A Rapid Method to Isolate Highly Purified T or B Cells from Blood, Lymph Node or Spleen Samples For Use in Donor-Recipient Crossmatch Assays

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Introduction

The crossmatch assay is used as part of a pre-transplant immunologic risk assessment to determine the compatibility between donor-recipient pairs. Isolated T or B cells from the donor are mixed with recipient serum and the presence of donor-specific antibodies is detected through a complement-dependent killing assay (CDC-crossmatch) or by flow cytometry (flow crossmatch). Isolation of specific cell types can be time consuming, and multiple methods must often be validated in laboratories that receive a variety of tissue types.

We have developed a method (EasySep™ Direct) to isolate T or B cells directly from whole blood (WB) in 25 minutes, or lymph node (LN) and spleen samples in 11 minutes, without RBC lysis, sedimentation or density gradient centrifugation. Isolation of T or B cells using this method was tested on WB, peripheral blood mononuclear cells (PBMC) (model system for LN, which typically have few RBC) as well as on a suspension of PBMC/WB and a B cell line (model system for spleen, which has a high B cell content). Isolations can be automated using RoboSep™. This new method enables the isolation of highly purified T or B cells from multiple tissue sources using the same reagents, thus simplifying validation for a busy HLA laboratory.

Methods

Samples:

Whole Blood (WB): Peripheral blood, 24 or 48-hour post draw, was obtained commercially.

Mock Spleen: To mimic the cell composition found in human spleens, equal numbers of PBMCs were combined with the B cell line Nalm/6. WB from the same donor was then added back to the sample at a ratio of 80:1.

Mock Lymph Node: Previously frozen or freshly isolated PBMC were used as a surrogate for human lymph node samples.

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

EasySep™ Direct Cell Isolation Strategy: Unwanted cells, platelets and red blood cells (RBCs) were immunomagnetically labelled and then placed into an EasySep™ magnet. Labeled unwanted cells were retained in the magnet, while untouched T or B cells were poured or pipetted off (Figures 1 and 2).

Figure 1. EasySep™ Direct protocol to isolate T or B cells from whole blood or buffy coat samples

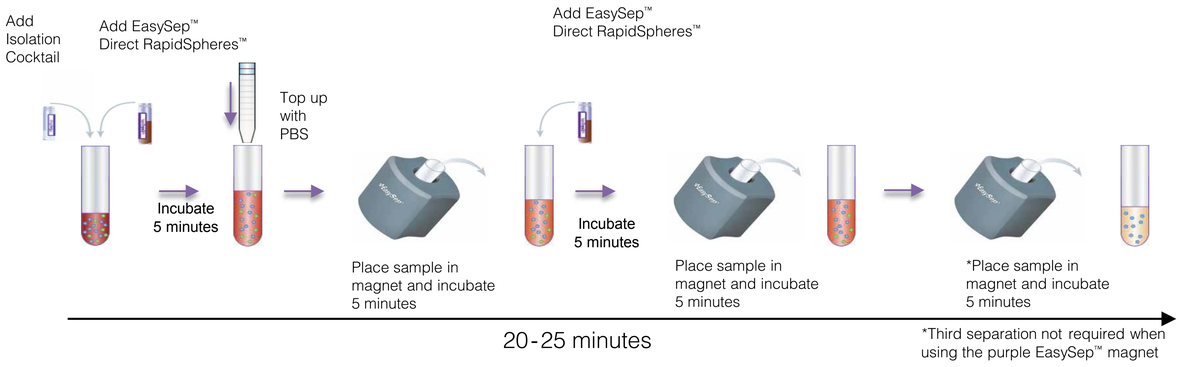
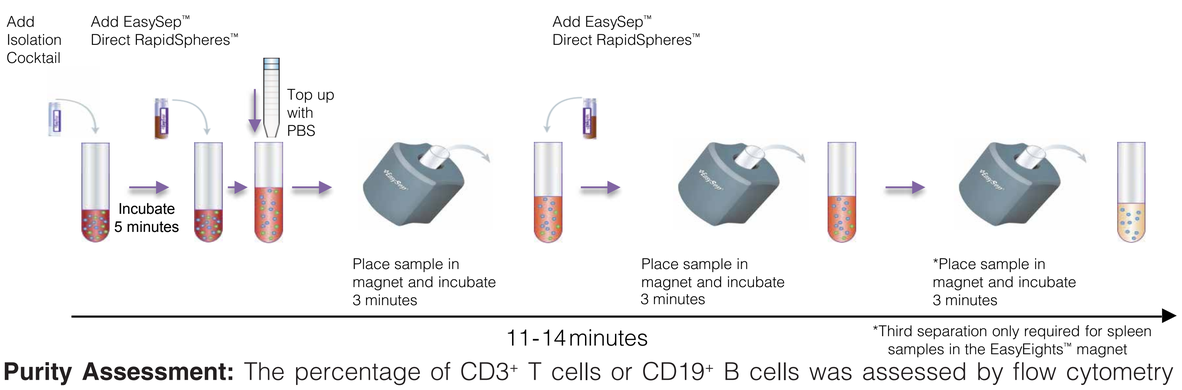


