Streamlined Automated Sample Preparation for Kappa/Lambda Light Chain Assessment Using Agilent NovoStation Sample Preparation System and NovoCyte Opteon Spectral Flow Cytometer

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Introduction

Accurate assessment of kappa/lambda (κ/λ) immunoglobulin light chain expression by flow cytometry remains technically challenging due to potential interference by cytophilic antibodies and serum immunoglobulins, particularly IgG, which can adhere to B cell surfaces and obscure true κ/λ signals. Thus, multiple prewashing steps are commonly recommended to remove interfering proteins, to reduce background and to improve resolution of κ/λ expression on peripheral blood B cells. However, prewashing can also introduce operational difficulties, including cell loss, reduce cell viability, and elevated background signals.

In this study, we employed the Agilent NovoStation sample preparation system to process specimens for a 12-color immunophenotyping panel. Whole blood samples were prepared either manually or via an automated workflow, which incorporated sequential prewashing, staining, lysis, and post-label washing, followed by acquisition on the NovoCyte Opteon spectral flow cytometer. Comparative analyses evaluated κ/λ resolution, cell recovery, population frequencies, and hands-on time.

Experimental

Study design

- Three normal donor peripheral blood samples.
- Unstained and single-stained reference controls, as well as triplicate multicolor samples (24 samples total), were prepared by manual and fully automated protocols.
- The Agilent NovoStation sample preparation system integrates the Agilent NovoCyte Opteon spectral flow cytometer, enabling automated, hands-free transition between sample preparation and data acquisition.
- All raw data were further analyzed using NovoExpress (v2.2.0) software.

Specificity	Fluorochrome	Clone	Source	Catalog number
CD45	PerCP	2D1	DAKO	PR701
CD3	PerCP-Cy5.5	SK7	Agilent	8931015
CD4	BUV496	RPA-T4	BD	741134
CD8	Pacific Blue	SK1	Biolegend	344718
CD7	PE-Cy5	CD7-6B7	Agilent	8931050
CD5	APC	L17F12	Agilent	8930230
CD16	Alexa Fluor 700	3G8	Biolegend	302026
CD19	PE-Cy7	HIB19	Agilent	8930015
Kappa	FITC	Polyclonal	Dako	F043401-8CN
Lambda	PE	Polyclonal	Dako	R043701-8CN
CD20	Spark YG 593	2H7	Biolegend	302367
CD10	APC-Fire750	HI10a	Agilent	8931036

Table 1. Reagents used for the 12-color immunophenotyping panel.

Workflow comparison

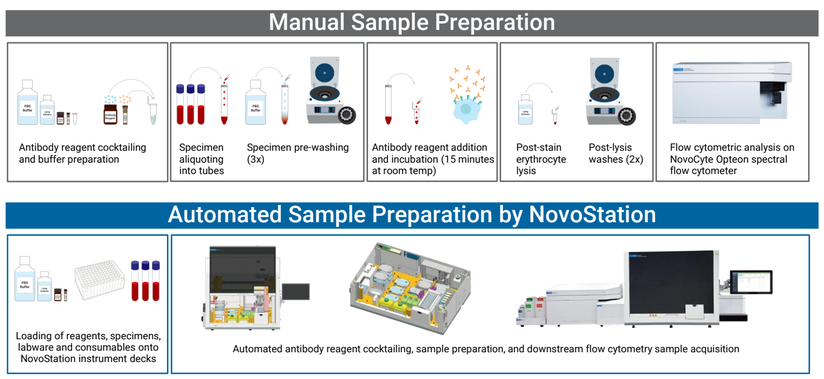


Figure 1. Workflow comparison between manual sample preparation (top) and automated sample preparation by NovoStation (bottom), which streamlines reagent cocktailing, specimen processing, antibody staining, erythrocyte lysis, and sample washing steps by performing them in a hands-off manner.

