A new and standardized method to isolate viable, high-quality PBMCs from patient blood samples

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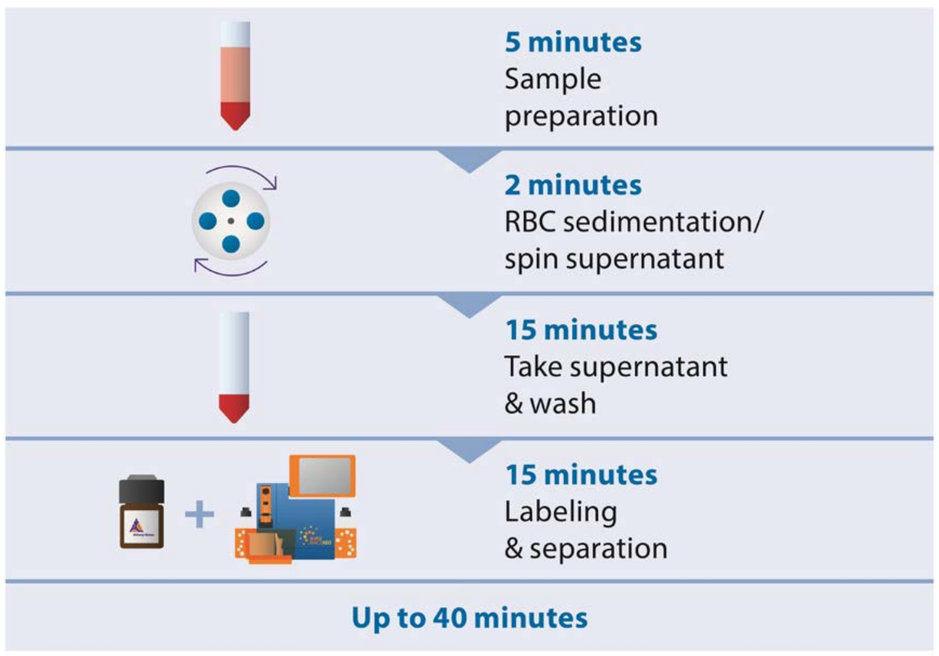
Introduction

With the rise of immunotherapy, monitoring immune responses in clinical trials is crucial. Peripheral blood mononuclear cells (PBMCs) are key analytes for flow cytometry, ELISpot, and transcriptomics, but traditional isolation methods are slow, manual, and require skilled personnel, often compromising sample integrity. We present a standardized, automation-friendly method that yields high-quality PBMCs, even from older samples. This approach minimizes hands-on time, enhances reproducibility, and improves immune monitoring and biomarker discovery in clinical and research settings.

Methods

1 Rapid, automated protocol for PBMC isolation

Figure 1



PBMCs were isolated from whole blood using density gradient centrifugation (Ficoll) or the StraightFrom® Whole Blood PBMC Isolation Kit. Erythrocytes were sedimented and removed using RBC removal antibodies, followed by granulocyte and erythrocyte depletion via magnetic separation (semi-automated or fully automated).

