Automated Versus Manual Sample Preparation for 25-Color Flow Cytometric Immunophenotyping of Human PBMCs with Agilent NovoStation

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Introduction

Flow cytometry enables high-dimensional immunophenotyping of peripheral blood mononuclear cells (PBMCs), but the preparation of complex antibody cocktails, as well as staining, incubation, washing, and fixation steps can often be labor-intensive and prone to variability. To address these challenges, we adopted the Agilent NovoStation automated sample preparation system for 25-color immunophenotyping and compared its performance with conventional manual workflows.

The NovoStation features integrated reagent and sample traceability, onboard cooling modules to preserve antibody stability, and precise liquid-level detection for accurate pipetting. It accommodates diverse biological sample types and reliably executes standard flow cytometry assay workflow steps, including staining, washing, fixation, and permeabilization. This platform also features native first-party integration with Agilent NovoCyte flow cytometers to enable automated acquisition of prepared samples.

In this study, 25-color antibody cocktails, single-stained controls, and multistained PBMC samples were prepared either manually or using the NovoStation sample preparation system, followed by acquisition on an integrated NovoCyte Opteon spectral flow cytometer. Comparative analyses assessed population percentages, resolution of immunophenotypic subsets, cell recovery, and hands-on time.

Experimental

Study design

- Sample: Human PBMC; unstained, single-color reference controls, and triplicate multicolor samples were prepared (30 samples total)
- Instruments: Agilent NovoStation sample preparation system and Agilent NovoCyte Opteon special flow cytometer, integrated for automated, hands-free sample-to-result workflow
- Sample acquisition and data analysis software: NovoExpress V2.2.0

No.	Maker	Fluorochrome	Clone	No.	Maker	Fluorochrome	Clone
1	Vδ2 TCR	BUV395	B6	14	CCR7	BV785	G043H7
2	CD27	BUV496	M-T271	15	CD123	FITC	6H6
3	CD33	BUV563	WM53	16	CD19	PerCP-eF710	J3-129
4	CD56	BUV615	NCAM16.2	17	CD24	PE	ML5
5	CD11c	BUV661	B-ly6	18	CXCR3	PE-Dazzle594	G025H7
6	IgD	BUV737	IA6-2	19	CD38	PE-Cy5	HIT2
7	CD8	BUV805	SK1	20	CD4	PE-AF700	S3.6
8	CD25	BV421	M-A251	21	γδ TCR	PE-Cy7	B1
9	CD45	BV510	HI30	22	CD127	APC	A019D5
10	CD3	BV570	UCHT1	23	CCR6	APC-R700	11A9
11	CD16	BV605	3G8	24	Amine	Zombie NIR	NA
12	HLA-DR	BV650	L243	25	CD45RA	APC-Cy7	HI100
13	CD14	BV711	MφP9				

Table 1. Reagents used for the 25-color immunophenotyping panel.

Workflow comparison

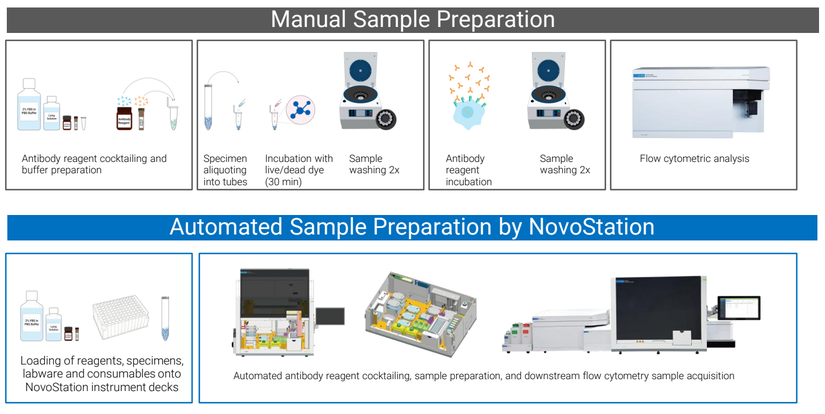


Figure 1. Workflow comparison between manual sample preparation (top) and automated sample preparation (bottom) using the Agilent NovoStation system, which streamlines this 25-color assay by greatly reducing hands-on assay steps.

