

Method comparison and clinical classification agreement between Freelite and N-Latex serum-free light chain assays for clinical laboratory implementation

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ABSTRACT

Background: Measurement of serum free light chains (SFLC) is an essential component in the diagnosis and monitoring of plasma cell dyscrasias. Two analytical platforms are widely used in clinical laboratories: the Freelite assay, which employs polyclonal antibodies with turbidimetric detection, and the N-Latex assay, which uses monoclonal antibodies with nephelometric measurement. Because these assays differ in antibody design, calibration, and analytical detection principles, numerical concentrations and κ/λ ratios may not be directly interchangeable across platforms. This study aimed to evaluate the analytical agreement and clinical classification concordance between the Freelite and N-Latex assays to assess the feasibility of implementing the N-Latex method in routine laboratory practice.

Materials and methods: A cross-sectional method-comparison study was performed using 33 paired archived residual serum samples obtained from routine clinical testing. Free κ and λ concentrations were measured using both Freelite and N-Latex assays on the same specimens. Analytical agreement was assessed using the Wilcoxon signed-rank test, Spearman correlation, Passing-Bablok regression, and Bland-Altman analysis. κ/λ ratios were interpreted according to assay-specific reference intervals, and categorical agreement was evaluated using Cohen's kappa coefficient.

Results: Freelite produced significantly higher κ concentrations than N-Latex (median 43.0 vs 38.9 mg/L, $p=0.0002$), whereas λ concentrations did not differ significantly. Strong correlations were observed for both κ ($p=0.897$) and λ ($p=0.951$); however, correlation alone was not sufficient to demonstrate quantitative interchangeability. Passing-Bablok regression demonstrated proportional bias for κ measurements, with Freelite yielding higher values at elevated concentrations and the 95% confidence interval of the slope not including 1.0. Bland-Altman analysis revealed wide limits of agreement for κ values at extreme concentrations, indicating clinically relevant numerical differences between methods. When κ/λ ratios were interpreted categorically, using assay-specific reference intervals, concordant classification was observed in 30 of 33 samples (91%), corresponding to substantial agreement ($\kappa=0.79$).

Conclusions: The Freelite and N-Latex assays demonstrate strong correlation but exhibit systematic quantitative differences, particularly for κ measurements at high concentrations. Although κ/λ ratio interpretation is largely concordant, the two assays should not be considered directly interchangeable for absolute numerical interpretation or longitudinal monitoring. Implementation of the

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Introduction

Quantification of serum-free light chains (SFLC), comprising free kappa (κ) and lambda (λ) immunoglobulin light chains, plays an important role in the screening, diagnosis, and monitoring of plasma cell dyscrasias, including multiple myeloma (MM), monoclonal gammopathy of undetermined significance (MGUS), and AL amyloidosis.^{1,2} These disorders are characterized by clonal proliferation of plasma cells that produce excessive amounts of either κ or λ light chains, resulting in an imbalance of the κ/λ ratio. The International Myeloma Working Group (IMWG) recommends SFLC measurement as part of the standard diagnostic algorithm, particularly in non-secretory or light-chain-only myeloma, where conventional electrophoretic techniques may fail to detect monoclonal protein production.^{3,4}

The κ/λ ratio is especially useful for distinguishing monoclonal from polyclonal immunoglobulin production and therefore serves as an important indicator of clonality. An elevated ratio suggests κ -restricted monoclonality, whereas a decreased ratio indicates λ -restricted monoclonality. In routine clinical practice, SFLC results are interpreted alongside serum protein electrophoresis, immunofixation electrophoresis, and bone marrow examination to establish a diagnosis or to monitor disease progression and treatment response.⁵ Furthermore, SFLC measurements may support disease monitoring and treatment-response assessment in selected plasma cell disorders, particularly oligosecretory multiple myeloma, nonsecretory multiple myeloma, Bence Jones multiple myeloma, and AL amyloidosis.⁶

Among the commercially available assays for SFLC measurement, the Freelite assay and the N-Latex assay are the two platforms most widely used in clinical laboratories. The Freelite assay employs polyclonal antibodies with turbidimetric detection, whereas the N-Latex assay uses monoclonal antibodies measured by nephelometry.^{7,8} Because these assays differ in antibody composition, epitope recognition, and analytical detection methods, numerical concentrations of κ and λ may vary between platforms, which can subsequently influence the calculated κ/λ ratio.⁹ Polyclonal antibodies used in Freelite may bind multiple epitopes on free light chains, whereas monoclonal antibodies used in N-Latex provide greater analytical specificity but may fail to recognize certain conformational variants.

