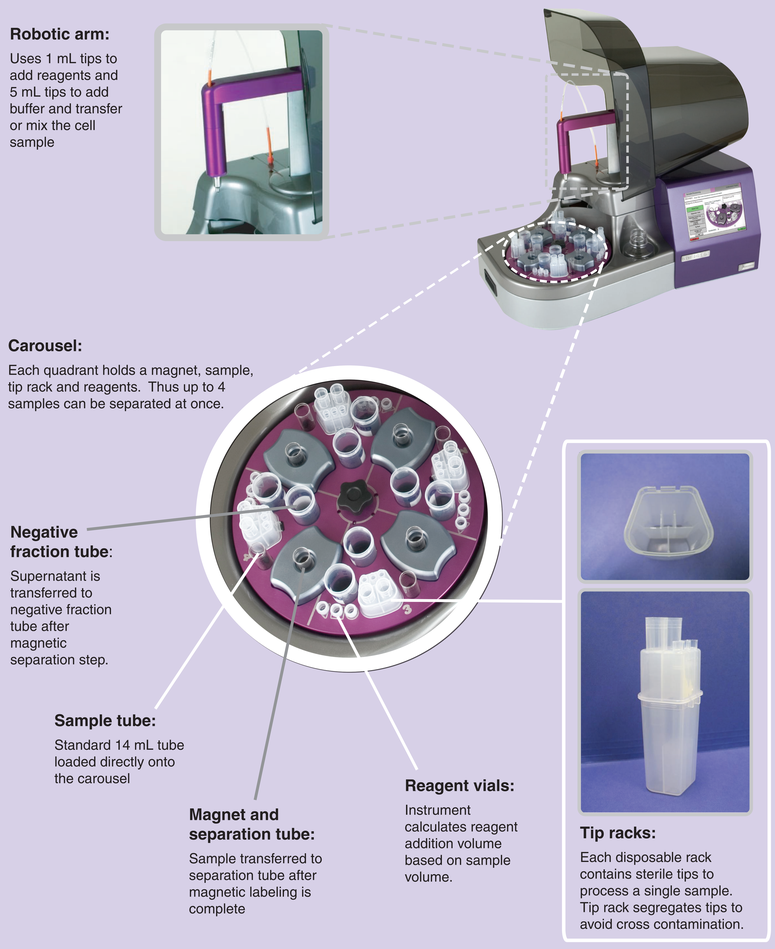


Objective

Fully automate the purification of hematopoietic cells from multiple blood and bone marrow samples.

Figure 3. Automated cell separator (RoboSep®)

The instrument fully automates both positive and negative selection by performing all immunomagnetic labeling steps directly in the sample tube and then transferring the sample to a magnet for the separation steps.



Results

Table 1. Automated Positive Selection of CD34$^+$ Cells

Cord blood (CB) and mobilized peripheral blood (MPB) CD34$^+$ cells were automatically isolated from previously frozen samples. For both sample types, CD34$^+$ cells were enriched to high purity. Manual separations that were performed in parallel for a subset of the automated separations below showed comparable purity and recovery (data not shown).

Sample type	% CD34$^+$ in start	% CD34$^+$ in enriched	% Recovery CD34$^+$ cells
CB (n = 9)	1.2 ± 0.4	96.6 ± 3.1	45 ± 9
MPB (n = 4)	0.7 ± 0.1	96.7 ± 3.1	45 ± 13

Table 2. Automated Negative Selection of Hematopoietic Progenitors

Hematopoietic progenitors were automatically enriched by depleting cells expressing any of CD2, CD3, CD11b, CD11c, CD14, CD16, CD19, CD24, CD56, CD66b, and glycophorin A. Manual separations that were performed in parallel for a subset of the automated separations below showed comparable purity and recovery (data not shown). For all 3 sample types, colony-forming-cells (CFC) were enriched about 40-fold.

Sample type	% CD34$^+$ in start	% CD34$^+$ in enriched	% Recovery CD34$^+$ cells	Fold-enrichment of CFC	% Recovery of CFC
CB (n = 3)	2.3 ± 1.3	60.4 ± 13.8	39 ± 20	40 ± 8	47 ± 15
MPB (n = 4)	1.1 ± 0.2	54.0 ± 9.9	44 ± 9	34 ± 14	31 ± 14
BM (n = 4)	4.7 ± 3.1	47.5 ± 7.5	N.A.	47 ± 10	71 ± 13

Conclusions

- Successful automation of both positive and negative selection
- Automatically processes up to 4 tissue samples at once
- Potential to automatically isolate multiple cell subsets from the same sample by combining positive and negative selection