Results and Discussion

Biological analytical comparison of manual and NovoStation workflows

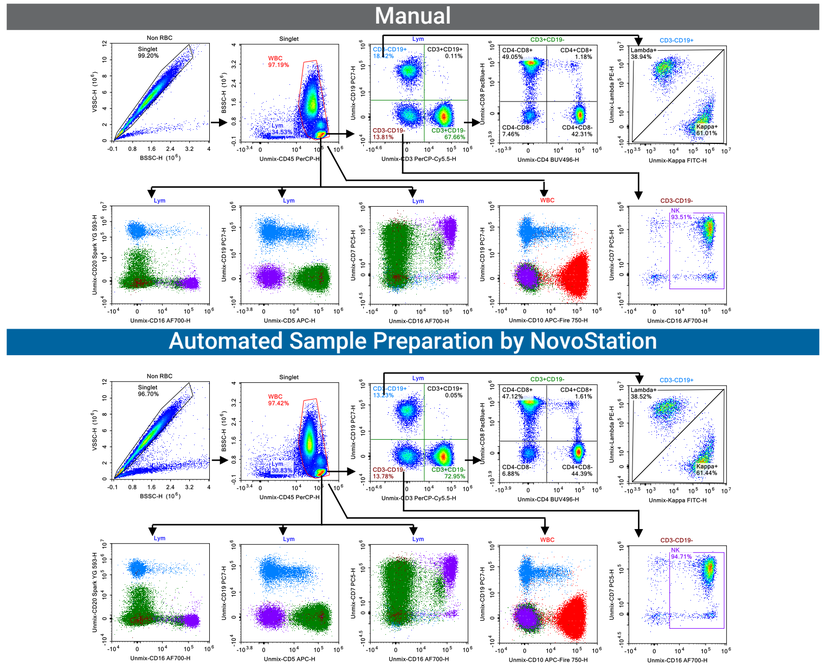


Figure 2. Scatter and dot plots showing the gating strategy to identify immune cell populations from human blood, showing comparable resolution in gated populations between manual (top) and NovoStation-automated (bottom) sample processing.

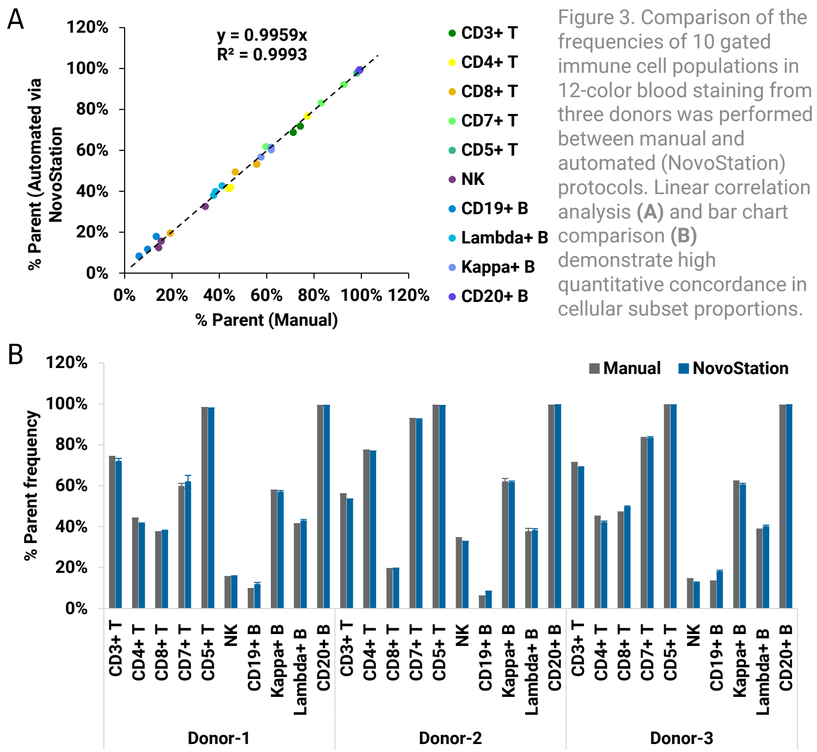


Figure 3. Comparison of the frequencies of 10 gated immune cell populations in 12-color blood staining from three donors was performed between manual and automated (NovoStation) protocols. Linear correlation analysis **(A)** and bar chart comparison **(B)** demonstrate high quantitative concordance in cellular subset proportions.

