Maxpar Human Immune Monitoring Panel Kit Validation Studies

Introduction

Whether researchers are following the immune response to treatments for cancer, infection or autoimmune disease or searching for biomarkers of disease onset and progression, they need a comprehensive, reliable method to monitor changes in immune cell populations.

The Maxpar® Human Immune Monitoring Panel Kit is a groundbreaking single-tube approach to immune profiling research enabling identification and enumeration of all key immune cell subsets in human peripheral blood mononuclear cells (PBMC) with Helios™, a CyTOF® system. The 29-marker mass cytometry panel expands the breadth of immune cell identification beyond current flow cytometric approaches, providing a reliable tool for immune monitoring studies that minimizes use of reagents, time and precious sample while maximizing actionable information.

In addition to the antibody panel (provided individually packaged), the kit contains all other reagents needed for sample staining, as well as:

- a step-by-step, detailed protocol for cell staining and sample collection on a Helios or CyTOF 2-to-Helios mass cytometry system;
- a Helios acquisition template specifically designed for use with the kit;
- access to both manual (Cytobank) and automated (GemStone™ by Verity Software House) third-party data analysis solutions.

The antibodies included in the kit undergo rigorous QC and are tested as a panel, ensuring reproducible performance and lot-to-lot consistency in population identification. We describe here the performance evaluation of the Maxpar Human Immune Monitoring Panel Kit (Cat. No. 201324), including measurements of panel repeatability; reproducibility across multiple samples, technicians and instruments; and an assessment of the impact on immune cell population resolution due to steric hindrance. Taken together, the results of this study demonstrate that the kit provides a repeatable, reproducible method for deep immune profiling studies of PBMC and that the full 29-marker panel functions as well as smaller panels for immune cell identification.

Relevant Fluidigm Documents

- Maxpar Human Immune Monitoring Panel Kit Cell Staining and Data Acquisition Protocol (PN PRD027)
- Approach to Bivariate Analysis of Data Acquired Using the Maxpar Human Immune Monitoring Panel Kit Technical Note (PN 400270)

Background

The 29 markers in the Maxpar Human Immune Monitoring Panel Kit include all those recommended by the Human ImmunoPhenotyping Consortium (HIPC)[1] plus six additional markers that enable identification of γδ T cells and granulocytes and better definition of certain T cell subsets (Table 1). The kit also includes reagents needed to stain for single, viable cells. Up to 40 immune cell populations (Table 2) were identified with the manual bivariate gating strategy found in the Premium Cytobank public experiment Fluidigm Maxpar Human Immune Monitoring Panel Kit 201324 Gating Example v1.0[2] and the application note Approach to Bivariate Analysis of Data Acquired Using the Maxpar Human Immune Monitoring Panel Kit[3] (PN 400270).

The Maxpar Human Immune Monitoring Panel Kit gating strategy was developed based on the Human ImmunoPhenotyping Consortium (HIPC) consensus panel [Maecker et al. *Nature Reviews Immunology* (2012)][1] and professional input from Verity Software House.

Table 1. Maxpar Human Immune Monitoring Panel Kit antibodies

Antibody (Clone)	Metal
Anti-Human CD45 (HI30)	Y89
Anti-Human CD196/CCR6 (G034E3)	141Pr
Anti-Human CD19 (HIB19)	142Nd
Anti-Human CD127/IL-7Ra (A019D5)	143Nd
Anti-Human CD38 (HIT2)	144Nd
Anti-Human IgD (IA6-2)	146Nd
Anti-Human CD11c (Bu15)	147Sm
Anti-Human CD16 (3G8)	148Nd
Anti-Human CD194/CCR4 (L291H4)	149Sm
Anti-Human CD123/IL-3R (6H6)	151Eu
Anti-Human TCRgd (11F2)	152Sm
Anti-Human CD185/CXCR5 (RF8B2)	153Eu
Anti-Human CD3 (UCHT1)	154Sm
Anti-Human CD45RA (HI100)	155Gd
Anti-Human CD27 (L128)	158Gd
Anti-Human CD28 (CD28.2)	160Gd
Anti-Human CD66b (80H3)	162Dy
Anti-Human CD183/CXCR3 (G025H7)	163Dy
Anti-Human CD161 (HP-3G10)	164Dy
Anti-Human CD45RO (UCHL1)	165Ho
Anti-Human CD24 (ML5)	166Er
Anti-Human CD197/CCR7 (G043H7)	167Er
Anti-Human CD8 (SK1)	168Er
Anti-Human CD25 (2A3)	169Tm
Anti-Human CD20 (2H7)	171Yb
Anti-Human HLA-DR (L243)	173Yb
Anti-Human CD4 (SK3)	174Yb
Anti-Human CD14 (M5E2)	175Lu
Anti-Human CD56 (NCAM16.2)	176Yb