Several comparative studies have reported limited numerical agreement between the two assays, although clinical interpretation based on κ/λ ratios is often broadly

N-Latex assay is feasible in routine clinical laboratories provided that assay-specific reference intervals, appropriate dilution protocols, and antigen-excess precautions are applied, and clinicians are informed about expected differences when transitioning between analytical platforms.

concordant.¹⁰⁻¹² The lack of assay standardization remains a major challenge in SFLC testing. At present, no internationally accepted reference standard for SFLC quantification exists, which prevents direct comparability or interchangeability of results across analytical platforms.^{13,14} Consequently, the Clinical and Laboratory Standards Institute (CLSI) recommends that laboratories perform local method comparison and validation studies before implementing a new measurement procedure, as outlined in guideline EP09-A3.¹⁵

The clinical implications of assay variability should not be underestimated. Differences in SFLC values between analytical platforms may lead to discrepancies in disease interpretation, particularly when results fall close to diagnostic or therapeutic thresholds. These challenges may be further amplified in patients with renal impairment, in whom altered clearance of free light chains may affect assay performance and the interpretation of the κ/λ ratio.^{16,17} Therefore, careful interpretation of SFLC results is required, especially when laboratories transition from one assay platform to another.

At our institution, SFLC testing has historically been performed using the Freelite™ assay through a reagent-rental arrangement that included analyzer support. However, recent procurement policy changes have made continuation of this model increasingly difficult, prompting evaluation of alternative assays that could provide comparable analytical performance while remaining compatible with the laboratory's existing analytical infrastructure.

The N-Latex FLC assay has emerged as a potential alternative platform. Its monoclonal antibody-based design may offer advantages in analytical specificity and reproducibility.¹⁸ Nevertheless, before introducing a new assay into routine clinical practice, it is essential to determine whether the candidate method produces results that are analytically comparable with those generated by the existing platform and whether clinical interpretation remains consistent.

Importantly, this challenge is not unique to a single laboratory. Clinical laboratories worldwide may need to replace or transition between SFLC assay platforms due to procurement policies, instrument availability, or infrastructure changes. A recent systematic review by Ho *et al.* highlighted substantial variability in SFLC assay performance across studies and emphasized the importance of local validation before introducing a new platform into routine clinical use.¹⁹ However, data evaluating assay comparability during real-world

laboratory transitions remain limited.

Given the central role of SFLC testing in the diagnosis and monitoring of plasma cell disorders, and the potential clinical impact of assay-related discrepancies, we conducted this study to evaluate the analytical agreement and clinical classification concordance between the established Freelite assay and the N-Latex assay. The aim of this study was to determine whether the N-Latex platform could be implemented as an alternative method in routine clinical laboratory practice while maintaining reliable interpretation of κ/λ ratios.

Materials and methods

Study design

This study was designed as a cross-sectional method-comparison study using archived residual serum specimens to evaluate the analytical agreement and clinical classification concordance between two serum free light chain (SFLC) assay systems.

Sample selection and handling

Residual serum specimens obtained from routine diagnostic testing at Maharaj Nakorn Chiang Mai Hospital, Faculty of Medicine, Chiang Mai University, were used for the analysis. Specimens were originally collected during standard phlebotomy procedures, and the remaining aliquots were stored at -20°C after completion of the requested clinical testing, in accordance with routine laboratory storage protocols. Samples were thawed once for analysis and were not subjected to repeated freeze-thaw cycles. For the purposes of this validation study, all specimens were anonymized and de-identified before analysis. No additional blood was drawn from patients, and no direct patient contact occurred.

Samples were eligible for inclusion if sufficient serum volume was available to perform paired κ and λ SFLC measurements using both assays on the same specimen. Hemolyzed samples and specimens with insufficient residual volume were excluded. Based on Freelite κ/λ ratio interpretation, which represented the routine method used at our institution, 25 of 33 samples were classified as normal and 8 as abnormal. Thus, most included specimens represented routine clinical samples with normal κ/λ ratio classification, while a smaller subset represented abnormal or borderline classifications.

Sample size justification

A total of 33 paired serum samples were available during the validation period and were therefore included in the study. The Clinical and Laboratory Standards Institute (CLSI) guideline EP09-A3 recommends using 40-100 patient samples for method-comparison studies when feasible. Therefore, the present study included fewer samples than recommended for a comprehensive method-comparison validation.

The limited sample number reflected the specialized nature and relatively low routine testing volume of SFLC assays, as well as the requirement for sufficient archived residual serum volume to perform paired measurements on two analytical platforms. Although the number of specimens was below the recommended CLSI range, the included samples covered a wide analytical concentration range, including physiological and markedly elevated free light chain values. Accordingly, this study should be interpreted as a preliminary real-world local implementation validation rather than definitive evidence of full analytical equivalence between the two assays.

Assay methods and instruments

Paired measurements of serum free κ and λ light chains were performed on the same serum specimens using two automated immunoassay platforms according to the respective manufacturers' instructions.