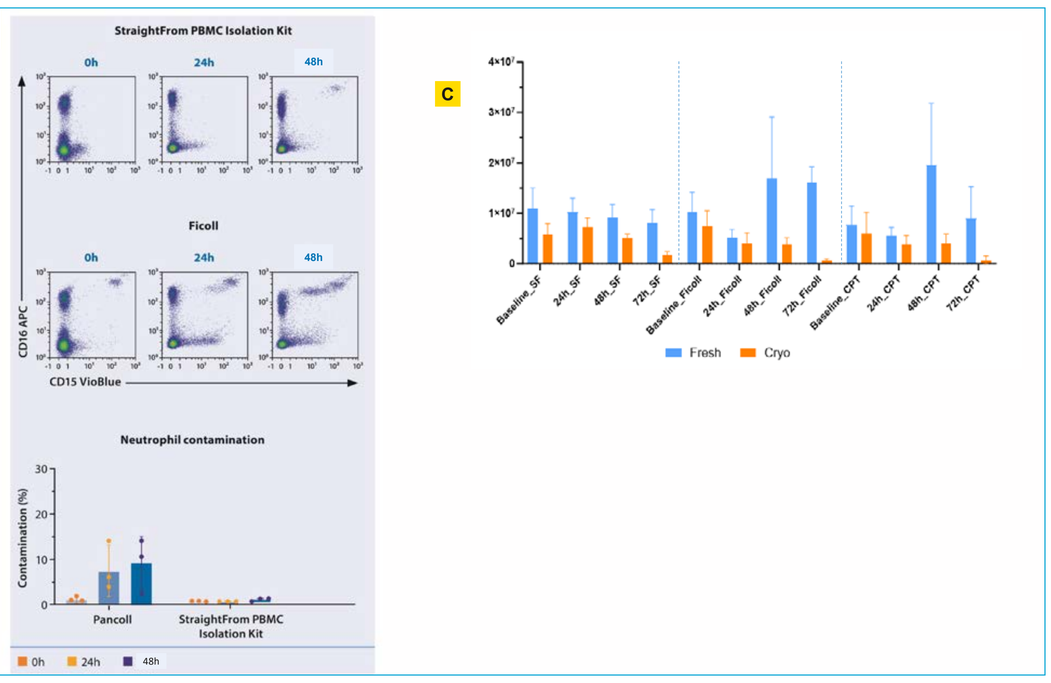
Results

1 Optimized PBMC isolation for delayed sample processing

Figure 2 (A, B) compares the neutrophil contamination of PBMCs isolated using the StraightFrom PBMC Isolation Kit and the Ficoll method. Flow cytometry dot plots illustrate the presence of CD16+CD15+ neutrophils at different time points (0h, 24h, and 48h) following blood storage at room temperature. The results demonstrate that Ficoll-isolated PBMCs show a substantial increase in neutrophil contamination over time, whereas PBMCs isolated using the StraightFrom PBMC Isolation Kit maintain minimal contamination levels. The bar graph quantifies this contamination, showing a significant accumulation of neutrophils in the Ficoll-isolated samples compared to the StraightFrom method.

PBMCs were generated from 10 mL of blood using the StraightFrom® Kit (SF), Ficoll, and CPT tubes at 0h (Baseline), 24h, 48h, and 72h post-blood draw (Fig. 2C). Cell yields were assessed in fresh and cryopreserved samples. Over time, Ficoll and CPT tubes showed increased neutrophil contamination, leading to artificially increased cell counts. In contrast, the StraightFrom® Kit maintained stable and reproducible yields, ensuring reliable PBMC isolation for both on-site and centralized processing, even with shipment delays.

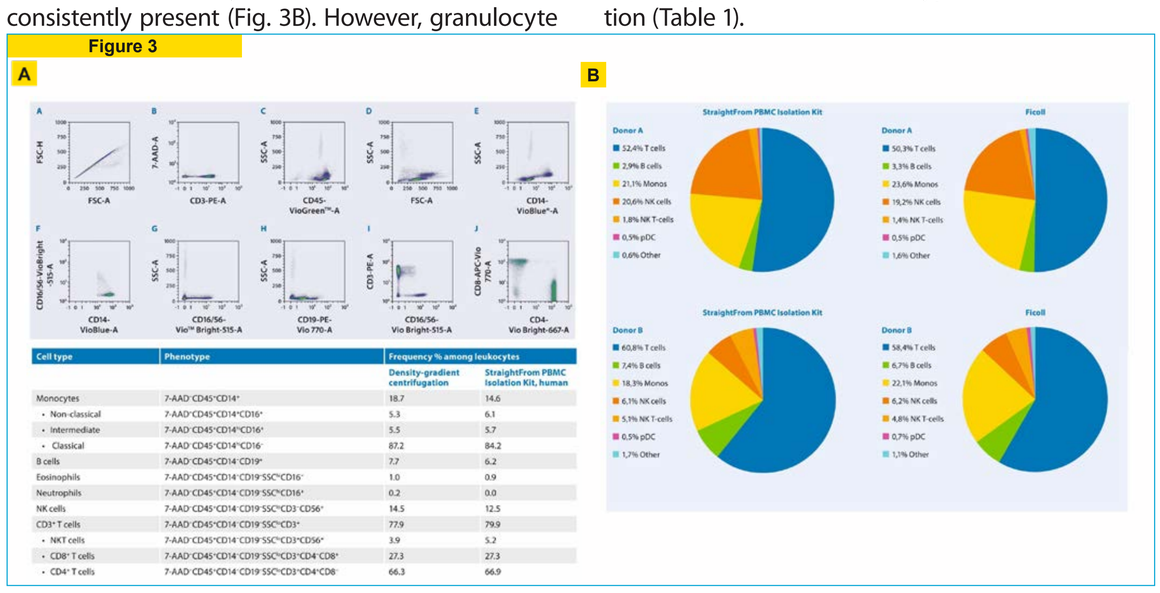
Figure 2



2 Composition of PBMCs

PBMCs were analyzed using the 8 Color Immunophenotyping Kit on a MACSQuant X analyzer (Fig.3 A). Regardless of the isolation method used, the expected PBMC populations—including lymphocytes (T, B, and NK cells), monocytes, and dendritic cells—were consistently present (Fig. 3B). However, granulocyte contamination varied between methods. The StraightFrom PBMC Isolation Kit effectively depleted granulocytes and erythrocytes, whereas the density-gradient centrifugation method resulted in a significant presence of both cell types in the PBMC fraction (Table 1).