Figure 2. EasySep™ Direct protocol to isolate T or B cells from spleen or lymph node samples

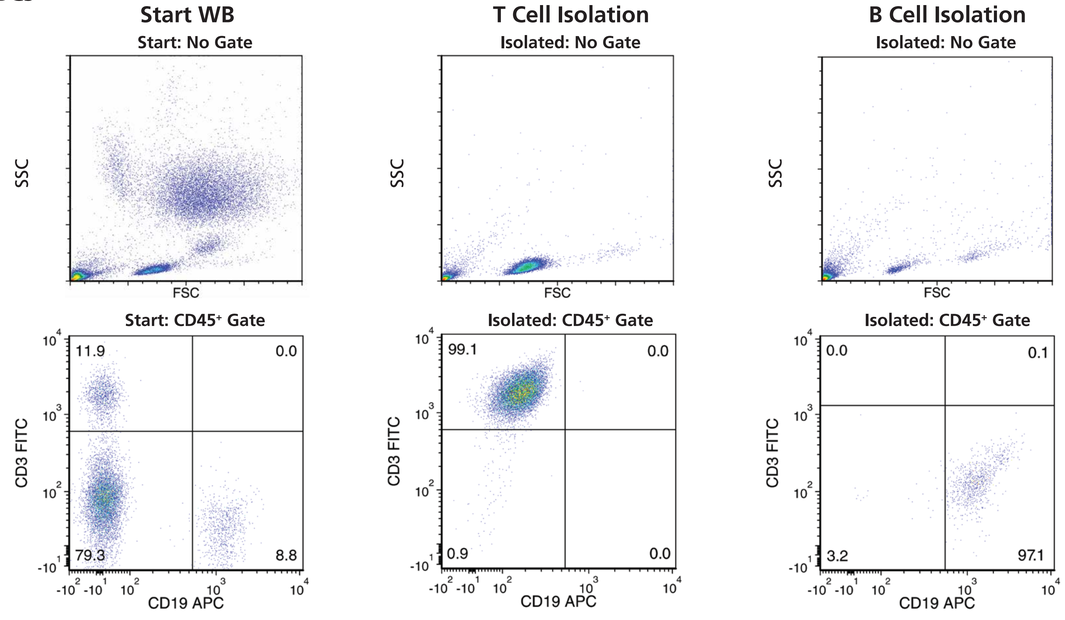


Purity Assessment: The percentage of $CD3^+$ T cells or $CD19^+$ B cells was assessed by flow cytometry using fluorochrome-conjuaged antibodies against CD45 and CD3 (for T cells) or CD45 and CD19 (for B cells). Cells were stained with the viability dye 7AAD and gated on $CD45^+$ events.

Assessment of Cell Recovery: The number of $CD3^+$ T cells or $CD19^+$ B cells per mL of whole blood, or per 5 x 10^7 cells for mock spleen or lymph node samples were determined by cell counting using a hemocytometer.