Table 2. Immune populations identified by manual gating with the Premium Cytobank public experiment Fluidigm_Maxpar Human Immune Monitoring Panel Kit_201324_Gating Example_v1.0[2]

Immune Populations	
Lymphocytes, dendritic cells (DCs), monocytes	CCR4+ CD4 Treg Memory
Total granulocytes	Th1 cells
CD3+ T cells	Th2 cells
Alpha beta T cells	Th17 cells
CD8 T cells	Total B cells
CD8 T cells, naive	Naive B cells
CD8 T cells, central memory (CM)	B cells, IgD- memory
CD8 T cells, effector memory (EM)	B cells, IgD+ memory
CD8 T cells, terminal effector (TE)	Transitional B cells
CD8 T cells, activated	Plasmablasts
CD4 T cells	Total monocytes
CD4 T cells, naive	Non-classical monocytes
CD4 T cells, central memory (CM)	Classical monocytes
CD4 T cells, effector memory (EM)	Total DCs
CD4 T cells, terminal effector (TE)	CD11c- CD123+ pDC
CD4 T cells, activated	CD11c+CD123- mDC
Total CD4+ Treg	Total NK cells
Total CCR4+ CD4+ Treg	CD16- NK cells
CCR4+ CD4 Treg naive	CD16+ NK cells
Total CCR4+ CD4+ Treg activated	Gamma-delta T cells

Materials and Methods

PBMC Preparation

Frozen human PBMC, isolated by leukapheresis, were purchased from Cellular Technology Ltd. Following the ImmunoSpot® CTL PBMC Thawing Protocol[4], 10 mL of 1X CTL Anti-Aggregate Wash™ Supplement (Cellular Technology Ltd.) was prepared for each PBMC vial thawed. PBMC vials were thawed at 37 °C, washed in 1X anti-aggregate wash, and re-suspended in serum-free RPMI 1640 supplemented with L-glutamine and penicillin-streptomycin for cell counting. Cells were counted on a TC20™ Automated Cell Counter (Bio-Rad Laboratories) using Trypan Blue viability dye (Bio-Rad Laboratories).

Cell Staining

Cells were stained using the Maxpar Human Immune Monitoring Panel Kit (Cat. No. 201324) according to the manufacturer's instructions[5].

Sample Acquisition

Sample acquisition was performed 18–24 hours post staining on a Helios system or CyTOF 2-to-Helios system with CyTOF Software version 6.7.1014, according to the Human Immune Monitoring Panel Kit Protocol[3]. All instruments were equipped with a WB Injector and samples acquired in Cell Acquisition Solution (CAS). Following instrument tuning and bead sensitivity test, the system was pre-conditioned with CAS. Cells were acquired at a concentration of 1 x 10^6 cells/mL using the Maxpar Human Immune Monitoring Panel Kit acquisition template (Hu_ImmuneMonitoring_Panel_201324_Acq.tem). A minimum of 300,000 events were acquired per file at a typical acquisition rate of 250–425 events/second.

Data Processing and Data Analysis

Following cell acquisition, the raw FCS files were normalized using the default settings on CyTOF Software 6.7.1014. The normalized FCS files were uploaded to Cytobank for analysis. The gating strategy in the public experiment titled Fluidigm_Maxpar Human Immune Monitoring Panel Kit_201324_Gating Example_v.10[2] was imported. Each gate was manually reviewed and adjusted according to the Approach to Bivariate Analysis of Data Acquired Using the Maxpar Human Immune Monitoring Panel Kit Technical Note[3]. The population event counts were exported using Cytobank's Export Statistics page[6].

Frequencies were calculated for each immune population as a function of the total live, single-cell population gated as described in the Approach to Bivariate Analysis of Data Acquired Using the Maxpar Human Immune Monitoring Panel Kit Technical Note[3].

Experimental Design

Immune cell populations with frequencies of 5% or greater were evaluated in the following experiments.

Repeatability

Repeat measurement variation was assessed using a single PBMC donor stained in triplicate by one technician. Each staining replicate was acquired in triplicate on a Helios system, for a total of 9 FCS files. This experiment was repeated on a CyTOF 2-to-Helios system to assess instrument variation.

