Innovative Cell Isolation for HLA Labs

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The HLA (Human Leukocyte Antigen) complex is the human counterpart to the Major Histocompatibility Complex (MHC) initially discovered in mouse studies of tumor transplantation and graft compatibility. Since the late 1960s, research on human organ and tissue transplantation has reinforced the importance of cell surface antigens expressed on purified hematological cell preparations in predicting and improving graft survival and transfusion efficiency. Today, the role of HLA antigens is of critical importance in transplant and transfusion medicine.

The HLA genes are encoded in a cluster on the short arm of chromosome 6. They encode the highly polymorphic family of HLA antigens, which are intricately involved in the immune response cascade. The majority of clinically-relevant HLA molecules fall within the Class I and Class II types, which are immunoglobulin-like glycoproteins comprised of two dissimilar protein chains. HLA Class I antigens have a broad distribution and are expressed on most nucleated cells, platelets and reticulocytes. The HLA Class II antigens in contrast, are expressed on a narrower range of cells including B cells and dendritic cells but are not normally found on T cells or platelets. The density of HLA antigen expression, both Class I and Class II, is not uniform between individuals or across cell types and antigen expression may be modulated by environmental and other stimuli.

The specific and varied distribution of HLA antigens often necessitates cell preparations of high purity and specificity when studying the HLA system. As it is often important to study cell preparations that have been minimally activated during the enrichment process, isolating cells for HLA analysis has always been a challenge. The isolation procedure must not excessively damage the cells or alter antigen expression thereby minimizing the incidence of false positives. The isolation procedure must also be cost effective, efficient and fast, even when working with difficult blood samples like old or cadaveric samples. This review illustrates two cell isolation systems, developed by STEMCELL Technologies, which meet these requirements. These systems are particularly suitable for HLA labs as both are performed at room temperature and the reagents have a shelf-life of up to two years from the date of manufacture, which simplifies stock maintenance and minimizes the need to validate and calibrate new lots.

The standard procedure for isolating mononuclear cells from blood samples is to eliminate the red blood cells (RBCs) and granulocytes with a density gradient centrifugation. At the end of the centrifugation, the mononuclear cells are collected at the density gradient medium: plasma interface. This simple technique can be modified slightly to isolate specific populations of the mononuclear cells, such as B or T cells. RosetteSep™ is a reagent that contains a combination of specific monoclonal antibodies designed to eliminate all mononuclear cells except the desired cell types by crosslinking them to RBCs which pellet during centrifugation. The blood is incubated with the RosetteSep™ reagent for 20 minutes at room temperature, and then the sample is layered on top of the density gradient medium, followed by a standard density gradient centrifugation. The unwanted cells, crosslinked to the RBCs, are pelleted and removed. The highly pure cells of interest are collected at the density gradient medium: plasma interface (e.g. ≥ 95% T cells). RosetteSep™ is available for many cell types, including T cells, B cells, total lymphocytes, NK cells, monocytes, and many more. For older blood samples in which granulocytes become less dense and do not pellet as effectively in density gradient centrifugations, granulocytes are specifically targeted for removal by an anti-CD66b antibody

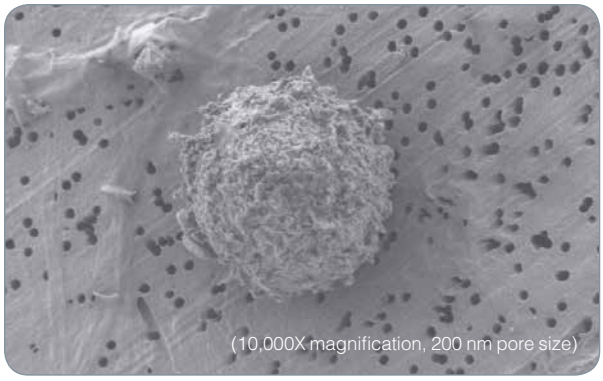


FIGURE 1. Scanning Electron Micrograph of a B cell Isolated using EasySep™ Positive Selection

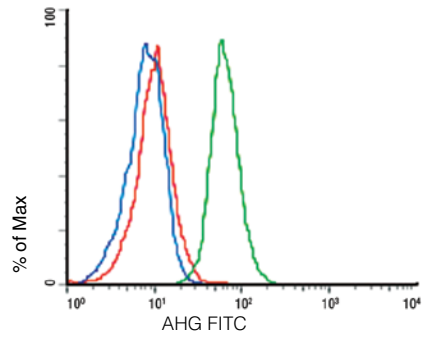
contained in each RosetteSep™ cocktail, making it possible to work with three-day old or refrigerated samples. Because the unwanted cells are targeted for depletion, cells isolated by RosetteSep™ are untouched and ready for subsequent downstream applications.

