Evaluating PBMC Usage in Clinical Trials: A Comparative Analysis of Cell Populations with Whole Blood in Cytek® Aurora

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Abstract

Immune monitoring of patients enrolled in clinical trials for drug development is of pivotal importance to support evaluation of drug safety and efficacy. The ability to develop high parameter panels with spectral flow cytometry, allows for a deeper characterization of patient samples and for an exhaustive picture of immune system dynamic in response to a specific drug treatment.

Peripheral blood mononuclear cells (PBMC) are increasingly utilized in clinical trials as surrogates for whole blood (WB) samples to monitor immune responses and disease progression. This study aims to evaluate the comparability of PBMC data with traditional blood data, focusing on major cell populations. Experiments were performed using the Cytek cFluor™ Immunoprofiling 14-color kit to characterize T cells, B cells, natural killer (NK) cells, monocytes, and their subsets. PBMCs were isolated from blood obtained from the same donors and collected simultaneously. PBMCs were tested within one week after isolation. Samples were processed in triplicate and acquired on the Cytek Aurora 5-laser instrument. The mean relative percentages of various cell populations were compared between PBMC and WB samples, with an acceptance criterion of less than 20% difference. Note that total leukocytes in PBMC and WB differ due to the cellular composition of the two matrix types. Reportable populations relative to "% of Leukocytes" were not considered for this comparison. Initial validation of this panel with WB showed that all primary populations were within the acceptance criteria of ≤25% CV for repeatability and reproducibility, except for rare populations. Precision data from the initial validation were compared to the PBMC data for further analysis. Our findings indicate that the majority of cell populations, including T cells, B cells, and monocytes, are within the acceptance criteria. However, notable exceptions were observed in the NK cell populations (CD56-CD16+) and the naïve B cell population (CD19+CD27-IgD+). These findings highlight the potential and limitations of using PBMCs as reliable surrogates for blood samples in clinical trials. While most cell populations demonstrate consistent patterns, discrepancies in NK cell and naïve B cell populations necessitate further investigation to understand the underlying mechanisms and optimize PBMC usage for accurate immune profiling. The choice of matrix for clinical sample testing should be based on the intended use of the data and the specific populations of interest to be monitored in patients. Our study underscores the importance of rigorous validation of PBMC data to ensure the reliability of clinical trial outcomes and advance the precision of immunological assessments in medical research. This assay is validated for exploratory assessment.

Method

Reference control:
PBMC was used in single color staining and unstained for reference control.

Antibody cocktail:
Antibody cocktail was prepared for these experiments. Note that ViaDye + cFluor BYG667 + cFluor BYG575 were added separately.

Sample processing:
WB from 3 apparently healthy donors was collected in Cyto-Chex® tubes and in Sodium citrate CPT tubes. Cyto-Chex® tubes were kept refrigerated (2-8°C) until processing (approximately 2 hours). CPT tubes were processed to purify PBMC and cryopreserved in liquid nitrogen until day of testing (approximately 4 days).

Sample Staining and acquisition:
NOTE: Antibody volumes were based on the Cytek cFluor™ IP Kit 14 Color except for ViaDye Red which was titrated in the lab.

1. 100uL WB or 100 µL of PBMC (1 million cells) was added into a staining tube (12mm x 75mm tube).
2. First, 5µL of ViaDye Red was added to the tube and incubated for 15min at room temperature (RT).
3. 3 mL of stain buffer was added, vortexed briefly, and centrifuged at 400 x g for 5 min at RT.
4. After decanting the supernatant, cell pellet was resuspended in 100uL stain buffer (BD Pharmingen stain buffer with BSA, 554657).
5. cFluor BYG667 anti-IgD was added and incubated for 15min at RT.
6. cFluor BYG575 anti-CCR7 was added and incubated for 15min at RT.
7. Appropriate volume from the antibody cocktail was added and incubated for 20min at RT.
8. 3 mL of stain buffer was added, vortexed briefly, and centrifuged at 400 xg for 5min at RT.
9. After decanting the supernatant, cell pellet was resuspended in 250uL fixation buffer (BD Cytofix, 554655).
10. After the incubation for 15 min at 2°C-8°C, 3mL of stain buffer was added, vortexed briefly and centrifuged at 400 xg for 5min at RT.
11. After decanting the supernatant, cell pellet was resuspended in 300uL stain buffer.
12. Cells were acquired on Cytek Aurora instrument.