The existing method was the Freelite assay (The Binding Site Ltd., Birmingham, UK), which employs polyclonal antibodies in a turbidimetric format and was performed on the Optilite analyzer. Calibration and internal quality control procedures were conducted according to routine laboratory operating protocols. Samples exceeding the analytical measurement range were automatically diluted by the analyzer and reanalyzed to obtain reportable concentrations.

The candidate method was the N Latex FLC assay (Siemens Healthineers, Germany), which utilizes monoclonal antibodies with nephelometric detection and was performed on the BN II Nephelometer. Manufacturer-provided reference intervals were applied (κ 6.7-22.4 mg/L, λ 8.3-27.0 mg/L, and κ/λ ratio 0.31-1.56). Specimens exceeding the reportable range were manually diluted using the manufacturer-supplied diluent and reanalyzed to ensure that measured values fell within the analytical measurement range.

For results below the analytical measurement range, values were not numerically extrapolated beyond the validated lower limit. Such results were reported according to the laboratory reporting convention as below the lower reportable limit, with interpretation based on the κ/λ ratio and the paired light-chain result. For results above the analytical measurement range, dilution and repeat measurement were performed before final reporting. Results were released only when the final measured value after dilution was within the validated reportable range and dilution recovery was considered acceptable according to routine quality procedures.

To minimize pre-analytical variability, both assays were performed on the same set of paired serum specimens. Internal quality control was assessed using commercial free light chain control materials at both normal and pathological concentration levels on each platform. During the validation period, the observed

coefficients of variation for internal quality control materials were <5% for both assays.

Detection and management of antigen excess or Hook effect

Because antigen excess can produce falsely low immunoassay results in specimens with very high analyte concentrations, laboratory procedures included specific steps for identifying and managing suspected hook-effect specimens. Antigen excess was considered when the measured SFLC concentration was unexpectedly low or inconsistent with the κ/λ ratio, serum protein electrophoresis, immunofixation findings, total immunoglobulin results, renal status, or clinical suspicion of plasma cell dyscrasia.

When antigen excess was suspected, the specimen was repeated after appropriate dilution using manufacturer-recommended diluent. For N-Latex testing on the BN II platform, built-in antigen-excess protection was used where available, and manual or serial dilution was performed for specimens with unexpectedly low results, markedly abnormal κ/λ ratios, or values near or above the measuring interval. The diluted result was multiplied by the dilution factor and accepted only if the measured concentration fell within the validated analytical range. If dilution nonlinearity or clinical inconsistency was observed, the result was flagged for cautious interpretation, and confirmatory testing using an alternative or orthogonal method was considered.

Statistical analysis

Statistical analyses were performed using MedCalc (MedCalc Software Ltd., Ostend, Belgium) and STATA version 16.0 (StataCorp, College Station, TX, USA). The distribution of κ and λ free light chain concentrations was evaluated using the Shapiro–Wilk test. Because the data were not normally distributed, non-parametric statistical methods were applied. Continuous variables were summarized using medians and interquartile ranges (IQR). Paired κ and λ concentrations obtained from the two assay methods were compared using the Wilcoxon signed-rank test. The association between measurements obtained from the two assays was evaluated using Spearman's rank correlation coefficient (ρ). Analytical agreement between the methods was further assessed using Passing-Bablok regression and Bland-Altman analysis.

Passing-Bablok regression, a non-parametric regression method robust to outliers, was used to evaluate potential systematic bias between the two measurement procedures. Regression equations and 95% confidence intervals (CI) for slope and intercept were calculated. For interpretation, a slope confidence interval including 1.0 and an intercept confidence interval including 0.0 were considered supportive of absence of proportional and constant bias, respectively. Because SFLC assays provide quantitative results, correlation alone was not considered sufficient

evidence of agreement or interchangeability.

Bland-Altman analysis was performed by plotting the difference between methods (Freelite minus N-Latex) against the mean of the two measurements. Mean bias and 95% limits of agreement (mean $\pm$ 1.96 standard deviations) were used to assess the magnitude of systematic and random variation between methods. Because an extremely high-concentration specimen affected the visual scaling of scatter and Bland-Altman plots, close-up plots excluding the highest-concentration specimen were prepared to better visualize the lower and mid-concentration ranges while retaining the full-range analysis for overall interpretation.

For clinical interpretation, each sample's κ/λ ratio was classified as normal or abnormal according to the method-specific reference intervals (0.26-1.65 for Freelite and 0.31-1.56 for N-Latex). Concordance in classification between the two assays was evaluated using Cohen's kappa coefficient (κ), which measures agreement beyond chance. Kappa values were interpreted according to conventional thresholds: values greater than 0.80 indicate near-perfect agreement, 0.61-0.80 substantial agreement, 0.41-0.60 moderate agreement, 0.21-0.40 fair agreement, and $\leq$ 0.20 slight agreement.

