
Grievink HW\textsuperscript{1}, Luisman T\textsuperscript{1,2}, Kluft C\textsuperscript{1}, Moerland M\textsuperscript{2}, Malone KE\textsuperscript{1}.

\begin{itemize}
  \item \textsuperscript{1}1 Good Biomarker Sciences, Leiden, the Netherlands.
  \item \textsuperscript{2}2 Centre for Human Drug Research, Leiden, the Netherlands.
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Abstract

Routine techniques for the isolation of human peripheral blood mononuclear cells (PBMCs) include density centrifugation with Ficoll-Paque and isolation by cell preparation tubes (CPTs) and SepMate tubes with Lymphoprep. In a series of experiments, these three PBMC isolation techniques were compared for cell recovery and viability, PBMC population composition, and cell functionality, aiming to provide a starting basis for the selection of the most appropriate method of PBMC isolation for a specific downstream application. PBMCs were freshly isolated from venous blood of healthy male donors, applying the different techniques in parallel. Cell recovery and viability were assessed using a hemacytometer and trypan blue. Immunophenotyping was performed by flow cytometry. Cell functionality was assessed in stimulated (100 ng/mL staphylococcal enterotoxin B [SEB]) and unstimulated 24 hours PBMC cultures, with cytokine production and lactate dehydrogenase (LDH) release as readout measures. PBMC isolation by SepMate and CPT resulted in a 70\% higher recovery than Ficoll isolation. CPT-isolated populations contained more erythrocyte contamination. Cell viability, assessed by trypan blue exclusion, was 100\% for all three isolation techniques. SepMate and CPT isolation gave higher SEB-induced cytokine responses in cell cultures, for IFN\(\gamma\) and for secondary cytokines. IL-6 and IL-8 release in unstimulated cultures was higher for CPT-isolated PBMCs compared to Ficoll- and SepMate-isolated PBMCs. LDH release did not differ between cell isolation techniques. In addition to criteria such as cost and application practicalities, these data may support selection of a specific PBMC isolation technique for downstream analysis.