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 Nalm6. 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-conjugated 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

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

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

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.