McNemar's test was additionally used to evaluate whether systematic directional disagreement in classification was present. All statistical tests were two-tailed, and a $p < 0.05$ was considered statistically significant.

Data protection and ethical considerations

This study involved the secondary use of anonymized leftover clinical serum specimens and therefore posed no more than minimal risk to participants. No direct patient contact occurred, and no identifiable personal or clinical information was accessed. All laboratory data were coded and handled in accordance with institutional data protection procedures. Based on these conditions, the study qualified for a waiver of informed consent under ethical guidelines governing the secondary use of human biological materials.

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University (Study Code: PAT-2568-0329).

Results

A total of 33 paired serum samples (N=33) were analyzed using both the Freelite and N-Latex assays. The measured concentrations of free κ and λ light chains covered a wide analytical range, reflecting the inclusion of specimens with both physiological and markedly elevated values. Freelite κ concentrations ranged from 15.8 to 7,130 mg/L, whereas N-Latex κ concentrations

ranged from 12.6 to 2,690 mg/L. For λ measurements, Freelite values ranged from 7.3 to 1,170 mg/L, while N-Latex values ranged from 8.3 to 607 mg/L.

The distribution of free light chain concentrations measured by the two assays is summarized in Table 1. The median κ concentration measured by Freelite was 43.0 mg/L (IQR 34.1-92.0), which was significantly

higher than the median κ concentration measured by N-Latex (38.9 mg/L; IQR 16.7–68.7) (Wilcoxon signed-rank test, $p=0.0002$). In contrast, the median λ concentration measured by Freelite (50.3 mg/L; IQR 25.0-89.1) was slightly lower than that obtained using N-Latex (55.5 mg/L; IQR 29.2-111.5), although the difference was not statistically significant ($p=0.117$).

Table 1. Distribution of serum-free light chain concentrations measured by Freelite and N-Latex assays (N=33).

Analyte	Median (IQR) mg/L		p value*
	Freelite	N-Latex	
Free κ FLC	43.0 (34.1-92.0)	38.9 (16.7-68.7)	0.0002
Free λ FLC	50.3 (25.0-89.1)	55.5 (29.2-111.5)	0.117

Note: *values compared using the Wilcoxon signed-rank test.

Despite the differences in absolute concentrations, strong correlations were observed between the two assays. The Spearman rank correlation coefficient was $\rho=0.897$ for κ and $\rho=0.951$ for λ (both $p<0.001$), indicating strong monotonic relationships between the measurements obtained from the two analytical platforms. However, the correlation coefficients did not exceed 0.975, and correlation alone was not considered sufficient to establish quantitative interchangeability between assays.

Passing-Bablok regression analysis was performed to further evaluate the relationship between methods.

For κ measurements, the regression equation was $N\text{-Latex} = -13.7 + 0.76 \times \text{Freelite}$ (slope 95% confidence interval, CI: 0.56-0.90). Because the 95% CI for the κ slope did not include 1.0, proportional bias was observed, with N-Latex yielding lower κ values than Freelite, particularly at higher concentrations. For λ measurements, the regression equation was $N\text{-Latex} = 3.9 + 0.96 \times \text{Freelite}$ (slope 95% CI: 0.84-1.09). The 95% CI of the λ slope included 1.0, suggesting better quantitative alignment for λ than for κ measurements. Scatter plots illustrating these relationships are shown in Figure 1.

