

Automated Sample Preparation for Kappa/Lambda Light Chain Assessment

Streamlined immunophenotyping workflow using the Agilent NovoStation sample preparation system and Agilent NovoCyte Opteon spectral flow cytometer for enhanced efficiency and reproducibility

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Abstract

Accurate assessment of kappa and lambda (κ/λ) immunoglobulin light chain expression by flow cytometry is technically challenging due to potential inaccuracies in manual sample preparation, including inconsistent cell lysis and interfering proteins that obscure true κ/λ signals. This study used the Agilent NovoStation sample preparation system to automate sample preparation steps—such as prewashing to remove cytophilic antibodies and immunoglobulins, staining, erythrocyte lysis and post-staining washes—before acquisition on the Agilent NovoCyte Opteon spectral flow cytometer with a 12-color immunophenotyping panel. Automated processing yielded comparable κ/λ resolution and B cell population characterization compared to manual methods while significantly reducing hands-on steps and cutting hands-on time from 89.4 to 6.8 minutes. The NovoStation system integration with the NovoCyte Opteon enabled automated, hands-free transition between sample preparation and data acquisition. These results demonstrate that automated sample preparation substantially improves workflow efficiency and reproducibility for κ/λ light chain assessment in immunophenotyping applications.

Introduction

Flow cytometry is a vital technique for immunophenotyping and characterizing immunoglobulin light chain expression. In particular, assessment of kappa and lambda (κ/λ) light chain expression on B cell populations is commonly used for determining B cell clonality, distinguishing reactive from neoplastic B cell populations, and supporting the characterization of B cell lymphoproliferative disorders. Reliable κ/λ assessment remains technically challenging, particularly when manual sample preparation protocols are used. A key limitation of manual processing is variability in sample preparation steps, including erythrocyte lysis and washing, which can leave residual plasma proteins and cell-bound immunoglobulins on B cell surfaces. These residual cytophilic antibodies and serum immunoglobulins can mask or interfere with the detection of surface κ and λ light chains, reducing the resolution and reliability of light chain analysis.

To address these issues, multiple prewashing steps are recommended to remove unwanted proteins and immunoglobulins from B cells. However, performing these sequential washes manually is difficult and can lead to inconsistent cell recovery, reduced cell viability, and elevated background signals. Additionally, the significant hands-on labor requirements limit the reproducibility and throughput of manual protocols. These limitations underscore a critical need for a standardized, reproducible, and efficient approach to sample preparation that maintains analytical accuracy while reducing technical variability and operational burden.

Automated sample preparation systems offer a promising solution to address these challenges by enabling consistent, hands-free execution of complex multistep protocols. The integration of automated sample preparation with modern flow cytometry platforms could substantially improve the reliability of κ/λ light chain assessment while simultaneously reducing hands-on time and minimizing human error.

This study evaluates the performance of the Agilent NovoStation sample preparation system coupled with the Agilent NovoCyte Opteon spectral flow cytometer for comprehensive immunophenotyping and κ/λ light chain analysis. It compares analytical outcomes and operational efficiency with conventional manual protocols.

Experimental

Study design

Three normal donor peripheral blood samples were evaluated to compare manual and automated sample preparation workflows. Unstained and single-stained reference controls, as well as triplicate multicolor samples, were prepared using both manual and fully automated protocols, totaling 24 samples.

Sample preparation

Whole blood samples were processed using two distinct methodologies: manual sample preparation and automated preparation via the NovoStation system. The manual sample preparation workflow required sequential execution of multiple steps. Antibody cocktails were prepared manually and added to samples. Blood samples were manually aliquoted and subjected to three wash cycles to remove interfering proteins. Lysing reagent was added manually, followed by two additional wash cycles.

The automated workflow used the NovoStation system to replicate all the essential steps associated with this assay workflow to ensure accurate κ/λ assessment, including antibody cocktail preparation, specimen prewashing, antibody incubation, erythrocyte lysis, and post-lysis wash steps prior to flow cytometry analysis. A comparison of the sample preparation protocols is illustrated in Figure 1.