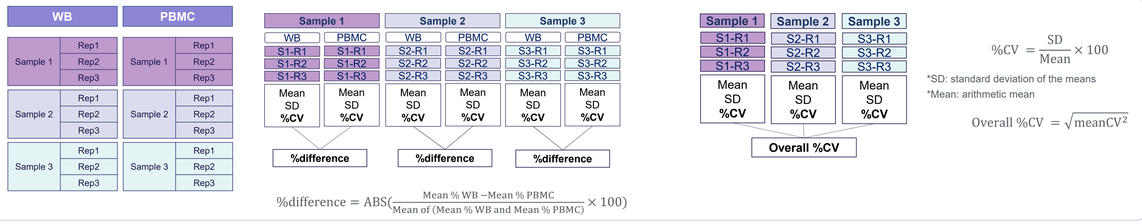
Reportable List

Reportables	Relative %
NKT cells	% NK cells
Monocytes	% Leukocytes
Classical	% Monocytes
Intermediate	
Non-classical	
B cells	% Lymphocytes
Naïve	% B cells
Memory	
IgD+ Memory	
NK cells	% Lymphocytes
$CD56^{high}$	% NK cells
$CD56^{dim}$	
CD56- CD16+	
Lymphocytes	% Leukocytes
T cells	% Lymphocytes
CD4+ T Cells	% T cells
Naïve	% CD4+ T cells
Central Memory (CM)	
Effector Memory (TEM)	
RA+ Effector memory (TEMRA)	
T regulatory T cells	
CD8+ T cells	% T cells
Naïve	% CD8+ T cells
Central Memory (CM)	
Effector Memory (TEM)	
RA+ Effector memory (TEMRA)	