Results and Discussion

Flow cytometry data comparisons between manual and automated workflow

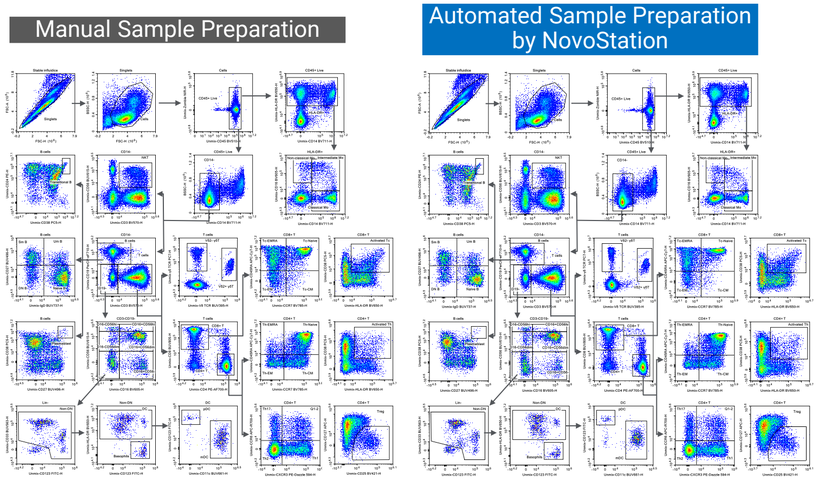


Figure 2. Scatter and dot plots showing the gating strategy to identify 44 immune cell populations from human PBMCs, showing comparable resolution in each population between manual (left) and automated (right) methods.

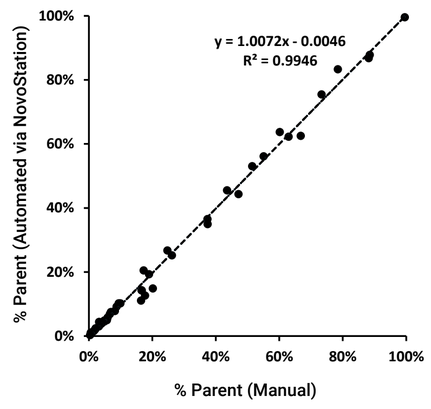


Figure 3. Comparison in % parent frequencies of 44 gated immune cell populations from 25-color PBMC staining between manual and automated (NovoStation) methods, illustrating the quantitative concordance in results.

% Parent	Manual		NovoStation	
	Average	SD	Average	SD
CD45+ Live	99.53%	99.56%	0.03%	0.02%
CD14-	88.50%	0.36%	87.78%	0.61%
HLA-DR+	37.41%	0.22%	34.92%	0.74%
Non-classical Mo	4.84%	0.08%	4.49%	0.05%
Intermediate Mo	1.06%	0.10%	1.20%	0.14%
Classical Mo	26.15%	0.72%	25.18%	1.23%
NKT	5.87%	0.10%	5.74%	0.16%
CD3-CD19-	9.95%	0.17%	10.18%	0.08%
B cells	16.63%	0.37%	14.27%	0.34%
T cells	73.31%	0.34%	75.44%	0.27%
Vδ2- γδT	3.86%	0.02%	3.83%	0.07%
Vδ2+ γδT	3.13%	0.02%	2.99%	0.08%
CD4+ T	55.06%	0.15%	56.14%	0.43%
CD8+ T	37.38%	0.13%	36.52%	0.49%
Activated Th	4.00%	0.24%	3.99%	0.28%
Th-CM	17.68%	0.25%	12.62%	0.10%
Th-EM	18.95%	0.15%	19.28%	1.06%
Th-EMRA	3.23%	0.16%	4.38%	0.69%
Th-Naive	60.14%	0.02%	63.71%	0.38%
Activated Tc	16.61%	0.77%	14.27%	0.61%
Tc-CM	0.56%	0.16%	1.03%	0.82%
Tc-EM	8.75%	0.02%	9.16%	0.07%
Tc-EMRA	43.55%	0.17%	45.53%	1.01%
Tc-Naive	47.13%	0.30%	44.29%	2.37%
Th1	24.72%	0.19%	26.69%	0.31%
Th2	62.91%	0.22%	62.26%	0.37%
Th17	5.65%	0.12%	4.93%	0.39%
Treg	6.91%	0.20%	7.50%	0.19%
Transitional B	1.94%	0.05%	2.02%	0.20%
Plasmablast	0.38%	0.01%	0.29%	0.03%
IgD-CD27- B	9.41%	0.67%	10.21%	0.30%
Swiched memory B	66.74%	1.79%	62.49%	1.34%
Naive B	6.59%	0.24%	6.82%	0.25%
Unswiched memory B	17.27%	1.09%	20.48%	0.94%
CD16-CD56dim	4.83%	0.49%	4.86%	0.44%
CD16-CD56hi	2.10%	0.22%	2.39%	0.22%
CD16+CD56-	16.43%	0.43%	11.05%	0.17%
CD16+CD56dim	51.46%	1.39%	53.01%	1.87%
CD16+CD56hi	1.59%	0.12%	1.61%	0.11%
Basophils	20.13%	2.07%	14.84%	0.89%
DC	78.43%	2.08%	83.30%	0.83%
mDC	88.16%	0.69%	86.75%	0.44%
pDC	8.18%	0.05%	7.83%	0.11%