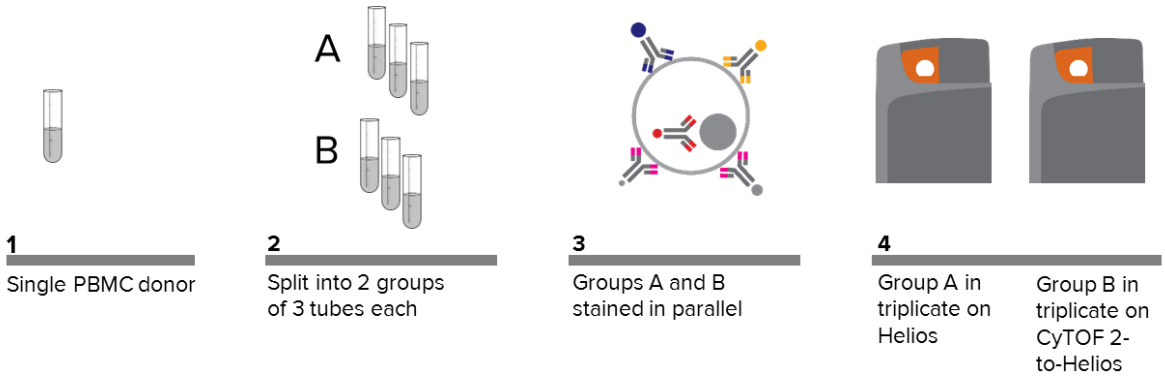


Figure 1. Workflow for assessing repeatability of the Maxpar Human Immune Monitoring Panel Kit with one PBMC donor

Reproducibility

Measurement variation was assessed using five PBMC donors, five technicians, and two instruments. Each technician prepared one staining replicate of each PBMC donor. Each specimen was acquired on two instruments, a Helios system and a CyTOF 2-to-Helios system, over two days, one instrument per day.

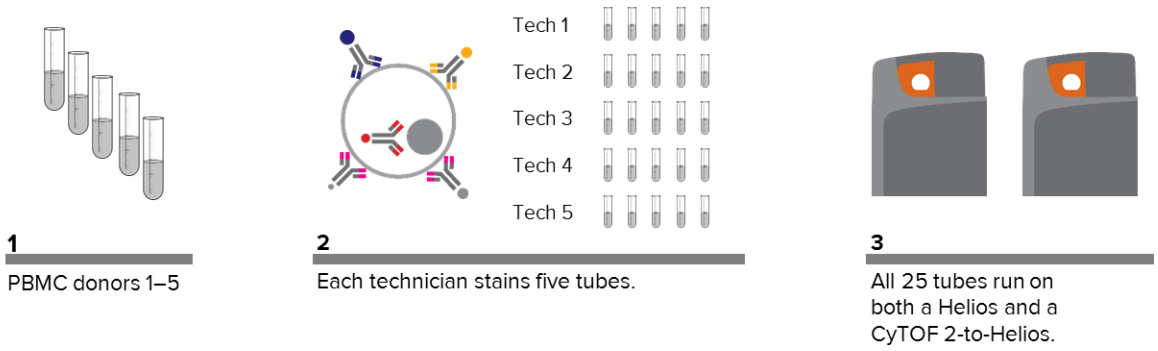


Figure 2. Workflow for assessing reproducibility of the Maxpar Human Immune Monitoring Panel Kit with multiple PBMC donors and multiple technicians

Immune Population Resolution

The Maxpar Human Immune Monitoring Panel Kit was assessed for consistent population identification and enumeration by comparing the staining results using the entire kit panel to smaller subpanels of immune cell markers. A single technician stained each of four different PBMC specimens with the full panel and with three subpanels of 10–12 antibodies (Table 3) to detect the specific major immune populations listed in Table 4 (T cells, B cells, and monocytes). Specimens were acquired on a Helios and a CyTOF 2-to-Helios system. See Figure 3 for workflow.

Table 3. Antibody subpanels used for immune population resolution test

T Cell Subpanel	
Antibody (Clone)	**Metal**
Anti-Human CD45 (HI30)	Y89
Anti-Human CD19 (HIB19)	142Nd
Anti-Human TCRgd (11F2)	152Sm
Anti-Human CD3 (UCHT1)	154Sm
Anti-Human CD45RA (HI100)	155Gd
Anti-Human CD66b (80H3)	162Dy
Anti-Human CD197/CCR7 (G043H7)	167Er
Anti-Human CD8 (SK1)	168Er
Anti-Human CD4 (SK3)	174Yb
Anti-Human CD14 (M5E2)	175Lu