SepMate™ is a specialized tube that significantly reduces the time necessary for density gradient separations. The SepMate™ tube contains a unique insert that prevents the density gradient medium and blood sample from mixing. The density gradient medium is pipetted through a central hole in the insert, and the sample rapidly pipetted on top of the insert. This eliminates the need to layer the sample directly over the density gradient medium, a time-consuming and highly laborious step. Centrifugation can be carried out with the brake on, further reducing the total time necessary for separation. After centrifugation, the supernatant containing purified cells is simply poured into a new tube. SepMate™ can be used on its own to isolate mononuclear cells in 15 minutes, or combined with RosetteSep™ to isolate untouched and highly purified cell subsets directly from whole blood in 25 minutes.

EasySep™ is a powerful column-free immunomagnetic cell isolation system that can rapidly isolate highly purified cell populations from virtually any type of sample. EasySep™ is available for either depletion of unwanted cells (negative selection) or selection of specific cells of interest (positive selection). EasySep™ negative selection targets and depletes unwanted cells, leaving the cells of interest untouched. Cell isolations can be performed either on mononuclear cell suspensions or directly on whole blood that has been treated with a hetastarch-based agent designed to reduce the red blood cell burden of the sample prior to EasySep™ separation.

The latter method has the advantage of being rapid (< 1 hour), does not require density gradient centrifugation or additional RBC lysis steps, and produces a high yield of highly enriched, viable cells. With EasySep™ positive selection, the cells of interest (from mononuclear cell preparations or whole blood samples) are labeled with tiny magnetic particles, and purities of 99% are possible for many cell types including $CD3^+$ T cells and $CD19^+$ B cells. The EasySep™ Magnetic Particles are extremely small when compared with the size of a cell, and are invisible when viewed under the microscope (Figure 1). The small magnetic particle size reduces the risk of membrane shearing and damage to the cell compared with other magnetic isolation systems. In addition, the particles are flow cytometry-compatible and can be immediately used in any application, including serological typing and flow cytometric crossmatch analysis (Figure 2, Table 1). EasySep™ is available for many cell types, including T cells, B cells, total lymphocytes, NK cells, monocytes and many more.

If working with numerous samples, or to save a significant amount of hands-on time, cell isolations can be fully automated with RoboSep™, which uses EasySep™ reagents to label and separate cells. RoboSep™ is an ideal tool for busy labs as it can simultaneously process up to 4 independent samples with only 5 minutes of hands-on time. This saves technician time, and decreases staff fatigue. Most importantly, sample cross-contamination is eliminated as each sample is handled with a unique set of disposable tips.



Positively selected cells were incubated without serum (blue), with negative serum (red) and with positive serum (green), and stained with FITC-conjugated goat anti-human globulin (AHG).

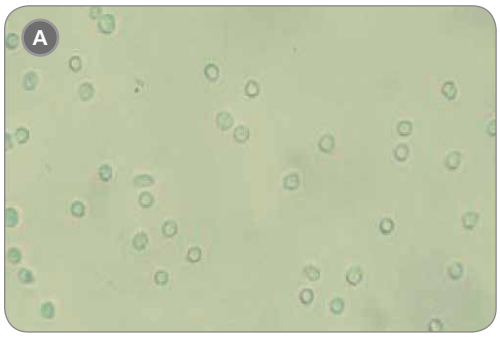
FIGURE 2. Flow Cytometric Crossmatch (FCXM) Analysis Following EasySep™ HLA Whole Blood CD3 Positive Selection

Chimerism analysis is crucial to monitoring post-transplant outcome and determining appropriate post-transplantation therapy.[1-8] Investigating chimerism within specific cell subsets is an increasingly common practice that can increase the sensitivity of assays and provide clinically valuable information.[9-11] For these purposes, chimerism labs require methods for isolating highly purified cells for clear and reliable downstream analysis. EasySep™ positive selection kits are available for isolating a number of relevant cell populations: for example, myeloid versus lymphoid cells, or individual cell populations such as $CD3^+$, $CD14^+$, $CD15^+$, $CD19^+$, $CD33^+$, $CD34^+$, $CD56^+$ and many more (Table 1). Cells can be isolated directly from whole blood or buffy coat, which saves time and minimizes sample handling. Because chimerism analysis is typically performed using small samples, lineage-specific analyses often require isolation of more than one cell type from a single starting sample. Sequential isolation protocols are available to maximize cell recovery from these samples, and can be carried out manually using EasySep™ or via walk-away automation using RoboSep™.