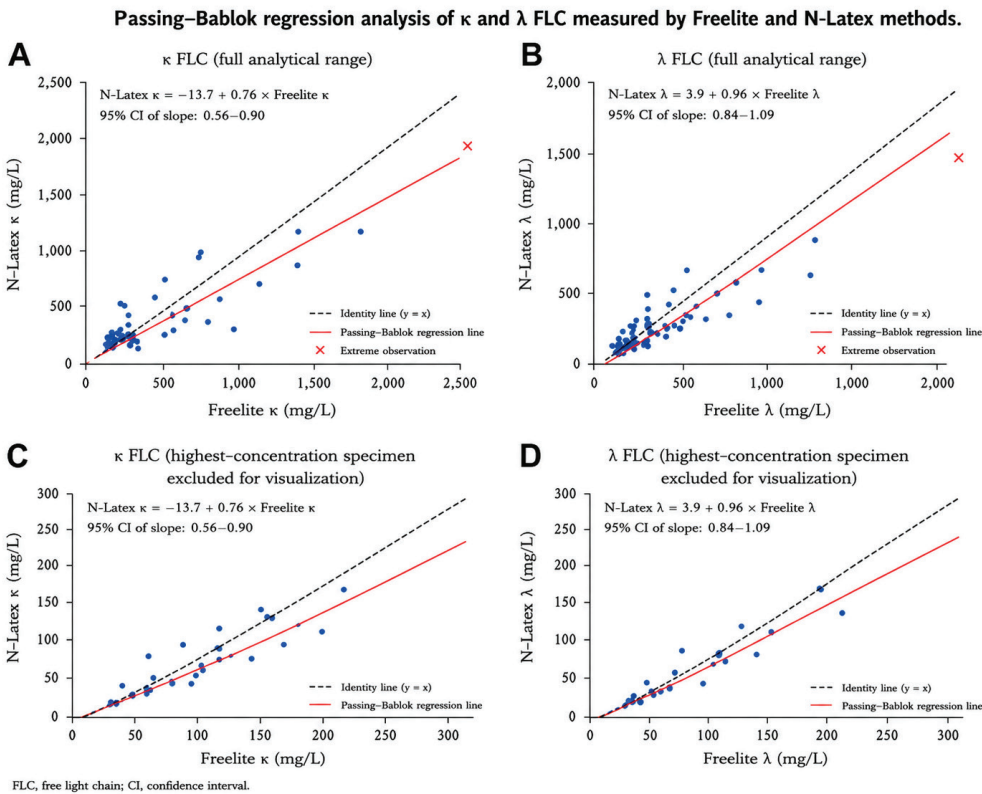


Figure 1. Passing-Bablok regression plots comparing Freelite and N-Latex serum free light chain measurements. A and B; κ and λ measurements across the full analytical range, C and D: excluding the highest-concentration specimen for visualization of the lower and mid-concentration ranges. The identity line represents $y=x$, and the regression line represents the Passing-Bablok fit.

Agreement between the two measurement methods was further evaluated using Bland-Altman analysis. For κ measurements, the mean difference (Freelite minus N-Latex) was +236 mg/L, with 95% limits of agreement ranging from -1,703 to +2,176 mg/L. For λ measurements, the mean bias was +20.7 mg/L,

with 95% limits of agreement ranging from -233 to +274 mg/L. The wide limits of agreement, particularly for κ , indicate that individual numerical results may differ substantially between assays and should not be used interchangeably for patient monitoring. Bland-Altman plots illustrating these results are presented in Figure 2.