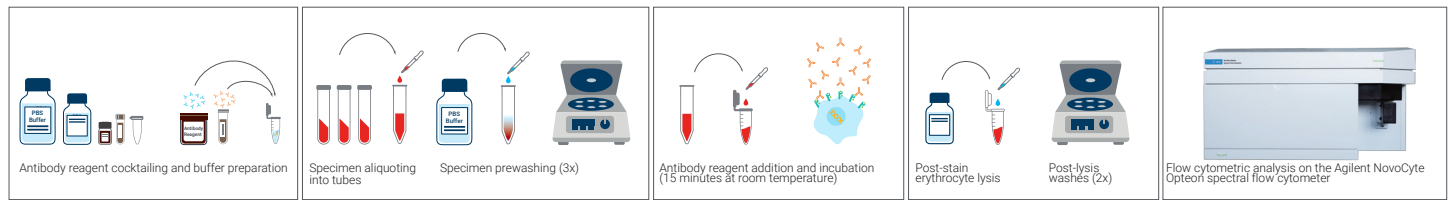
Antibody staining panel

A 12-color immunophenotyping panel was employed to enable comprehensive characterization of lymphocyte populations and κ/λ light chain expression. The reagents used for the panel are listed in Table 1. This combination of markers enabled identification of T cell subsets, B cell populations, and κ/λ light chain-expressing cells within a single tube.

Flow cytometric acquisition and analysis

Samples were acquired on the NovoCyte Opteon spectral flow cytometer. The NovoStation system enabled automated, hands-free transition between sample preparation and data acquisition, eliminating the need for manual sample transfer and intermediate handling steps. All raw data were analyzed using Agilent NovoExpress software, version 2.2.0.

Manual sample preparation



Automated sample preparation using the Agilent NovoStation system

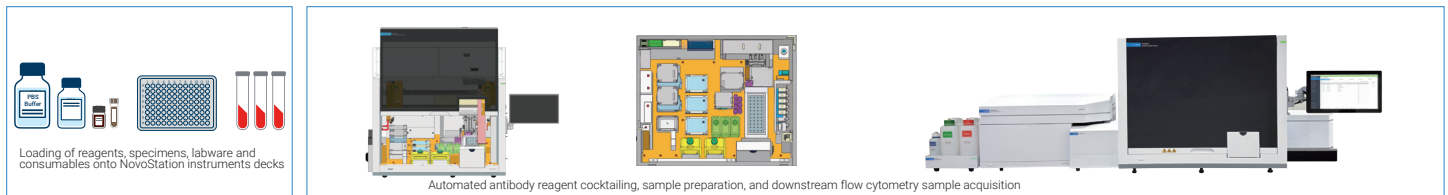


Figure 1. Workflow comparison between (top) manual sample preparation and (bottom) automated sample preparation by the Agilent NovoStation sample preparation system. The automated workflow streamlines reagent cocktail preparation, specimen processing, antibody staining, erythrocyte lysis, and sample washing through a standardized, hands-off process.

Immune cell populations were identified using a sequential gating strategy. Initial gating identified lymphocytes based on forward and side scatter properties, followed by CD45 expression. Subsequent gates identified CD3+ T cells, CD4+ T cells, CD8+ T cells, NK cells, CD19+ B cells, the κ and λ light chain expression pattern in B cells, and the expression patterns of CD16, CD20, CD5, CD10, and CD7 across lymphocyte subsets.

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

Specificity	Fluorochrome	Clone	Source	Part 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	CD7-687	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

Comparative analyses evaluated κ/λ resolution, population frequencies, stain index, fluorescence signal distributions, and hands-on time between manual and automated workflows.

