

Rapid, Automated Lymphocyte Isolation Directly from Whole Blood with EasySep™ Direct

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INTRODUCTION

Automation is an important requirement for streamlining and standardizing technical procedures, such as cell isolation, in a routine laboratory. We have previously developed RoboSep™-S, the first instrument to fully automate the cell labeling and cell isolation steps of an immunomagnetic cell isolation procedure. RoboSep™-S can isolate up to four individual samples at one time and single-use disposable filter tips eliminate the possibility of sample cross-contamination.

We have recently developed EasySep™ Direct, a rapid method to isolate highly purified lymphocytes directly from whole blood without the need for any pre- or post-cell processing steps. This study describes automation of the EasySep™ Direct procedure using RoboSep™-S to isolate T cells, B cells and total lymphocytes (T and B cells combined). Performance (cell purity and recovery) is directly compared to using the manual silver EasySep™ magnet in head-to-head experiments.

METHODS

Sample Preparation: Peripheral blood, 24- or 48-hour post draw, was obtained commercially.

Cell Isolation: T cells, B cells, or total lymphocytes were isolated using EasySep™ Direct HLA Crossmatch T Cell Isolation Kit (Catalog #19671 or 89671 for CE-IVD), EasySep™ Direct HLA Crossmatch B Cell Isolation Kit (Catalog #19684 or 89684 for CE-IVD), or EasySep™ Direct Total Lymphocyte Isolation Kit (Catalog #19655), respectively.

EasySep™ Direct Manual Isolation Strategy using "The Big Easy" EasySep™ Magnet (silver magnet, Catalog #18001): Unwanted cells, platelets, and red blood cells (RBCs) were immunomagnetically labeled and then placed into the silver EasySep™ magnet. Labeled unwanted cells were retained in the magnet, while untouched T cells, B cells, or total lymphocytes were poured off (Figure 1).

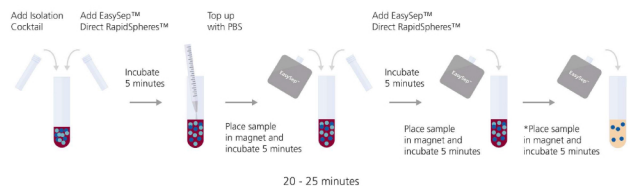


FIGURE 1: EasySep™ Direct Isolation Procedure Using the Silver EasySep™ Magnet

EasySep™ Direct Automated Isolation Procedure Using RoboSep™-S
Whole blood was loaded into RoboSep™-S (Catalog #21000) following the on-screen user-guided interface, and unwanted cells were targeted for depletion by addition of an antibody cocktail and magnetic particles. After cell labeling, the sample was moved to the separation tube in the magnet by the robotic arm of the instrument. Cells of interest were then transferred to a collection tube, while unwanted cells were retained in the magnet.

Typical RoboSep™-S Protocol



FIGURE 2: RoboSep™-S

RoboSep™-S Protocol	1 Sample	4 Samples
T Cells	22 minutes	40 minutes
B Cells	26 minutes	41 minutes
Total Lymphocytes	22 minutes	40 minutes

TABLE 1: Time Required to Isolate Cells from 1 or 4 Whole Blood Samples Using RoboSep™-S

Purity Assessment: The percentage of CD3+ T cells, CD19+ B cells, or total lymphocytes (CD3+ and CD19+ cells combined) was assessed by flow cytometry using fluorochrome-conjugated antibodies against CD45 and CD3 (for T cells), CD45 and CD19 (for B cells), or CD45, CD3, and CD19 (for total lymphocytes). Cells were stained with the viability dye 7-AAD and gated on live CD45+ events.

Assessment of Cell Recovery: The number of CD3+ T cells, CD19+ B cells, or total lymphocytes (T and B cells combined) per mL of whole blood was determined by cell counting using a hemocytometer.