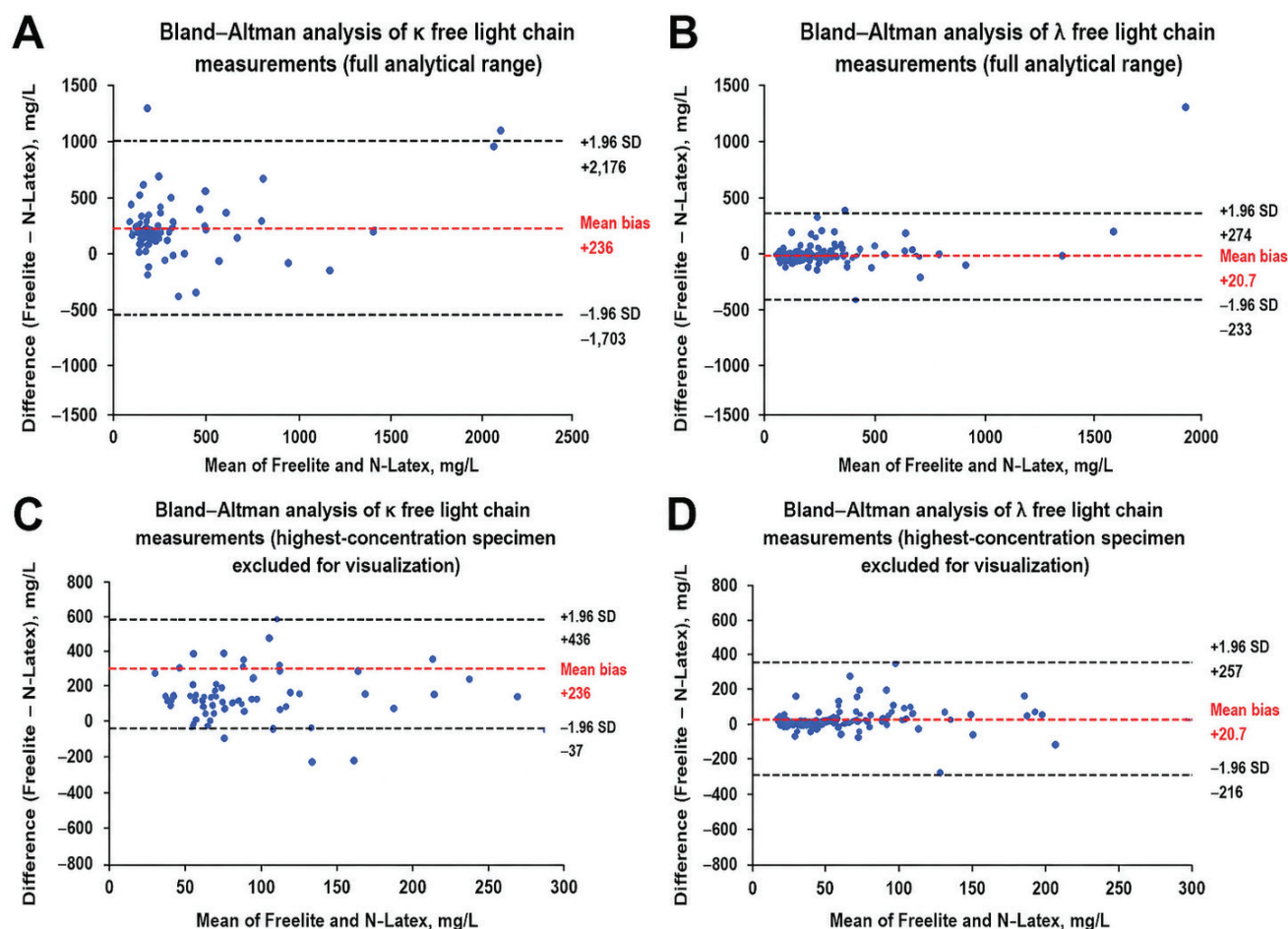


Figure 2. Bland-Altman plots comparing Freelite and N-Latex serum free light chain measurements. A: κ free light chain measurements across the full analytical range, B: λ free light chain measurements across the full analytical range, C: κ free light chain measurements after exclusion of the highest-concentration specimen for visualization purposes only, D: λ free light chain measurements after exclusion of the highest-concentration specimen for visualization purposes only. Red dashed lines indicate the mean bias, and black dashed lines indicate the 95% limits of agreement.

For clinical interpretation, κ/λ ratios from both assays were categorized as normal or abnormal according to assay-specific reference intervals. Using Freelite reference limits, 25 of 33 samples (75.8%) were classified as normal and 8 (24.2%) as abnormal. Using N-Latex reference limits, 26 samples (78.8%) were classified as normal and 7 (21.2%) as abnormal.

Overall concordance between the two assays was observed in 30 of 33 samples (91%). The Cohen's kappa coefficient was 0.79, indicating substantial agreement in categorical interpretation. McNemar's test showed no statistically significant difference in classification between the two methods ($p > 0.99$). The contingency table for classification concordance is shown in Table 2.

Table 2. Concordance of κ/λ ratio classification between Freelite and N-Latex assays (N=33).

	N-Latex normal	N-Latex abnormal	Total
Freelite normal	24	1	25
Freelite abnormal	2	6	8
Total	26	7	33

Three samples showed discordant κ/λ ratio classifications between the two assays. Because both assays were performed on the same serum specimen, these discordances are unlikely to represent true biological variation and most likely reflect assay-related analytical or classification differences. In all cases, discrepancies occurred in samples with κ/λ ratios close to the respective reference interval thresholds or in specimens with elevated free light chain concentrations.

Details of the discordant cases are summarized in Table 3. In *Case A*, the Freelite κ/λ ratio was 1.669 (classified as abnormal), whereas the N-Latex ratio was 1.126 (within the reference range). In *Case B*, Freelite reported a κ/λ ratio of 1.71 (abnormal) while N-Latex reported 0.82 (normal). In *Case C*, Freelite produced a ratio of 0.375 (normal), whereas N-Latex produced a ratio of 0.248 (below the reference limit). No cases were observed in which N-Latex indicated a

markedly elevated κ/λ ratio while Freelite classified the ratio as normal. These findings suggest that discordant classifications occurred mainly near assay-specific decision limits rather than as major categorical disagreement.

In all three discordant cases, paired measurements were performed on the same serum specimens; therefore, the discordant classifications were unlikely to represent true biological variation. *Case A* and *Case B* were classified as abnormal by Freelite but normal by N-Latex because the N-Latex κ/λ ratios shifted into the reference interval. In *Case B*, the discrepancy was mainly driven by substantially lower κ concentration measured by N-Latex. In *Case C*, the N-Latex ratio shifted below the lower reference limit, mainly due to lower κ and higher λ values compared with Freelite. All discordant-case values were within the reportable analytical range.

Table 3. Discordant κ/λ ratio classifications between Freelite and N-Latex assays.