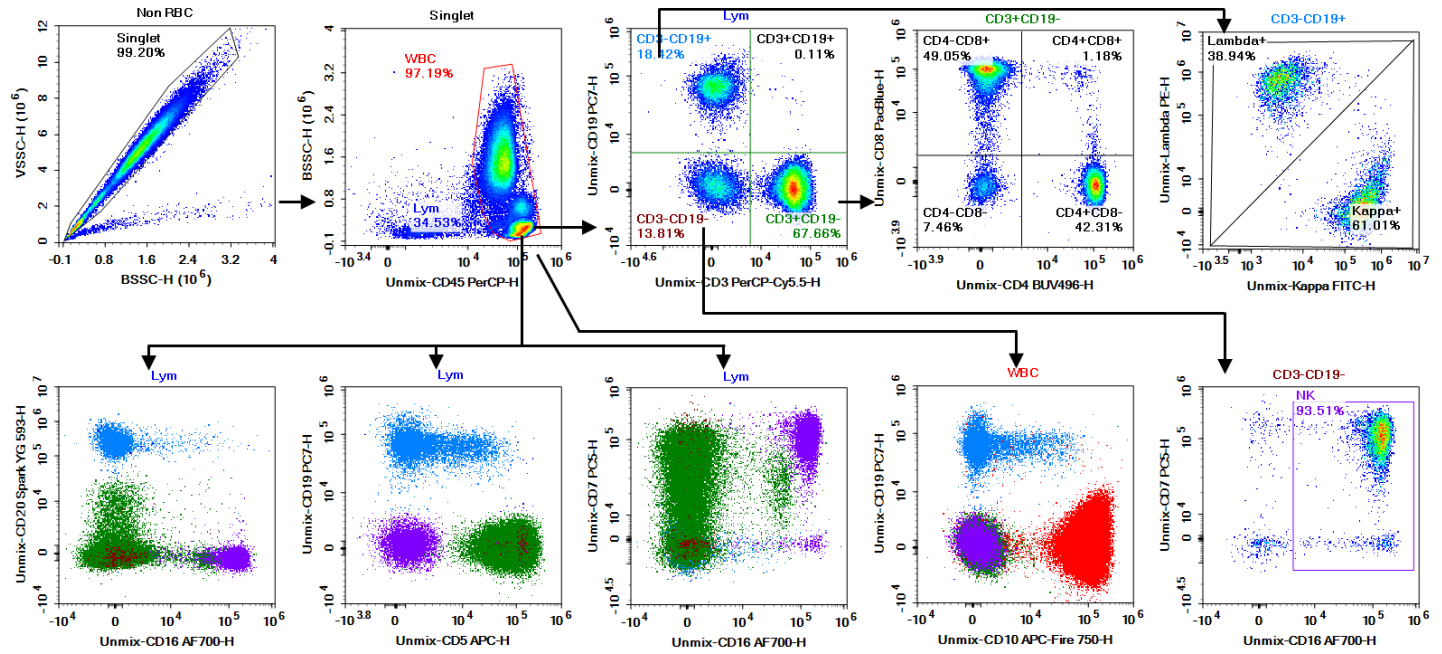
Results and discussion

Automated sample preparation using the NovoStation system was evaluated for its ability to streamline κ/λ light chain assessment in a 12-color immunophenotyping panel. Whole blood samples from three normal donors were processed using both manual and fully automated protocols, with comparative analyses focused on κ/λ resolution, cell recovery, population frequencies, and operational efficiency.

Immunophenotypic characterization and cell population analysis

Both manual and NovoStation-automated workflows successfully identified major lymphocyte subsets, including T cells, CD4+ T cells, CD8+ T cells, NK cells, and B cells. In addition, both workflows characterized κ and λ light chain expression in B cells and evaluated the expression patterns of CD16, CD20, CD5, CD10, and CD7 across lymphocyte subsets. Scatter and dot plot gating strategies demonstrated comparable marker resolution and population identification between the two methods (Figure 2).