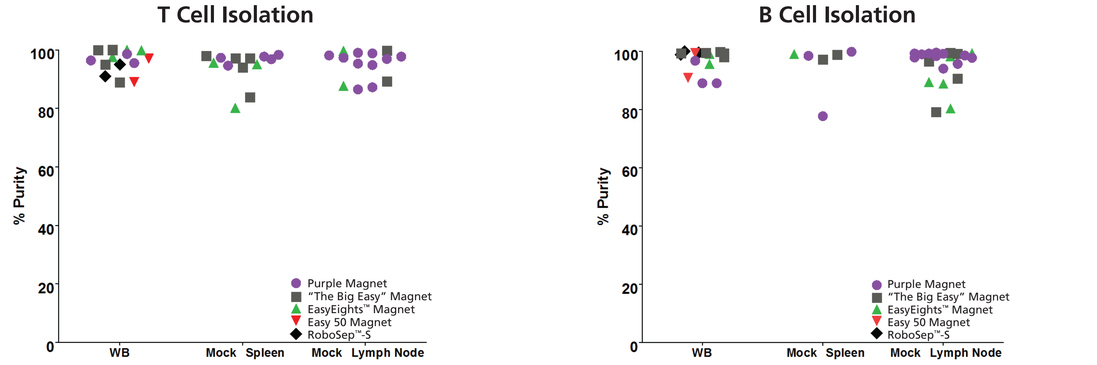
Results

Figure 3. Flow cytometric assessment of $CD3^+$ T cells or $CD19^+$ B cells before and after isolation from whole blood using EasySep™ Direct shows high purity of target cells with minimal contamination of RBCs



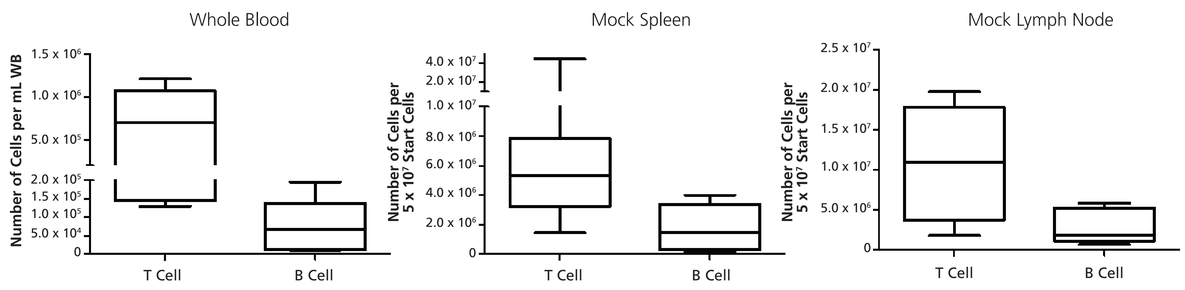
Top panels show FSC vs SSC (no gate), while bottom panels show staining with fluorochrome-conjugated anti-CD3 and anti-CD19 antibodies. Cells were gated on $CD45^+$ events. RBCs in the human WB start samples were lysed using ammonium chloride to determine starting cell frequencies. Isolated cell fractions were not lysed.

Figure 4. Average T cell or B cell purities following EasySep™ isolation from WB, mock spleen, or mock LN samples were above 94%



Isolations were performed on five different magnet platforms, including RoboSep™-S, the automated cell separator. T cell purities were 96 ± 4 (n = 14) from WB, 94 ± 6 (n = 13) from mock spleen, and 95 ± 5 (n = 14) from mock LN (mean ± SD). B cell purities were 97 ± 4 (n = 15) from WB, 95 ± 9 (n = 9) from mock spleen, and 95 ± 6 (n = 21) from mock LN samples.

Figure 5. Average number of T cells or B cells isolated from blood, mock spleen, and mock lymph node samples using EasySep™ Direct



On average, 6.5 x 10^5 T cells and 7.3 x 10^4 B cells were recovered from 1 mL of WB. Starting from 5 x 10^7 cells, 10 million T cells and 1.8 million B cells were recovered from mock spleen samples, while 10 million T cell and 2.8 million B cells were recovered from mock LN samples.

Summary

- T cells or B cells can be isolated from blood in as little as 20 minutes, and from spleen or lymph node samples in as little as 11 minutes.
- No density centrifugation, sedimentation or additional RBC removal is required.
- Starting with blood from normal, healthy donors, T cell or B cell purities between 89 - 99% can be achieved.
- On average, 650,000 T cells and 70,000 B cells can be recovered per mL of blood.