Case	Freelite κ (mg/L)	Freelite λ (mg/L)	Freelite κ/λ ratio	Freelite classification	N-Latex κ (mg/L)	N-Latex λ (mg/L)	N-Latex κ/λ ratio	N-Latex classification	Main driver of discordance	Measurement range status
A	43.4	26.0	1.669	Abnormal	33.9	30.1	1.126	Normal	Lower κ and higher λ by N-Latex shifted the ratio into the normal range	Within reportable range
B	117.0	68.4	1.711	Abnormal	42.0	51.3	0.819	Normal	Markedly lower κ by N-Latex was the main driver of normalization	Within reportable range
C	33.3	88.8	0.375	Normal	24.5	98.6	0.248	Abnormal	Lower κ and higher λ by N-Latex shifted the ratio below the lower reference limit	Within reportable range

Note: Freelite κ/λ ratio reference interval: 0.26-1.65. N-Latex κ/λ ratio reference interval: 0.31-1.56. Measurement range status indicates whether final reported values were within the reportable analytical range.

Discussion

In this method-comparison validation study, we evaluated the analytical and clinical agreement between the Freelite polyclonal serum free light chain (SFLC) assay and the N-Latex monoclonal SFLC assay. The results demonstrate strong overall correlation between the two methods but also reveal systematic quantitative differences, particularly for κ measurements at higher concentrations. In addition, a small proportion of samples showed discordant κ/λ ratio classifications. These findings are relevant for laboratories evaluating the interchangeability of SFLC assays during platform transitions.

From an analytical perspective, the Freelite assay tended to yield higher κ concentrations than the N-Latex assay, particularly at elevated levels. This pattern was reflected by the positive bias observed in the Bland-Altman analysis and by the regression slope below unity in the Passing-Bablok model. In contrast, λ measurements showed smaller differences between methods, with Freelite values tending to be slightly lower than those obtained with the N-Latex assay. Similar analytical patterns have been reported in previous comparative studies. For example, Daves *et al.* reported higher κ concentrations with Freelite and slightly higher λ concentrations with N-Latex, suggesting that differences in antibody composition and assay calibration may contribute to systematic variation between platforms.²⁰ The additional contribution of the present study is its focus on real-world local implementation during transition from a Freelite/Optilite workflow to an N-Latex/BN II workflow, including practical considerations for discordant classification, dilution procedures, antigen-excess management, and clinician communication.

The clinical relevance of the observed bias depends on the intended use of SFLC testing. For longitudinal monitoring, the mean κ difference of +236 mg/L and the wide limits of agreement are clinically important because an apparent change after switching assay platforms may reflect analytical bias rather than true biological change. The smaller λ bias of +20.7 mg/L is less likely to affect interpretation in many routine samples, but it may still be relevant when results are close to clinical decision thresholds or when small serial changes are being assessed.

The observed analytical differences likely reflect the distinct antibody designs used in the two assays. The Freelite assay uses polyclonal antibodies that recognize multiple epitopes on free light chains. This broader epitope recognition may allow detection of heterogeneous or polymerized light chain forms and may contribute to higher measured κ concentrations. In contrast, the N-Latex assay employs monoclonal antibodies directed against specific epitopes, which may provide greater analytical specificity but may detect certain light chain conformations differently. Differences in calibrator assignment and assay

architecture may also contribute to variation in measured concentrations.

The difference in detection principle may also influence assay performance. Turbidimetry measures reduction in transmitted light caused by immune-complex formation, whereas nephelometry measures light scattered by immune complexes. Nephelometry is generally considered more analytically sensitive for small particles and low concentrations because scattered light can be detected against a low background signal. This potential sensitivity advantage may support reproducibility in lower concentration ranges. However, it does not establish direct interchangeability, because SFLC assay performance is also influenced by antibody specificity, calibration, dilution linearity, and antigen-excess protection.

Another analytical consideration relates to the measurement of very high free light chain concentrations. Antigen excess (Hooke effect) can lead to falsely low results when samples exceed the analytical range and are not appropriately diluted. On the Optilite platform used for the Freelite assay, samples exceeding the measurement range are automatically diluted and reanalyzed. In contrast, nephelometric platforms such as the BN II analyzer may require manual dilution procedures to detect antigen excess. In the present dataset, the largest discrepancy in κ concentrations occurred in a sample with extremely elevated values, which may reflect such analytical limitations. These findings highlight the importance of establishing appropriate dilution protocols and verification procedures when implementing new SFLC assays. In practice, antigen excess should be suspected when SFLC results are unexpectedly low or inconsistent with the κ/λ ratio, serum protein electrophoresis, immunofixation findings, total immunoglobulin results, renal status, or clinical suspicion. In such cases, repeat testing with appropriate manual or serial dilution should be performed, and results should be accepted only when the measured concentration falls within the validated analytical range after dilution correction.

