

A rapid method for the isolation of eosinophils without Ficoll™ or RBC Lysis

Maureen A. Fairhurst, Melany Nauer, Terry E. Thomas
StemCell Technologies Inc., 570 W 7th Ave., Vancouver, BC, V5Z 1B3, Canada

Introduction

Eosinophils are polymorphonuclear cells that typically comprise 1-5% of blood leucocytes in non-allergic healthy humans. Allergic and asthmatic reactions result in activation of eosinophils with increase in cytokines and lytic enzymes in their cytoplasmic granules. Release of these components results in damage to target cells. Eosinophils are also important mediators of inflammatory responses against parasitic infections. Many protocols for eosinophil isolation stress the avoidance of ammonium chloride lysis as this reagent interferes with their antigen processing, cytokine responses and additionally alters cell morphology.

We describe a rapid and simple method for the enrichment of eosinophils from whole blood that does not require Ficoll or lysis steps and yields good purity, recovery and viability. Blood was collected in a blood collection tube containing heparin and red blood cell (RBC) were

removed by sedimentation using HetaSep. The use of HetaSep eliminated the steps of careful layering over Ficoll™ and subsequent RBC lysis.

The eosinophils were then enriched using immunomagnetic, column-free negative selection (EasySep®). Briefly, cells were labeled with a cocktail of antibodies targeting unwanted cells. These were then coupled to magnetic nanoparticles and the sample was placed in a magnet. The supernatant containing the unlabeled eosinophils was collected leaving labeled cells behind in the magnet. The whole separation procedure has been automated with the pipetting robot RoboSep®.

Purity of eosinophils was assessed by Wright's stained cytopspins and flow cytometry. Eosinophils were defined as CD45+CD66b+CD16- cells displaying high side scatter and low forward scatter.

Methods

HetaSep sedimentation for removal of RBC

Whole blood was collected in a blood collection tube containing heparin. One part HetaSep was added to 5 parts blood and mixed well. The tube was centrifuged for 5 minutes at 50 x g with the brake off. The tube was left to sit an additional 10 minutes at room temperature. The top plasma layer containing the nucleated cells was removed without disturbing the RBC layer. This fraction was washed twice with cold buffer (PBS with 2% FBS plus 1mM EDTA). At least one of these washes incorporated a slow spin (120 x g, 10 minutes) to remove platelets. Cells were resuspended at 5×10^7 cells/mL. The starting cell number per experiment was $1.8 - 5.0 \times 10^7$.

Figure 1. EasySep® Procedure for column-free cell enrichment

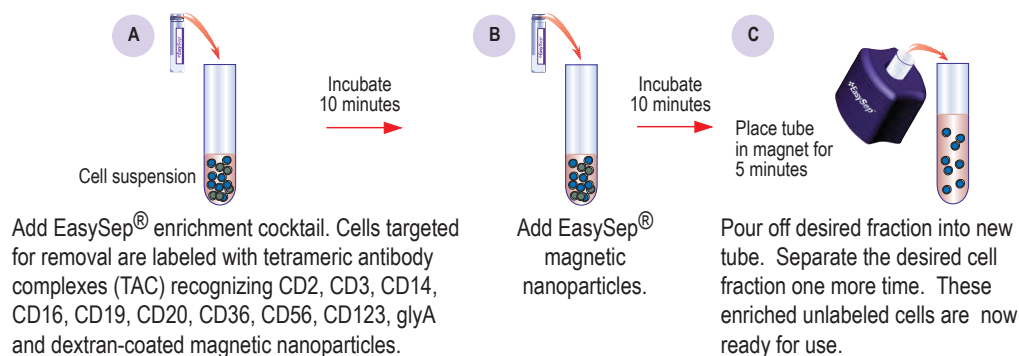
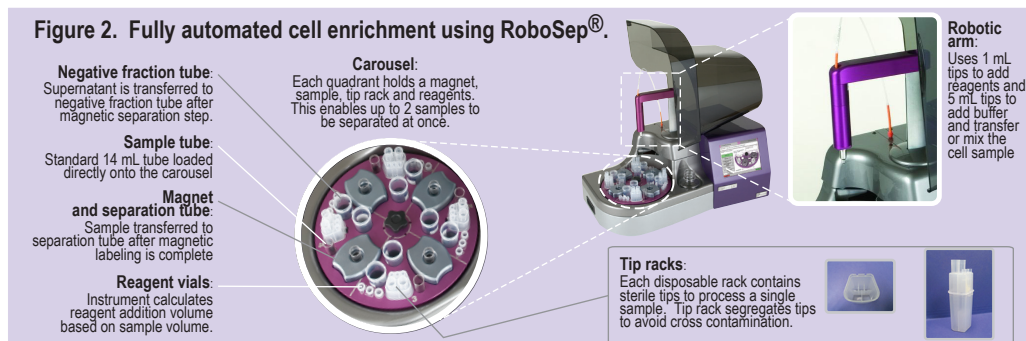


Figure 2. Fully automated cell enrichment using RoboSep®.