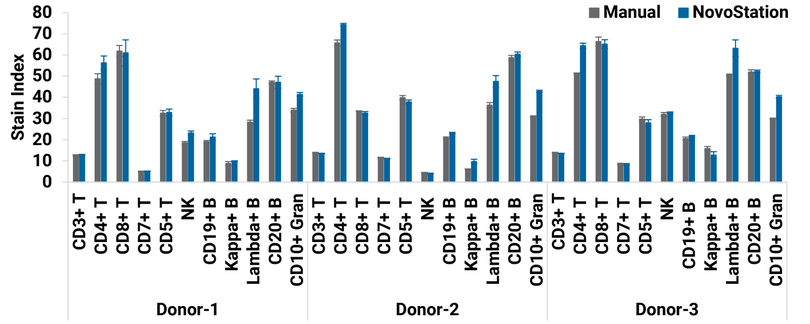


Figure 4. Comparison of Stain Index of each population in manual versus NovoStation prepared 12 color-stained samples from three donors.

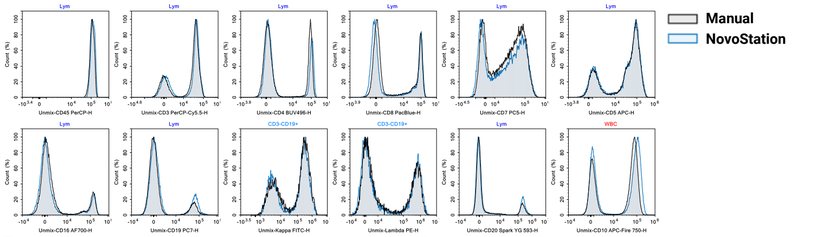


Figure 5. Histogram plot overlays for each of 12 fluorescent channels from manual versus NovoStation automated sample preparation methods.

Assay Step	Manual		NovoStation	
		Time (min)		Time (min)
Prepare Cocktail	Hands-On	6.9	Hands-On	1.5
			Hands-Off	9.9
Aliquot blood samples and Pre Wash (3x)	Hands-On	17.0	Hands-On	5.3
	Hands-Off	22.2	Hands-Off	162.7
Aliquot blood samples into tubes and add antibody for incubation	Hands-On	29.5		
	Hands-Off	15.0		
Add lysing solution and post-lysis washes (2x)	Hands-On	36.0		
	Hands-Off	31.0		
Total sample preparation workflow	Total Hands-On	89.4	Total Hands-On	6.8
	Total Hands-Off	68.2	Total Hands-Off	172.6
	Total	157.6	Total	179.4

Figure 6. The number of hands-on assay steps and length of hands-on time are both significantly reduced when using NovoStation automation to prepare the 12-color assay protocol, compared with manual methods.

Conclusions

- When performing κ/λ immunoglobulin light chain assessment, thorough specimen prewashing is necessary to minimize interference from cytophilic antibodies and serum IgG and ensure resolution of B cell populations
- When utilizing the Agilent NovoStation sample preparation system to automate a prewash/stain/lyse/wash 12-color assay protocol on human whole blood specimens, the number of hands-on steps was significantly reduced
- Sample processed using NovoStation automation yielded comparable resolution and characterization of Kappa and Lambda expressing populations, compared with those processed manually

References

O'Donahue, M.; Johnson, L.; Hedley, B.; Vaughan, E. Flow Cytometric Testing for Kappa and Lambda Light Chains. Sponsored and reviewed by ICCS Quality and Standards Committee. 2018.

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