Validation of a 15-Color Spectral Flow Cytometry Panel for Globally Harmonized Comprehensive Leukocyte Profiling in Human Whole Blood: Translational and Clinical Applications

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1. INTRODUCTION

Study needs — **Solutions**

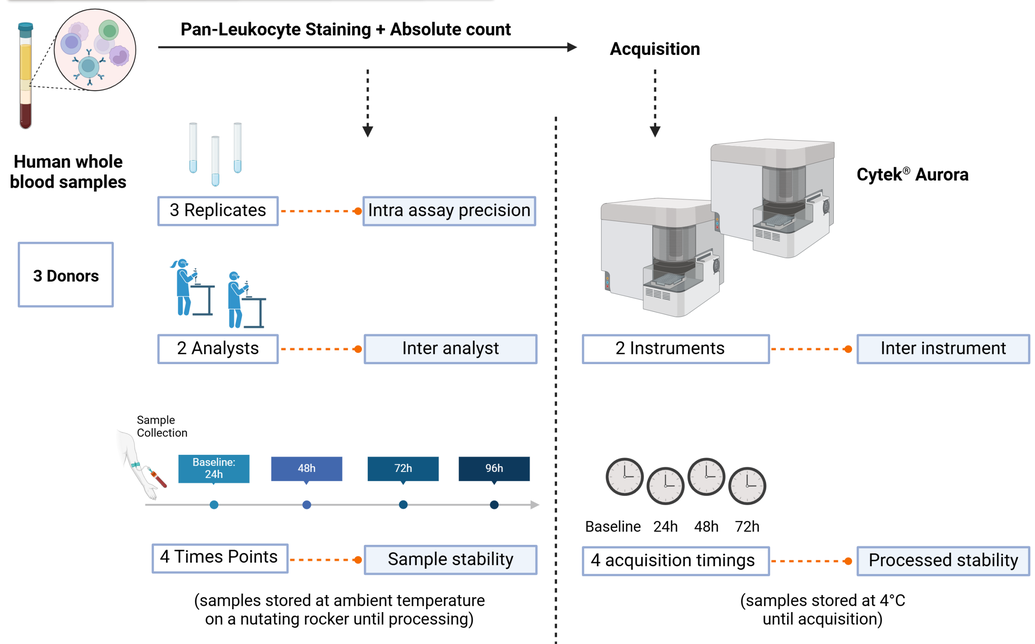
- **Immunomonitoring**
- **Reliable and reproducible data**
- **Clinical studies applications**

- **Validated flow cytometry Method**

Assessed populations

- **Neutrophils**
- **Eosinophils**
- **Basophils**
- **Hematopoietic Stem Cells (HSC)**
- **Monocyte subsets:** - Classical, - Intermediate, - Non-Classical
- **T cell subsets:** - CD4+, - CD8+
- **B cells**
- **NK cell subsets:** - early, - mature, - terminal
- **NKT cells**

