Background on the need to remove granulocytes prior to immunological analysis using ELISPOT:

Immunological monitoring is a critical component in clinical trials. The majority of samples for monitoring are derived from mononuclear cells (PBMC) within whole blood (WB). Typically, the WB samples are drawn and the blood is processed to remove red blood cells, granulocytes, and platelets, most commonly via Ficoll gradient separation. This process is somewhat labor intensive requiring dedicated laboratory space, equipment, and trained lab personnel. More often, the only solution is to ship the WB to a processing center for PBMC isolation and freezing, where the immune monitoring occurs at a later point of time. Functional integrity of the peripheral blood mononuclear cells (PBMC) declines with time from blood draw (1, 2), and it is well established that granulocytes play a major role in this functional decline (3, 4). The longer the storage time until processing of whole blood, the higher is the contamination degree of PBMC with granulocytes (3). During prolonged storage granulocytes become activated and change their buoyancy profile what leads to their less efficient separation during Ficolling. Activated granulocytes are known to suppress T-cell function by down modulating the signal transducing zeta chain of the CD3 molecule (5). Therefore, depletion of granulocytes and/or procedures that will lead to effective granulocyte removal and inhibition of their activation and its inhibitory effects on T-cells shortly after blood draw would extend the functional integrity of PBMC samples to provide meaningful clinical immunological monitoring data. An easy to integrate solution with a controllable and minimal work load is desirable. It would also be advantageous to deplete platelets at the same time.

Depletion of Granulocytes:

Quick Sep-CD15 are unique compared to current magnetic separation products on the market (Table 1). The particles work directly in undiluted whole blood and remove granulocytes very rapidly with quantitative yield of non-targeted immunological lymphocyte populations (Table 2; Figure 1; columns 1-2; 7-12). It is also important to note (Figure 1; columns 3-4) that monocytes are not depleted and are in fact enriched in spite of the fact that a subset of monocytes express CD15. Retention of monocytes is important since they are antigen presenting cells.
Quick Sep-CD15 Advantages

- Quick Sep-CD15 has the following advantages over existing technologies...
  - Very rapid reaction kinetics
  - High specific binding
  - Minimal non-specific binding
  - High recoveries of non-targeted lymphocytes for ELISPOT analysis
  - Works directly in undiluted whole blood and PBMC preparations

Table 2

Whole Blood Quick Sep-CD15 Depletion Procedure

- 50ul CD15 beads are washed and added to 1ml whole blood in 2ml tube
- Tube is rotated end-over-end for 5 minutes
- Tube is placed in magnetic holder for 1 minute
- Whole blood is transferred to a clean tube
- Whole blood samples are stained with CD4-FITC/CD8-PE/CD3-PerCPec5.5/CD56-APC and analyzed on a FACS Calibur
References:


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