B Cell Subpanel	
Antibody (Clone)	**Metal**
Anti-Human CD45 (HI30)	Y89
Anti-Human CD19 (HIB19)	142Nd
Anti-Human CD38 (HIT2)	144Nd
Anti-Human IgD (IA6-2)	146Nd
Anti-Human CD185/CXCR5 (RF8B2)	153Eu
Anti-Human CD3 (UCHT1)	154Sm
Anti-Human CD27 (L128)	158Gd
Anti-Human CD66b (80H3)	162Dy
Anti-Human CD24 (ML5)	166Er
Anti-Human CD20 (2H7)	171Yb
Anti-Human CD14 (M5E2)	175Lu

Monocytes/DC/NK Subpanel	
Antibody (Clone)	**Metal**
Anti-Human CD45 (HI30)	Y89
Anti-Human CD19 (HIB19)	142Nd
Anti-Human CD11c (Bu15)	147Sm
Anti-Human CD16 (3G8)	148Nd
Anti-Human CD123/IL-3R (6H6)	151Eu
Anti-Human CD3 (UCHT1)	154Sm
Anti-Human CD66b (80H3)	162Dy
Anti-Human CD161 (HP-3G10)	164Dy
Anti-Human CD20 (2H7)	171Yb
Anti-Human HLA-DR (L243)	173Yb
Anti-Human CD14 (M5E2)	175Lu
Anti-Human CD56 (NCAM16.2)	176Yb

Table 4. Populations identified by subpanels

T Cell Subpanel	B Cells Subpanel	Monocytes Subpanel
Total lymphocytes	Total lymphocytes	Total lymphocytes
Alpha beta T cells	CD3+ T cells	CD3+ T cells
CD3+ T cells	B cells	Total monocytes
CD4 T cells	Naive B cells	Classical monocytes
CD4 T cells, naive*		
CD8 T cells		
CD8 T cells, naive*		

*Naive CD4 and CD8 T cells were identified as CCR7+CD45RA+.

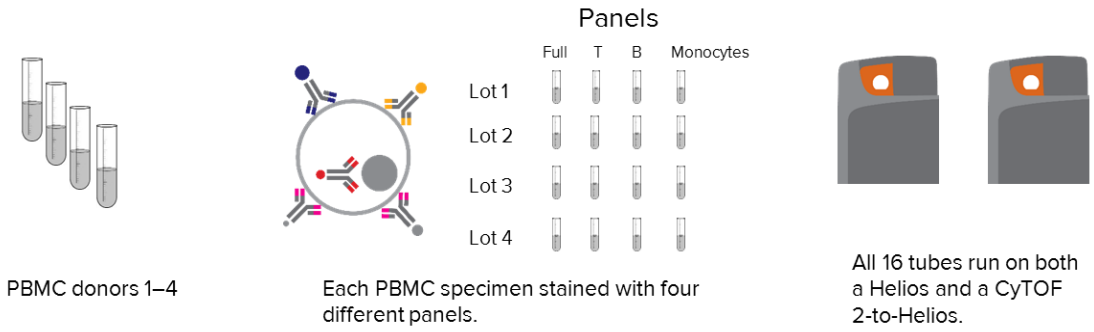


Figure 3. Workflow for assessing panel performance of the Maxpar Human Immune Monitoring Panel Kit with multiple PBMC donors and subpanels

Results

The studies described here were designed to evaluate the Maxpar Human Immune Monitoring Panel Kit for repeatability, reproducibility, and ability of the full panel to resolve populations as compared to smaller subpanels.

Repeatability

Repeatability is a measure of precision over replicate staining and acquisition of a single specimen and was measured as the coefficient of variation (CV) of the immune cell population frequencies. PBMC from a single donor were stained using the Maxpar Human Immune Monitoring Panel Kit and acquired in nine repeat measurements on two instruments, a CyTOF 2-to-Helios and a Helios. All populations measured had standard deviations (SD) of less than 2% and CVs of less than 20% for both instruments (Figure 4.) Overall, the panel kit shows a high level of repeatability in identifying immune cell populations and measuring population frequencies.