2. VALIDATION PROCESS

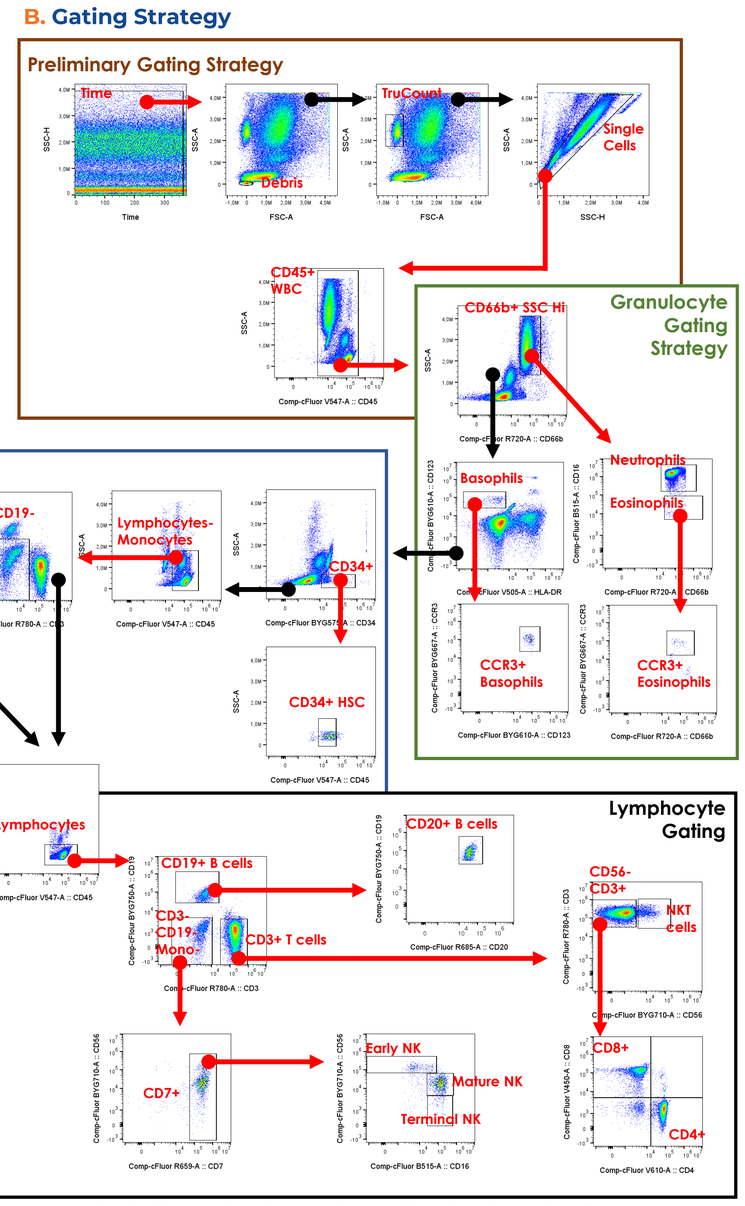


3. SPECTRAL FLOW CYTOMETRY PANEL OPTIMIZATION

A. 15-color pan-leukocyte panel

Target	Clone	Fluorophore
CD45	HI30	cFluor® V547
CD66b	G10F5	cFluor® R720
CD123	6H6	cFluor® BYG610
CD193 (CCR3)	5E8	cFluor® BYG667
CD34	4H11	cFluor® BYG575
CD14	MEM-15	cFluor® BYG781
CD16	3G8	cFluor® B515
HLA-DR	L243	cFluor® V505
CD7	CD7-6B7	cFluor® R659
CD19	HIB19	cFluor® BYG750
CD20	2H7	cFluor® R685
CD3	SK7	cFluor® R780
CD4	SK3	cFluor® V610
CD8	SK1	cFluor® V450
CD56	LT56	cFluor® BYG710

B. Gating Strategy



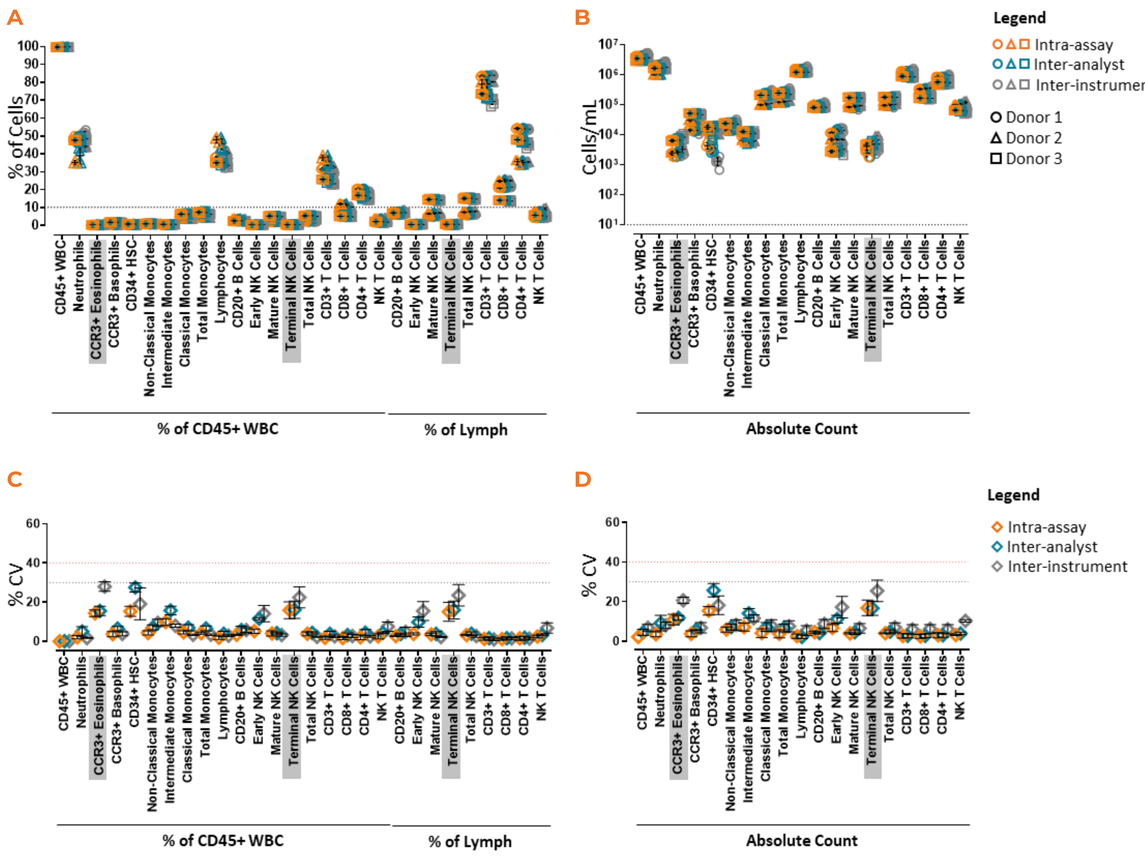
Red arrows *indicate the analysis focused on cells within the gate:*
Black arrows *indicate the analysis of cells outside the gate:*

Panel and Gating strategy used to identify the populations of interest.