Manual sample preparation



Automated sample preparation using the Agilent NovoStation system

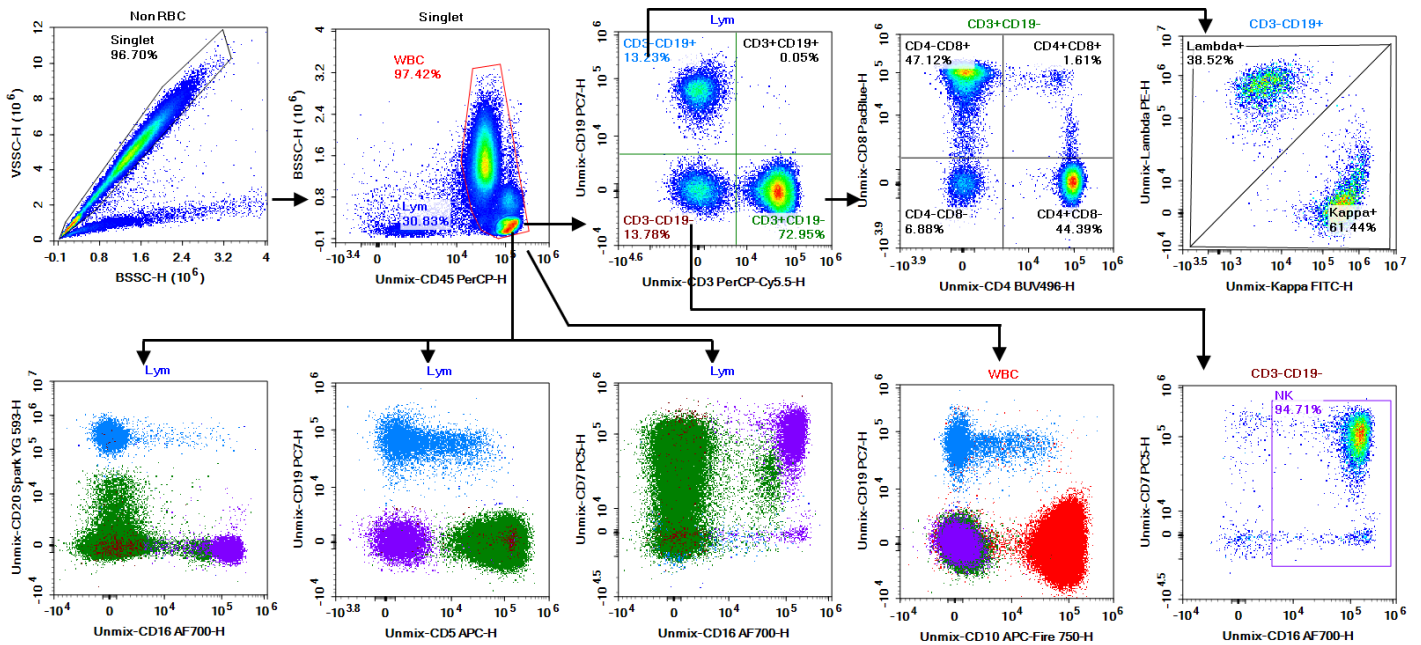


Figure 2. Gating strategy for identification of immune cell populations from whole blood samples. Representative scatter and dot plots from (top) manual and (bottom) Agilent NovoStation system-automated sample processing showing comparable resolution in gated populations between protocols.

The frequency distributions of these populations across the three donor samples showed consistent results between manual and automated processing, indicating that automation did not compromise the ability to resolve distinct lymphocyte subsets (Figure 3).