Figure 3

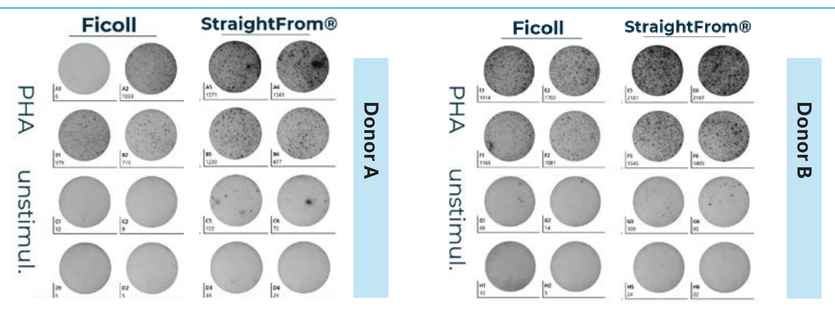


Cell type	Phenotype	Frequency % among leukocytes	
		Density-gradient centrifugation	StraightFrom PBMC Isolation Kit, human
Monocytes	7-AAD⁻CD45⁺CD14⁺	18.7	14.6
• Non-classical	7-AAD⁻CD45⁺CD14⁺CD16⁺	5.3	6.1
• Intermediate	7-AAD⁻CD45⁺CD14⁺CD16⁺	5.5	5.7
• Classical	7-AAD⁻CD45⁺CD14⁺CD16⁻	87.2	84.2
B cells	7-AAD⁻CD45⁺CD14⁻CD19⁺	7.7	6.2
Eosinophils	7-AAD⁻CD45⁺CD14⁻CD19⁻SSC^hiCD16⁻	1.0	0.9
Neutrophils	7-AAD⁻CD45⁺CD14⁻CD19⁻SSC^hiCD16⁺	0.2	0.0
NK cells	7-AAD⁻CD45⁺CD14⁻CD19⁻SSC^loCD3⁻CD56⁺	14.5	12.5
CD3⁺ T cells	7-AAD⁻CD45⁺CD14⁻CD19⁻SSC^loCD3⁺	77.9	79.9
• NKT cells	7-AAD⁻CD45⁺CD14⁻CD19⁻SSC^loCD3⁺CD56⁺	3.9	5.2
• CD8⁺ T cells	7-AAD⁻CD45⁺CD14⁻CD19⁻SSC^loCD3⁺CD4⁻CD8⁺	27.3	27.3
• CD4⁺ T cells	7-AAD⁻CD45⁺CD14⁻CD19⁻SSC^loCD3⁺CD4⁺CD8⁻	66.3	66.9

3 IFNγ ELISpot assay

ELISpot assay comparing Ficoll and StraightFrom methods for PBMC isolation following PHA stimulation. PBMCs from two donors (A and B) were isolated using either Ficoll density gradient centrifugation or the StraightFrom method. Cells were stimulated with phytohemagglutinin (PHA) and assessed for IFNg secretion via ELISpot. Each condition includes technical duplicates. Robust spot formation is observed with PHA, indicating strong immune activation, while negative controls show no spots, confirming assay specificity and baseline activity.

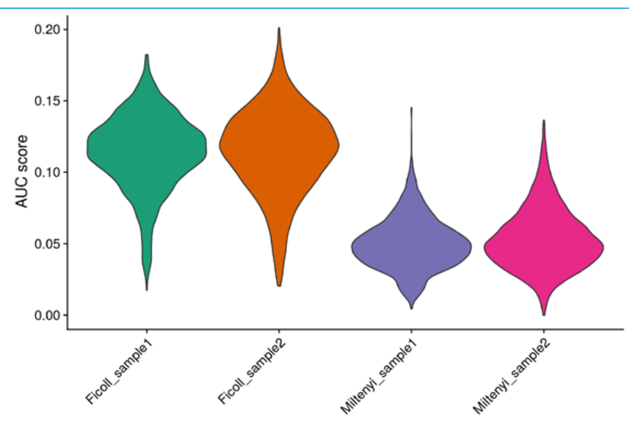
Figure 4



4 scRNA-sequencing

Violin plot showing the AUC scores for inflammatory response and autoimmune signature genes in scRNA-seq data (Fig.5). Samples processed with Ficoll (green, orange) exhibit elevated scores, indicating stress-induced gene expression. In contrast, samples processed with the automated Miltenyi workflow (purple, pink) show minimal activation of these pathways, highlighting the reduced cellular stress and improved sample integrity.

Figure 5



Conclusion

Miltenyi Biotec's StraightFrom PBMC Isolation Kit, based on MACS Technology, offers a reproducible and automatable solution for isolating high-quality PBMCs from patient blood samples. The kit supports a wide range of analysis methods, including flow cytometry, ELISpot, and single-cell sequencing. Designed for use in clinical trials, it enables consistent PBMC yields even from older blood samples and ensures reliable and reproducible processing, regardless of operator experience. Its efficiency, scalability and ease of use make it an essential tool for generating PBMCs both on-site and centralized laboratory settings.