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Results

Table 1. % Purity and % Recovery of Eosinophils enriched by negative selection using manual EasySep® or RoboSep®

start	% purity CD66b+CD16-		% recovery*	
	EasySep®	RoboSep®	EasySep®	RoboSep®
1.7 ± 1.0	94.0 ± 3.0	91.3 ± 4.0	69 ± 21	74 ± 20

Purities determined by flow cytometry. All samples gated on CD45⁺ viable (PI⁻) cells. Values are expressed as means ± 1 sd (n=11 EasySep®; n=7 RoboSep®). *Cell recoveries calculated from start cell suspension after HetaSep.

Figure 3. FACS plots before and after enrichment of eosinophils
(Plots show viable (PI⁻) cells gated on CD45.)

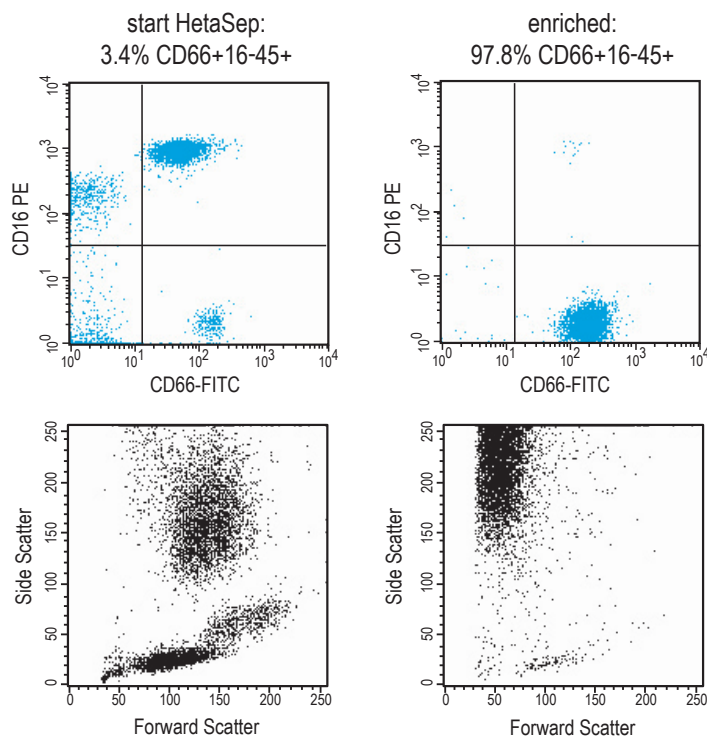
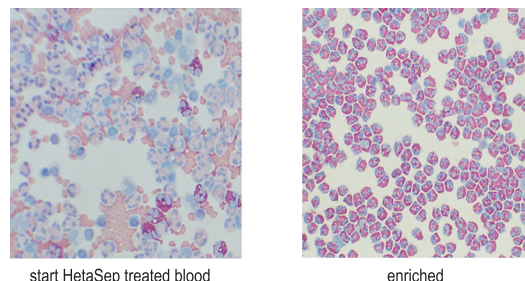


Figure 4. Eosinophil cytoplasmic granules show characteristic pink-red colour in Wright's stained cytopspins



Conclusions

- Eosinophils can be enriched from HetaSep treated whole blood using EasySep®. No columns are required. Entire procedure takes less than 2 hours.
- Eosinophil enrichment can be fully automated using RoboSep®.
- No layering over Ficoll™ or lysis step is required to achieve high purity, recovery and viability (>98%) of eosinophils.
- Discrepancy in cell purity data between flow and cytopsin data (95-100% purity data not shown) may be attributable to the number of cells analyzed (10,000 cells were counted during flow compared to 100 - 200 by microscopy).
- One tube of blood (5.5 mL) yields approximately 1.6×10^5 eosinophils by the described method (range of 21 - 51% recovery from whole blood start).
- Enriched cells have typical eosinophilic morphology with intact granules.

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StemCell Technologies Inc
The Cell Experts™ | www.stemcell.com

In North America
Tel: 1.800.877.0713
Fax: 1.604.877.0704
Toll Free Tel: 1.800.667.0322
Toll Free Fax: 1.800.567.2899
e-mail: info@stemcell.com

In Europe
Tel: +33 (0) 4 76 04 75 30
Fax: +33 (0) 4 76 18 99 63
e-mail: info@stemcellfrance.com

In the United Kingdom
Tel: +44 (0) 20 7537 7565
Fax: +44 (0) 20 7515 5408
Toll Free within UK:
Tel: 0800 731 27 14
Fax: 0800 731 27 13
e-mail: info@stemcellgb.com