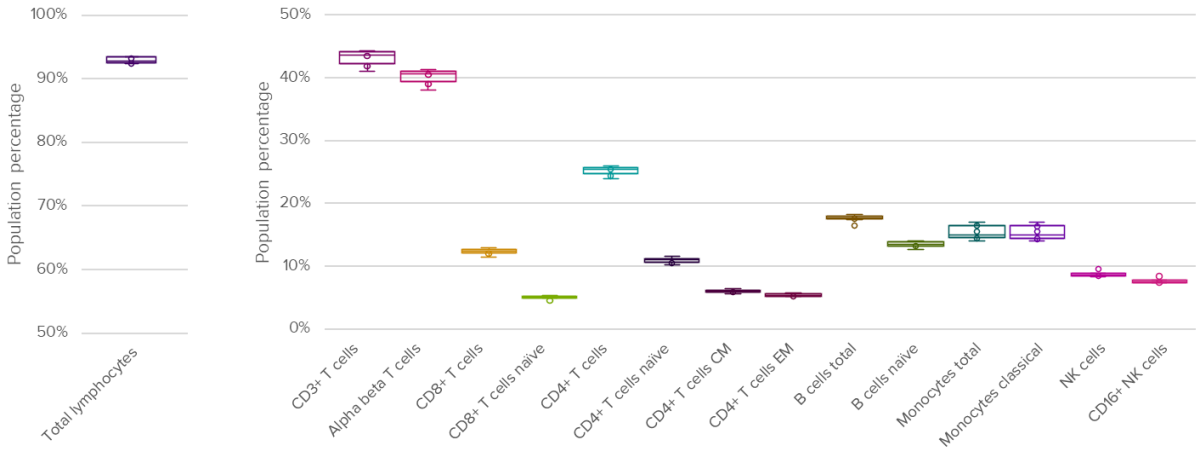
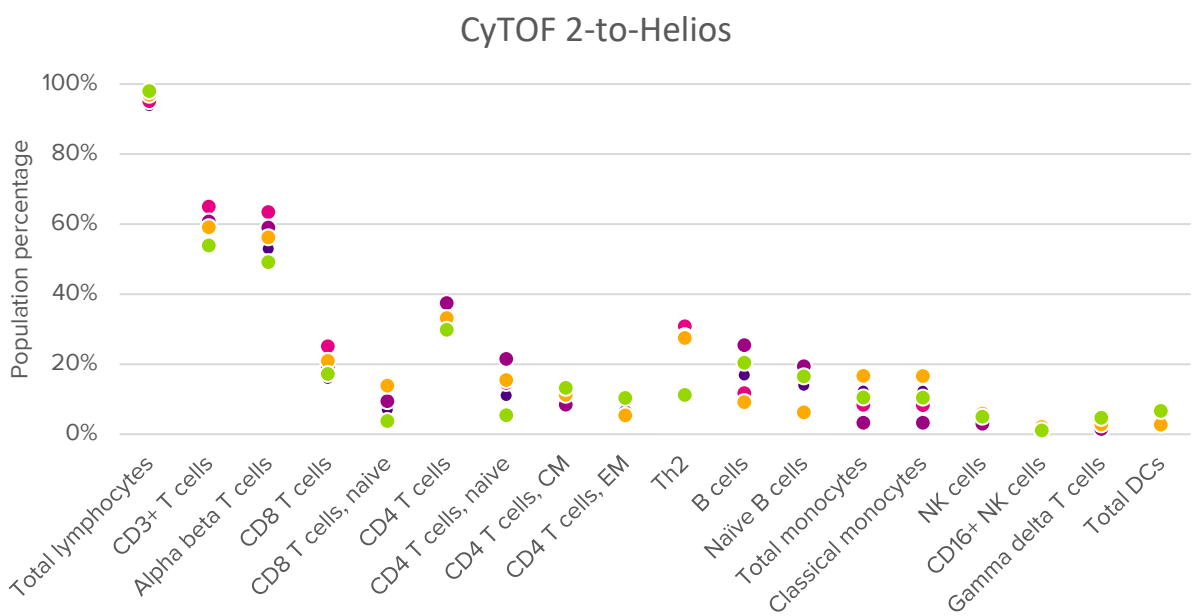


Figure 4. Median population percentages and interquartile ranges as determined for 15 immune cell subsets from a single PBMC donor collected on both a CyTOF 2-to-Helios and a Helios instrument. N = 18 measurements for each population

Reproducibility

To measure the reproducibility of cell frequency determinations, five PBMC donor specimens were stained by five different technicians and acquired on two instruments. The average population frequencies and CVs were calculated for each donor and instrument. When run on either instrument, populations with frequencies greater than 5% (Figures 5A and B) had CVs of less than 10%, except for central memory (CM) CD4 T cells and monocytes (total and classical), which still had CVs under 20% (Figures 5C and D). These results demonstrate that the Maxpar Human Immune Monitoring Panel Kit shows a high degree of reproducibility across multiple PBMC donors.