A) 15-color pan-leukocyte panel used for the validation.

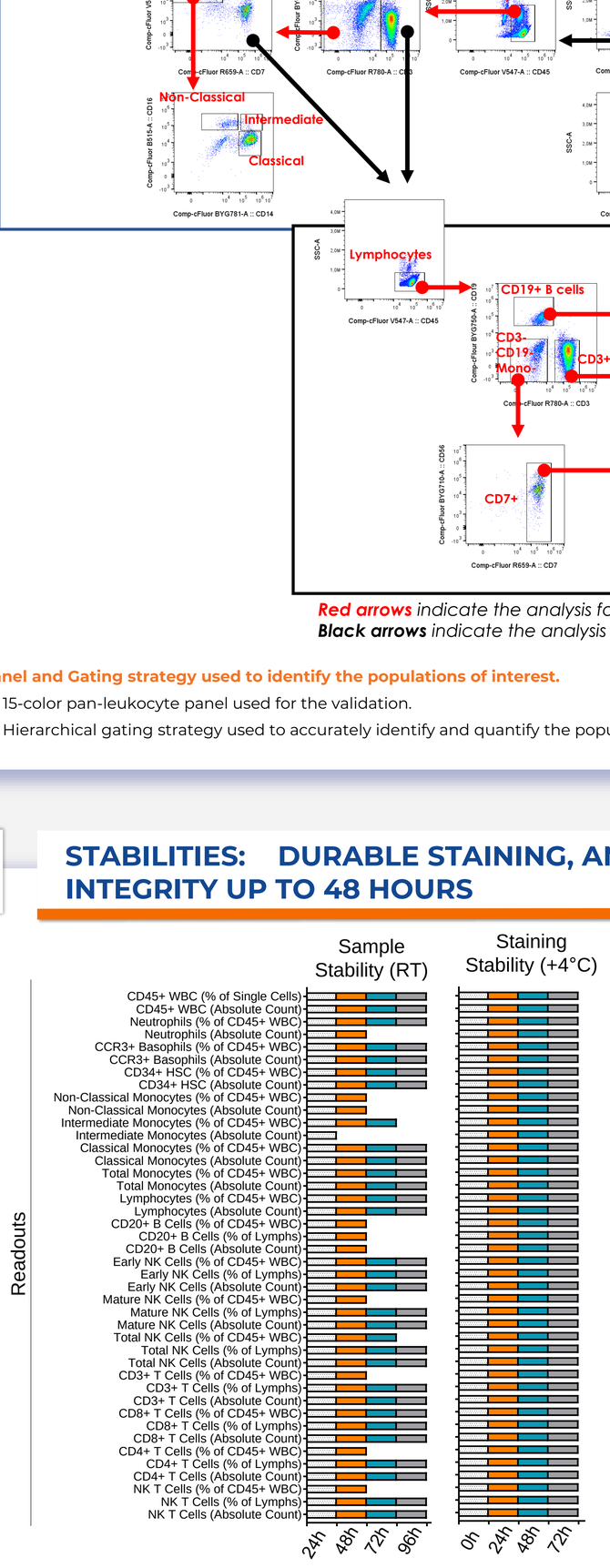
B) Hierarchical gating strategy used to accurately identify and quantify the populations of interest in each validation assay.

4. PRECISION: CONSISTENT PRECISION ACROSS ALL VALIDATION PHASES



Precision assessment of all readouts. A) Percentage of cells measured for each identified population (x-axis) relative to the total CD45+ WBC and the total lymphocytes for lymphocyte populations. **B)** Absolute count of cells for each identified population (x-axis), reported as cells/mL of whole blood. **C-D)** Mean % CV across all three donors for each readout, shown for both frequency (C) and absolute count (D). Readouts with less than 100 gated events are highlighted in grey. **E)** Pie charts for each validation assay, illustrating the proportion of readouts categorized into six levels of mean % CV measured in the three donors. The light orange line highlights values of % CV below 20%.

5. STABILITIES: DURABLE STAINING, AND RELIABLE SAMPLE INTEGRITY UP TO 48 HOURS



Sample and Staining Stability Results:

Each bar represents the latest time points at which each readout meets the acceptance criteria (30% mean percentage change for non-rare subsets and 40% for rare subsets).

The sections within each bar correspond to specific stability levels.

Failure to meet the acceptance criteria at any time point resulted in automatic disqualification for all subsequent time points.

6. CONCLUSIONS

Robust analytical method to investigate the main players of the immune system in human blood sample

- **Consistent & Robust Assays** → **Reliable Results**
- **Ready-to-use** → **Expedited and straightforward results**
- **Customizable** → **Adaptable for client's need**
- **Cross-validated** → **For multisite clinical studies**