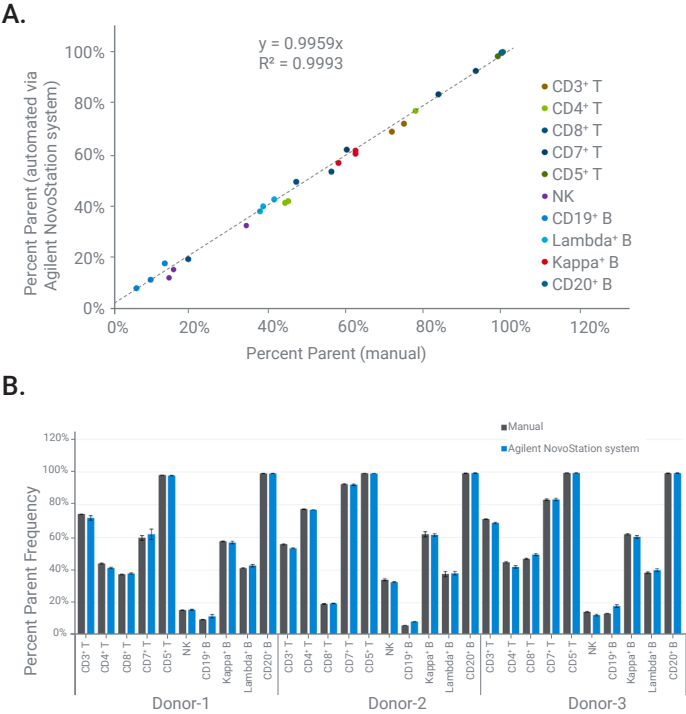


Figure 3. Frequency comparison of 10 immune cell populations across three healthy donors following manual or Agilent NovoStation system-automated sample preparation. (A) Linear correlation analysis and (B) bar chart comparisons demonstrate strong quantitative agreement between manual and automated workflows, confirming comparable characterization of immune cell subsets.

Stain index analysis for each marker revealed equivalent fluorescence signal quality between manual and NovoStation-prepared samples (Figure 4). The stain index, which quantifies the separation between positive and negative populations relative to background variation, is a key metric for assessing antibody staining quality. The comparable stain index values demonstrate that automated processing maintained the staining intensity and specificity required for accurate immunophenotyping.

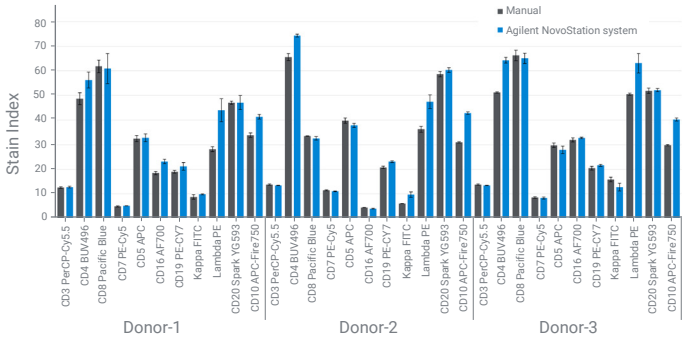


Figure 4. Stain index comparison between manual and Agilent NovoStation system-automated sample preparation. Stain index values for each of the 11 markers analyzed (excluding CD45) across three donors, demonstrating equivalent fluorescence signal quality and specificity between preparation methods.

Fluorescence signal performance

Histogram overlays across all 12 fluorochromes showed comparable signal distributions and background levels between manual and NovoStation-automated sample preparation methods (Figure 5). This consistency across fluorochromes indicates that the automated workflow preserved the optical properties and detection characteristics of the immunophenotyping panel, including the critical kappa and lambda detection. The maintenance of fluorescence signal quality across all fluorochromes is essential for multiparameter flow cytometry, where simultaneous detection of multiple markers enables comprehensive cellular characterization.