A



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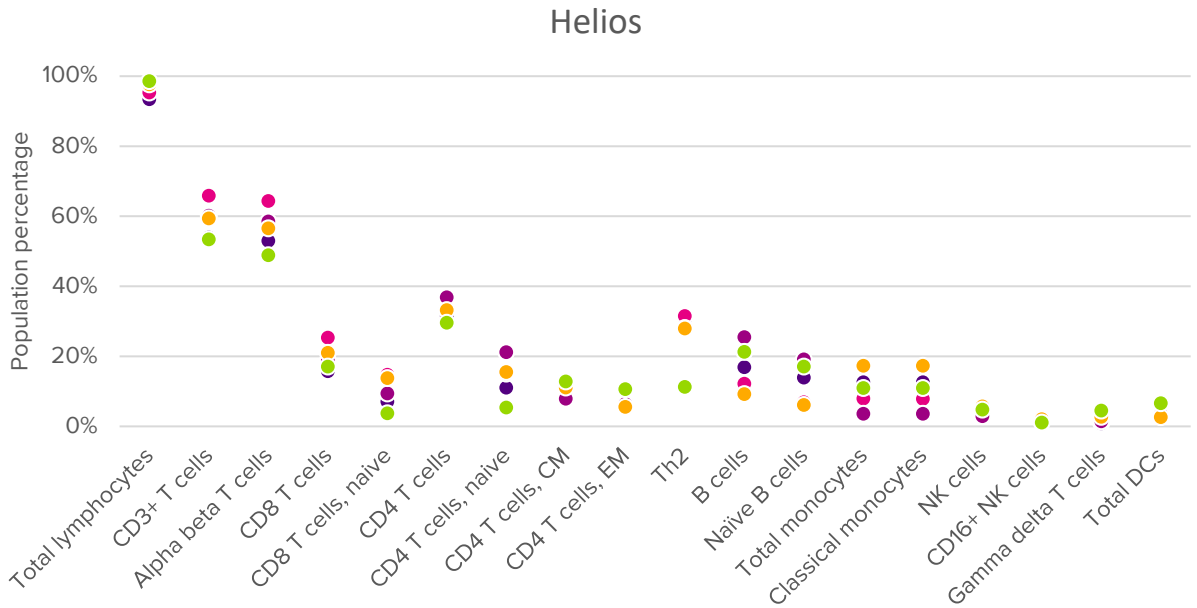