RESULTS

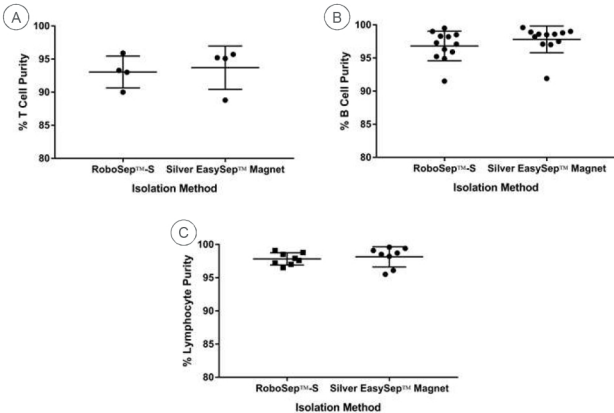


FIGURE 3: Similar Purities were Obtained in Head-to-Head Experiments Between Automated and Manual EasySep™ Direct Using RoboSep™-S or the Silver EasySep™ Magnet

Cells were isolated using either RoboSep™-S or the manual silver EasySep™ magnet in head-to-head experiments. (A) Following EasySep™ Direct T cell isolation, purities of 93.1 ± 2.4% and 93.7 ± 3.3% (mean ± SD) were obtained using RoboSep™-S or the manual silver EasySep™ magnet, respectively (n = 4). (B) Following EasySep™ Direct B cell isolation, purities of 96.8 ± 2.2% and 97.8 ± 2.0% (mean ± SD) were obtained using RoboSep™-S or the manual silver EasySep™ magnet, respectively (n = 12). (C) Following EasySep™ Direct total lymphocyte isolation, purities of 97.8 ± 0.9% and 98.1 ± 1.5% (mean ± SD) were obtained using RoboSep™-S or the manual silver EasySep™ magnet, respectively (n = 8).

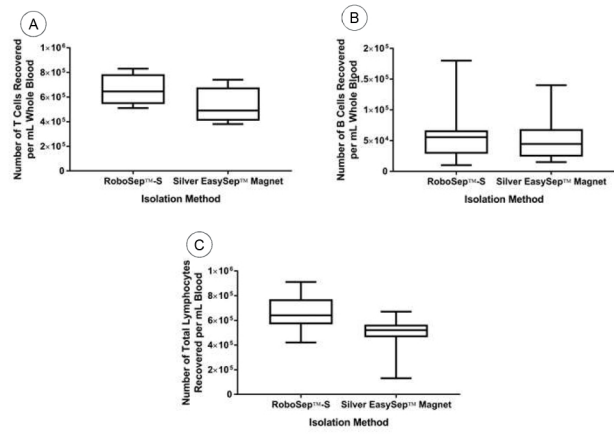


FIGURE 4: Similar or Better Cell Recovery from 1 mL of Blood Using EasySep™ Direct on RoboSep™-S

(A) Following EasySep™ Direct T cell isolation, 6.6 ± 1.3 × 10⁵ and 5.3 ± 1.5 × 10⁵ T cells (mean ± SD) were recovered from 1 mL of blood using RoboSep™-S or the manual silver EasySep™ magnet, respectively (n = 4). (B) Following EasySep™ Direct B cell isolation, 5.8 ± 4.4 × 10⁴ and 5.0 ± 3.5 × 10⁴ B cells (mean ± SD) were recovered from 1 mL of blood using RoboSep™-S or the manual silver EasySep™ magnet, respectively (n = 12). (C) Following EasySep™ Direct total lymphocyte isolation, 6.6 ± 1.5 × 10⁵ and 4.9 ± 1.6 × 10⁵ total lymphocytes (mean ± SD) were recovered from 1 mL of blood using RoboSep™-S or the manual silver EasySep™ magnet, respectively (n = 8).

Summary

- EasySep™ Direct isolation of T cells, B cells, and total lymphocytes directly from whole blood can be automated on RobSep™-S.
- Similar purity and cell recovery are obtained using EasySep™ Direct manually or using RoboSep™-S.
- The highly purified lymphocytes obtained using EasySep™ Direct can be used in crossmatch assays or other laboratory techniques.
- Automation enables standardization of the isolation procedure and frees up technologist time.