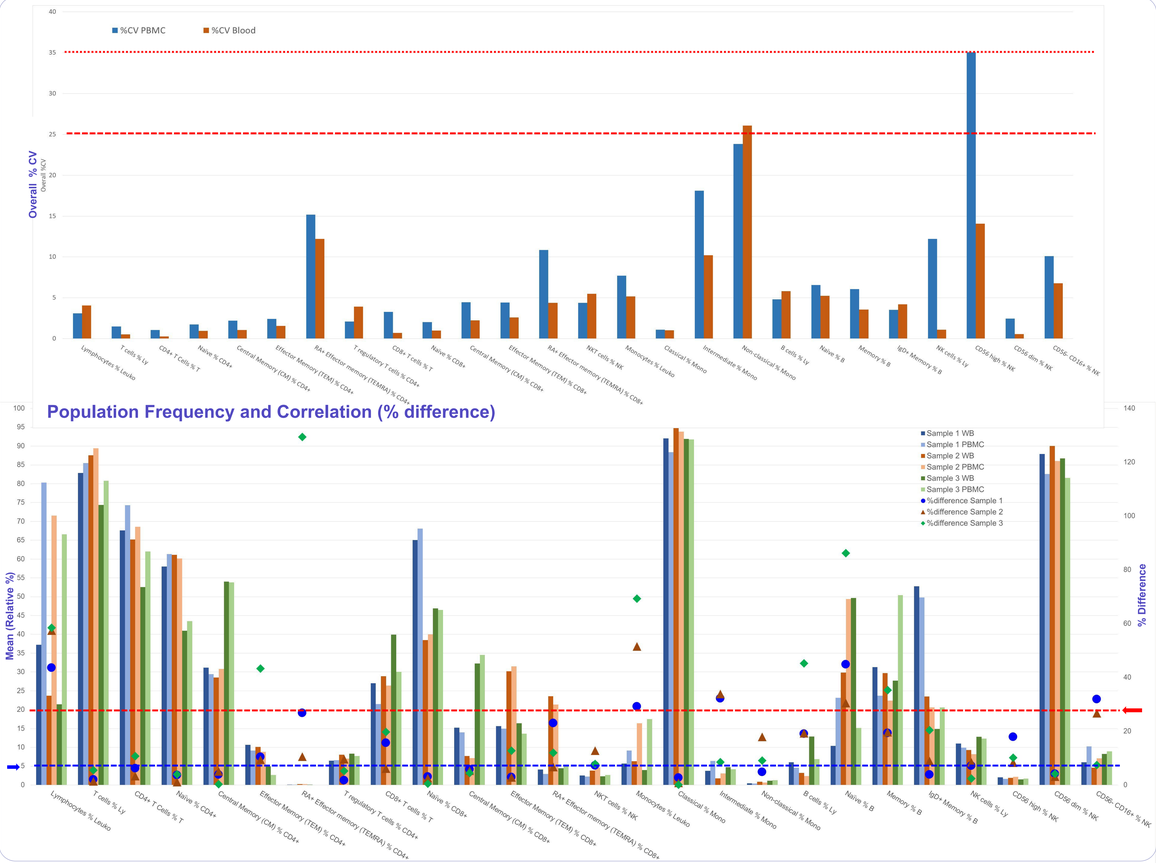
Panel Configuration

Violet		Blue		Red	
Marker	Fluorochrome cFluor	Marker	Fluorochrome cFluor	Marker	Fluorochrome cFluor
CD3	V420	CD8	B515	CD127	R659
CD14	V450	CCR7*	BYG575	CD16	R668
CD45	V547	IgD*	BYG667	CD56	R720
		CD45RA	B690	CD4	R780
		CD19	BYG710	viability	ViaDye Red
		CD25	BYG781	CD27	R840

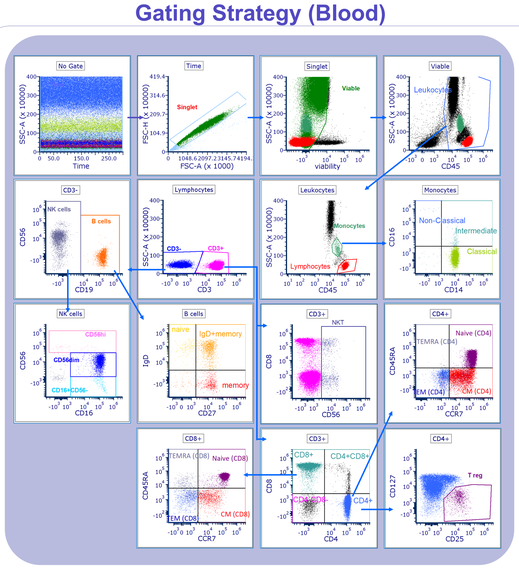
Experiment setup and calculation



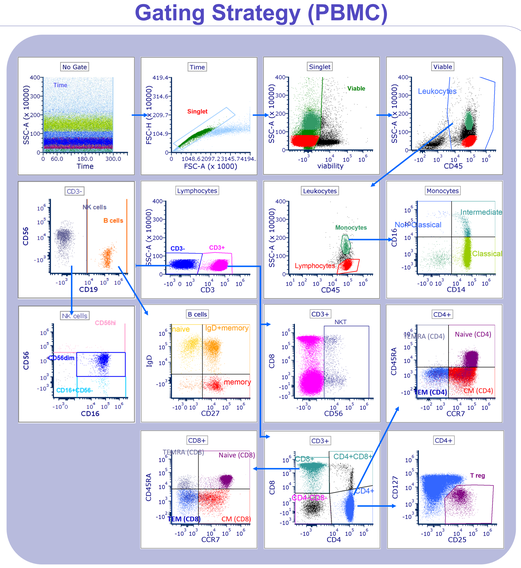
Results



Gating Strategy (Blood)



Gating Strategy (PBMC)



Conclusion

All the primary reportables are within the acceptance criteria for repeatability for both WB and PBMC. Non-classical monocytes and $CD56^{high}$ NK cells are rare population in both WB and PBMC and the %CV is <35% which is acceptable. Most populations correlate well between WB and PBMC. As seen in the plots, leukocytes population is different between WB and PBMC, notably granulocytes are absent in PBMC. Some of the subset populations and rare populations show high variability. Based on these data, both WB and PBMC are suitable for clinical sample testing. The choice of which matrix to use should be made based on the intended use of the data; WB would be preferred for holistic evaluation of all leukocytes, while PBMC would be preferred for lymphocytes and subsets.

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