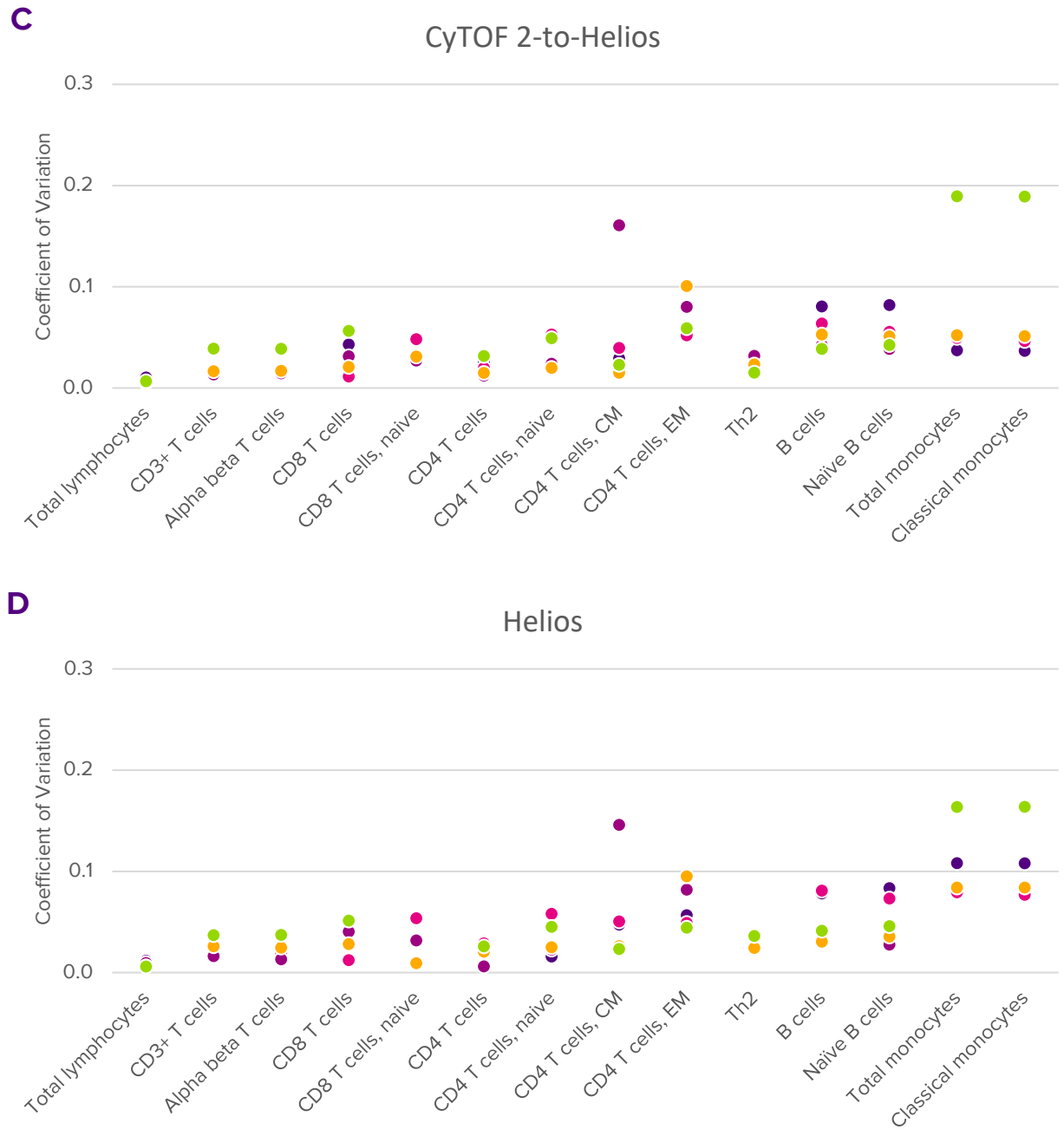

Figure 5. Reproducibility of the Maxpar Human Immune Monitoring Panel Kit. The average frequencies and CVs of major immune populations are depicted for each PBMC donor, for both CyTOF 2-to-Helios (A, C) and Helios (B, D). (See Experimental Design). Each donor is depicted with a different color **Donor 1, Donor 2, Donor 3, Donor 4 and Donor 5**).

Immune Population Resolution

This set of experiments was designed to determine whether high-multiplex mass cytometry staining would result in steric hindrance, thus impacting population identification and enumeration. The population resolution performance of the full 29-marker panel was compared to those of three smaller subpanels consisting of 10 to 12 markers that identify major immune populations. Four PBMC specimens were stained by one technician and acquired on two instruments (See Experimental Design).

The percentage of each specified population was plotted for all donors and instruments for the full versus partial panels and the R^2 values determined per population (Figure 6A, T cell subsets; 6B, monocyte and B cell subsets). The correlation (R^2) between the full and partial panel immune population frequencies was >0.95 for all subpopulations (Table 5). This high correlation demonstrates that the rigorous QC and titration of the antibodies used in the kit negates any theoretic effects on reliable immune cell population identification due to steric hindrance or the use of metal isotope-labeled antibodies.

Table 5. Correlation (R^2) values for identified populations across all PBMC donors

T Cell Subpanel	R^2
Total lymphocytes	0.953
Alpha beta T cells	0.982
CD3+ T cells	0.977
CD4 T cell	0.965
CD4 T cell, naive*	0.994
CD8 T cell	0.989
CD 8 T cell, naive*	0.997

Monocyte Subpanel	R^2
Total monocytes	0.976
Classical monocytes	0.974

B Cell Subpanel	R^2
B cells	0.994
Naïve B cells	0.997

*Naive CD4 and CD8 T cells were identified as CCR7+CD45RA+.

