

# RAPID GRANULOCYTE DEPLETION FROM LYMPHOCYTE PREPARATIONS

*R Siegel and E Klohe  
Inland Northwest Blood Center, Spokane, WA.*

## Introduction

Peripheral blood mononuclear cell (PBMC) preparations often contain a significant number of granulocytes and monocytes. Granulocyte contamination is particularly problematic when whole blood is shipped over long distances at cold temperatures or obtained from cadaveric organ donors treated with steroids. Removal of contaminating cells from lymphocytes ensures the optimal antigen : antibody ratio for HLA testing by preventing competitive absorption of HLA antibodies by non-lymphocytes. However, traditional purification steps increase preparation time and often compromise lymphocyte yield and viability.

We have devised a method for rapid depletion of granulocytes from lymphocyte preparations using Dynabeads™ (Dyna) coated with anti-CD15. In addition, monocytes may be efficiently removed from lymphocytes with anti-CD14 coated Dynabeads™. Simultaneous treatment of PBMCs with anti-CD14 and anti-CD15 coated Dynabeads™ results in lymphocyte preparations with a level of purity comparable to lymph node preparations. Dynabead™ treatment takes approximately *ten minutes*, and does not adversely affect lymphocyte yield or viability. This method is suitable for all downstream applications.

## Materials and Methods

Dynabeads™ are magnetized polystyrene beads coated with mouse IgM monoclonal antibodies directed against selected CD membrane antigens on target cell populations. CD15 beads (myeloid cell; product #111.17) bind human neutrophils, eosinophils, and monocytes (to a variable degree). CD14 beads (selectin positive cells; product #111.11) bind monocytes that may not be completely removed by treatment with CD15 beads. PBMCs were purified by negative selection of lymphocytes following magnet assisted removal of the Dynabead™: granulocyte/monocyte complexes.

To demonstrate the effect of Dynabead™ treatment, we compared five cell preparations from one donor. Fifty milliliters of whole blood were chilled at 4°C for one hour to simulate storage conditions/ temperatures encountered during distant shipment of samples in the winter months. Peripheral blood mononuclear cells were separated by Ficoll density gradient. An untreated sample was retained to represent the “worst case” scenario. Other aliquots of the PBMC preparation were treated with CD15 Dynabeads™, CD14 Dynabeads™, and

both beads simultaneously. Cells were also prepared from lymph nodes. Cell preparations were acquired using a FACScan flow cytometer (Becton Dickinson) and identified in FSC vs SSC dot plots using CellQuest™ software.

## Results

Dot plots with gated lymphocyte (R1), monocyte (R4) and granulocyte (R5) populations illustrate the percentage of leukocyte populations in different cell preparations. **Figure 1** is a cell preparation from lymph node containing virtually 100% lymphocytes (best case scenario). **Figure 2** represents cells obtained from shipped peripheral blood (worst case scenario). **Figures 3, 4 and 5** are cell preparations from the same peripheral blood sample, but treated with CD14 beads alone, CD15 beads alone and both CD14 and CD15 beads, respectively. All treatments result in a marked improvement in cell purity. Simultaneous treatment with CD14 and CD15 beads produces cell preparations that are nearly as pure as cells obtained from lymph nodes.

- Figure 1. Lymph Node Cell Preparation
- Figure 2. Peripheral Blood Mononuclear Cells
- Figure 3. CD14 Treatment
- Figure 4. CD15 Treatment
- Figure 5. CD14 + CD15 Treatment

## Conclusion

CD14 and/or CD15 Dynabeads™ may be used to efficiently and effectively remove contaminating monocytes and granulocytes from PBMCs. It is faster and easier to use than monoclonal antibody mediated lysis of contaminating cells, with no adverse effects on post-treatment lymphocyte viability. Grossly contaminated cell preparations may be purified to the level of lymph node preparations, without significant lymphocyte loss. This method has proven especially useful for whole blood samples shipped from distant locations for living donor kidney transplants. It is suitable for all routine HLA applications, including flow cytometric crossmatches and cytotoxicity assays.