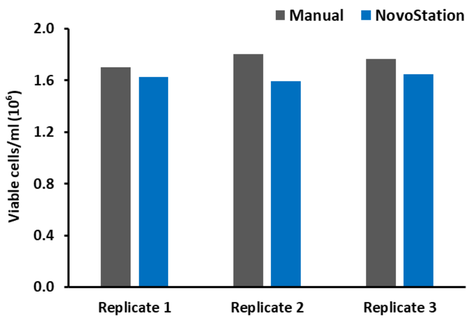


Figure 4. Automated sample processing using NovoStation (blue bars) offers comparable viable CD45+ cell counts in three replicate samples from the same donor, compared to manual methods (black bars) as measured using Zombie NIR dye.

Step	Manual		NovoStation	
		Time (min)		Time (min)
Prepare Cocktail	Hands-On	17.8	Hands-On	2.5
			Hands-Off	16.9
Aliquot PBMC samples and add Zombie NIR for incubation, then wash 2x	Hands-On	17.9	Hands-On	5.4
	Hands-Off	51.0	Hands-Off	166
Add antibody for incubation and wash 2x	Hands-On	49.8		
	Hands-Off	34.0		
Total	Hands-On	85.5	Hands-On	7.9
	Hands-Off	85.0	Hands-Off	182.9
	Hands-on & off	170.5	Hands-on & off	190.8

Figure 5. The number of hands-on assay steps and the length of hands-on time are both significantly reduced when using NovoStation automation to prepare 30 samples using a 25-color assay protocol, compared with manual methods.

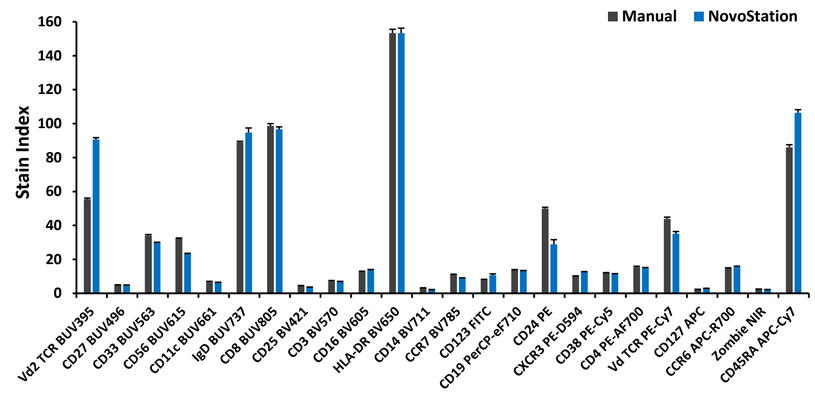


Figure 6. Stain index comparison of 24 fluorochromes from this panel (CD45 BV510 excluded) indicates comparable resolution in lymphocytes when sample processing is performed using NovoStation automation (blue bars), compared with manual method (black bars).

Conclusions

- We compared flow cytometry sample preparation methods between manual processing and automated processing (using Agilent NovoStation sample preparation system) of human PBMCs in the context of a 25-color immunophenotyping panel
- Results showed both sample preparation methods achieved comparable flow cytometry results with regard to antibody reagent stain index, resolution of 44 immune cell populations, and cell viability
- Utilizing the NovoStation enabled us to greatly reduce the number of hands-on steps and duration of hands-on time needed for performing this 25-color assay
- NovoStation can automatically initiate flow cytometric sample acquisition on a connected NovoCyte Opteon spectral flow cytometer, enabling a true hands-free “sample-to-answer” workflow

References

[1]Moncunill G, Han H, Dobaño C, McElrath MJ, De Rosa SC. OMIP-024: pan-leukocyte immunophenotypic characterization of PBMC subsets in human samples. Cytometry A. 2014 Dec;85(12):995-8.
[2]Maecker HT, McCoy JP, Nussenblatt R. Standardizing immunophenotyping for the Human Immunology Project. Nat Rev Immunol. 2012 Feb 17;12(3):191-200.

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