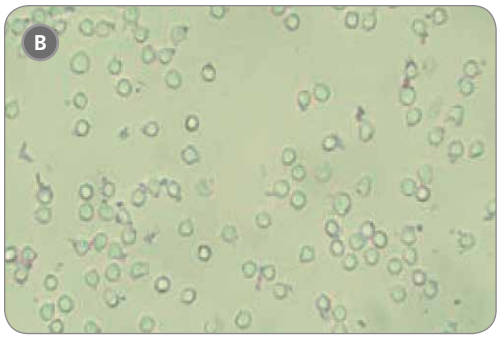
RosetteSep™ and EasySep™ provide cleaner cell suspensions when compared with other magnetic cell separation systems commonly used in HLA labs (Figure 3). The isolated cells show a brighter and more uniform fluorescence in serological HLA typing. Damage to the cells is limited during the isolation procedures, and as a result the risk of false positives is considerably reduced. Scoring is simplified, with less ambiguity, allowing for greater productivity and confidence.

"RosetteSep™ is very good at isolating the T/B cells & removing the platelets to make the cell preparations that we need to perform T/B - 3 color flow crossmatches.It gives us good viability and cell quantity without much manipulation."

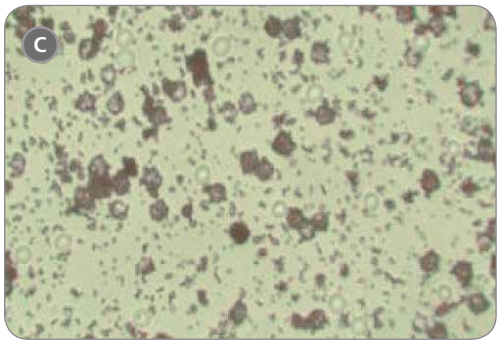
Dr. Mary Ann Head, Immunogenetic Transplant Laboratory, Oregon Health Sciences University



RosetteSep™ Enrichment. Cells are clean and untouched. (100X magnification)



EasySep™ Positive Selection. The tiny flow cytometry-compatible magnetic particles used in the EasySep™ procedure are invisible under the microscope. (100X magnification)



Other Magnetic Isolation Method. The large brown magnetic particles cover the cells and interfere with observation. (100X magnification)

FIGURE 3. Microscope comparison of three cell isolation methods.

T cells were isolated from blood samples using RosetteSep™ (A), EasySep™ Positive Selection (B), and another commercially available magnetic positive selection procedure often used in HLA labs (C). After isolation, the cell suspensions obtained were analyzed by microscopy.

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Kits Commonly Used for HLA Applications

CELL TYPE	SELECTION		SEROLOGY	FCXM[1]		CHIMERISM	
			Blood[2]	PBMC[3]	Blood[2]	PBMC[3]	Blood[2]
Total Lymphocytes	Negative		–	19961HLA	15263HLA[4] 19655 19961HLA	–	–
T Cells	Positive	CD2	–	18657HLA	18687HLA	–	–
		CD3	–	18051HLA	18081HLA	17851 18051HLA	18081HLA
	Negative		15061HLA[4]	17951 19051HLA	15061HLA[4] 19661 19951HLA	–	–
		Lymphoid ($CD3^+$)	–	–	–	–	15271HLA[4]
B Cells	Positive	CD19	–	–	–	17854 18054	18084
		CD19/CD20	–	–	–	–	18184HLA
	Negative		15064HLA[4]	17954 19054HLA	15064HLA[4] 19674 19954HLA	–	–
Myeloid Cells/ Granulocytes	Positive	CD15	–	–	–	–	18681HLA
		CD33	–	–	–	18257	18287HLA
		CD33/66b	–	–	–	–	18683HLA
		CD66b	–	–	–	–	18682
	Negative	Myeloid	–	–	–	–	15272HLA[4]
Monocytes	Positive	CD14	–	–	–	17858, 18058	18088
NK Cells	Positive	CD56	–	–	–	17855, 18055	18085HLA
	Negative		–	–	–	17955	–
Hematopoietic Progenitors	Positive	CD34	–	–	–	18056	18086

EasySep™/RoboSep™ kits RosetteSep™ kits EasySep™ Direct kits

1. Flow Cytometry Crossmatch. 2. Kit also works on other red blood cell containing samples (i.e. cord blood, buffy coat, bone marrow). 3. Peripheral Blood Mononuclear Cell; kit will also work on single cell suspensions of bone marrow, spleen, and lymph node. 4. This kit carries the CE marking.

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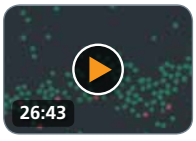

WEBINAR

Cell Separation Solutions for HLA and Chimerism Analysis

www.stemcell.com/HLAWebinar