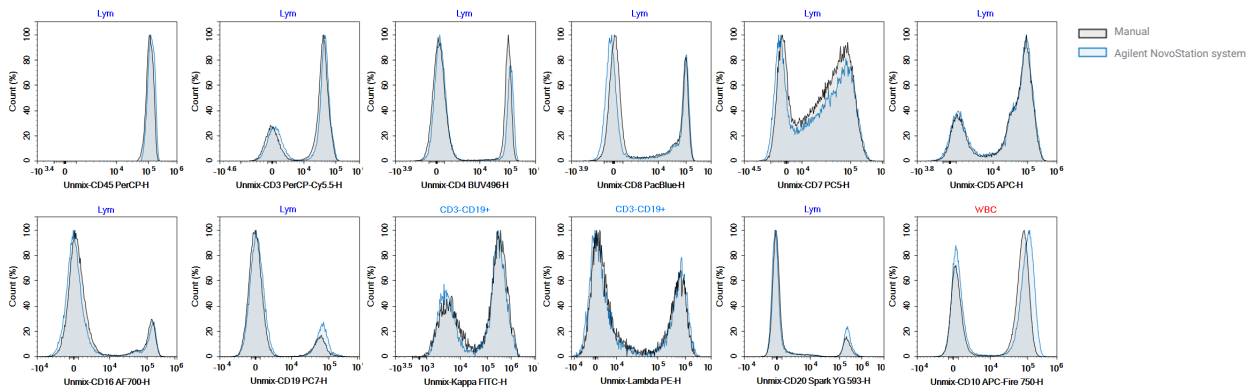


Figure 5. Overlay histograms for all 12 markers comparing manual versus Agilent NovoStation system-automated sample preparation. Comparable signal distributions and background levels were observed across all markers, demonstrating equivalent staining performance between the two methods.

Operational efficiency and hands-on time reduction

The NovoStation sample preparation system markedly enhances workflow efficiency by automating complex sample preparation steps, reducing manual steps and cutting hands-on time from 89.4 to 6.8 minutes (Table 2). This automation standardizes critical prewashing processes, ensuring consistent removal of interfering proteins while preserving cell integrity and viability. Additionally, integration with the NovoCyte Opteon spectral flow cytometer enables

a hands-free transition from preparation to data acquisition, minimizing human error and eliminating intermediate handling. Together, these features streamline the entire process, improve reproducibility, and free up valuable laboratory time.

Conclusion

This study demonstrates that the Agilent NovoStation sample preparation system streamlines kappa and lambda light chain assessment compared with manual preparation. The NovoStation system-processed samples produced resolution and characterization of kappa- and lambda-expressing B cell populations comparable to manual methods. The automated workflow reduces manual prewashing challenges, including cell loss and reduced viability, while supporting accurate κ/λ immunoglobulin light chain assessment by flow cytometry. Integration with the Agilent NovoCyte Opteon spectral flow cytometer enables a hands-free transition from preparation to acquisition, improving efficiency and reducing opportunities for human error.

Together, the maintained analytical performance and substantial reduction in hands-on time support the NovoStation sample preparation system as an effective solution for high-throughput immunophenotyping workflows requiring stringent specimen preparation. Automated sample preparation can improve the efficiency of complex flow cytometry assay workflows such as κ/λ light chain assessment, while still yielding high-quality data output.

References

1. O'Donahue, M.; Johnson, L.; Hedley, B.; Vaughan, E. *Flow Cytometric Testing for Kappa and Lambda Light Chains*. Sponsored and reviewed by the International Clinical Cytometry Society (ICCS) Quality and Standards Committee, **2018**.

Table 2. Workflow time comparison between manual and Agilent NovoStation system-automated sample preparation, illustrating the reduction in hands-on steps and total hands-on time across all assay steps.

Assay Step	Manual		Agilent NovoStation System	
		Time (min)		Time (min)
Prepare cocktail	Hands-on	6.9	Hands-on	1.5
			Hands-off	9.9
Aliquot blood specimens and prewash (3x)	Hands-on	17.0	Hands-off	5.3
	Hands-off	22.2		
Aliquot blood specimens into tubes and add antibody for incubation	Hands-on	29.5	Hands-off	150
	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	159.9
	Total	157.6	Total	166.7

Products used in this application

Agilent products

[Agilent NovoCyte Opteon spectral flow cytometer](#) 

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