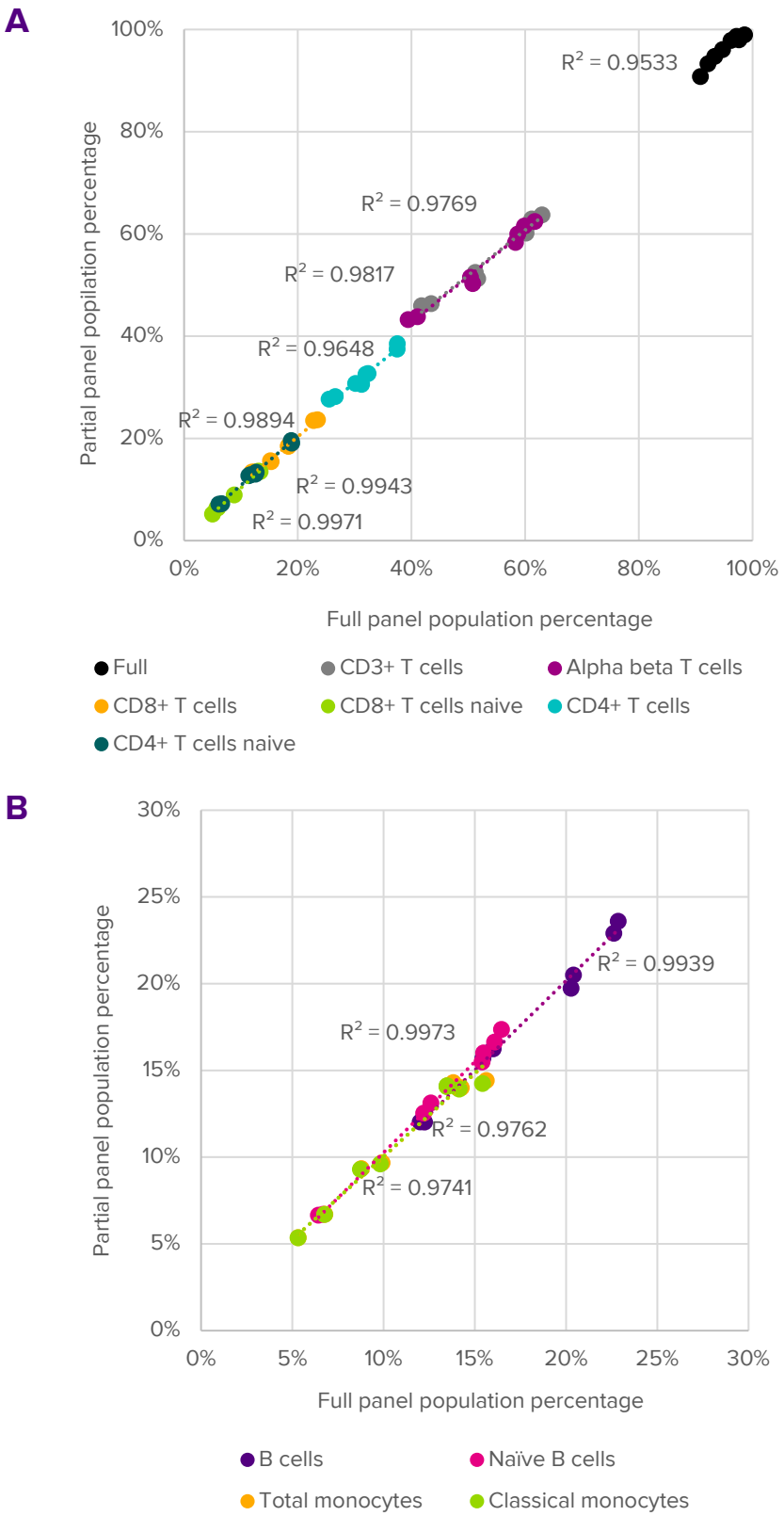

Figure 6. Immune population resolution performance of the Maxpar Human Immune Monitoring Panel Kit compared to partial panel sets. A, Total lymphocytes and T cell subsets. B. Monocyte and B cell subsets.

Discussion

Conclusions

These studies demonstrate that the Maxpar Human Immune Monitoring Panel Kit provides repeatable, reproducible identification and enumeration of a broad array of immune cell populations. In addition, the performance of the 29-marker panel compared to the small subpanels produces reliable identification of major immune populations, showing that signal interference from such a large panel does not impact immune cell population identification.

Additional Considerations

The Maxpar Human Immune Monitoring Panel Kit undergoes extensive internal quality control (QC) procedures ensuring that 1) antibodies in the kit are tested as a panel and do not require additional titration when used on PBMC from healthy nontreated subjects*, and 2) rigorous lot-to-lot panel QC is performed to ensure reproducible results. These measures save the user significant time, reagent, and use of precious samples as compared to designing, validating, and implementing an in-house developed panel.

The kit does lend itself, however, to customization because the antibodies are individually packaged and there are at least eight open channels for additional markers even when all 29 antibodies in the kit are used. Any addition of antibodies external to the kit requires re-titration and validation of performance.

The Maxpar Human Immune Monitoring Panel Kit is a powerful tool for deep immune profiling of peripheral blood mononuclear test samples and makes a valuable addition to the growing use of mass cytometry as a routine approach to immune monitoring in clinical research and translational research studies[7–16].

*A preliminary staining on noncritical samples is recommended to evaluate panel staining and determine if any minor antibody adjustments are needed.

References

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2 Fluidigm Maxpar Human Immune Monitoring Panel Kit 201324 Gating Example v1.0

3 Approach to Bivariate Analysis of Data Acquired Using the Maxpar Human Immune Monitoring Panel Kit Technical Note (PN 400270)

4 http://www.immunospot.com/resources/protocols/PBMC-thawing-protocol.htm

5 Maxpar Human Immune Monitoring Panel Kit Cell Staining and Data Acquisition Protocol (PN PRD027)

6 https://premium.cytobank.org/cytobank/experiments/151716/export/statistics

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