Despite the observed differences in absolute quantitation, the correlations between the two assays were strong. For most samples within typical clinical ranges, the two methods demonstrated consistent monotonic relationships, indicating that higher values measured by one assay generally corresponded to higher values measured by the other. Nevertheless, the proportional differences observed indicate that the two assays should not be considered directly interchangeable on an absolute numerical scale. Consistent with current laboratory practice guidelines, results obtained using different SFLC assays should not be used interchangeably for longitudinal monitoring of individual patients. Instead, laboratories should apply assay-specific reference intervals and maintain methodological consistency whenever possible during patient follow-up.

From a clinical perspective, agreement in κ/λ ratio classification was observed in 91% of samples, corresponding to substantial concordance ($\kappa=0.79$). The discordant cases observed in this study occurred in samples with κ/λ ratios close to the respective reference interval thresholds. In such borderline situations, relatively small analytical differences in κ or λ concentrations may shift the calculated ratio across the diagnostic cutoff. Similar findings have been reported in previous studies, where discrepancies between Freelite and N-Latex assays were observed in samples with ratios near decision limits.

In the present study, two discordant cases involved elevated κ/λ ratios detected by the Freelite assay but not by N-Latex, whereas one case involved a reduced ratio detected only by N-Latex. These observations are consistent with previous reports indicating that neither assay consistently identifies all abnormalities detected by orthogonal diagnostic methods such as immunofixation electrophoresis. Without independent confirmation using a reference method, it is not possible to determine which assay more accurately reflects the underlying disease status in these cases. Because paired measurements were performed on the same serum specimens, these discordant classifications are unlikely to represent true biological variation. They are more plausibly explained by assay-related analytical or classification differences near reference interval thresholds.

Importantly, none of the discordant cases in our cohort involved markedly abnormal free light chain concentrations that would meet established myeloma-defining thresholds. Instead, discrepancies occurred in samples with κ/λ ratios close to diagnostic cutoffs. Laboratories transitioning between assay platforms should therefore anticipate that a small proportion of results may shift between normal and abnormal classification, particularly among samples with borderline values. The expanded review of discordant cases showed that discrepancies occurred near assay-specific decision limits rather than as major categorical disagreements.

The presence of an extremely high-concentration specimen also influenced visualization of the scatter and Bland-Altman plots. Full-range plots were retained because high SFLC concentrations are clinically relevant in plasma cell disorders, while close-up plots excluding the highest-concentration specimen were added to improve visualization of the lower and mid-concentration ranges. This approach allows both complete analytical-range assessment and clearer visualization of the distribution of most specimens.

From an implementation perspective, our findings suggest that the N-Latex assay represents a feasible alternative to the Freelite assay within our laboratory environment. The assay can be performed on the BN II nephelometric platform already available in our laboratory, allowing integration into existing

workflows. However, clinicians should be informed that quantitative differences may occur when transitioning between assays. When a new assay is introduced, previously established patient baselines may shift due to analytical differences rather than biological change. Laboratories may therefore consider establishing new baseline values for patients undergoing longitudinal monitoring after the transition.

Limitations

This study has several limitations. Firstly, the sample size was relatively small ($N=33$), which was constrained by the availability of paired residual samples during the validation period. This number was below the 40-100 patient samples recommended by CLSI EP09-A3 for comprehensive method-comparison studies when feasible. Although the included specimens covered a wide analytical concentration range, the limited sample size reduces the ability to detect subtle analytical differences and may increase the influence of individual outliers. In addition, most samples had normal κ/λ ratio classifications, and abnormal specimens were underrepresented. Secondly, the study used leftover diagnostic samples; therefore, the sample population may not fully represent all clinical scenarios encountered in routine practice. Thirdly, discrepant results were not independently verified using reference diagnostic methods such as immunofixation electrophoresis or urine Bence-Jones protein analysis. Consequently, the study evaluates analytical agreement between assays rather than diagnostic accuracy relative to a clinical reference standard. Finally, the study focused primarily on comparative accuracy, and other analytical performance characteristics, including long-term precision, analytical sensitivity, lot-to-lot variation, dilution linearity, antigen-excess performance, and cost-effectiveness, were not evaluated in detail.

Conclusion

This method-comparison study demonstrates that the Freelite and N-Latex serum free light chain assays show strong overall correlation but exhibit systematic quantitative differences, particularly for κ measurements. These differences may influence κ/λ ratio interpretation when values are close to diagnostic thresholds. Nevertheless, categorical agreement between assays was substantial, with most samples yielding concordant clinical classifications. Our findings support the feasibility of implementing the N-Latex assay for routine SFLC testing, provided that assay-specific reference intervals, appropriate dilution protocols, and antigen-excess precautions are applied, and clinicians are informed about potential quantitative differences during the transition between analytical platforms. However, the two assays should not be considered directly interchangeable for absolute numerical interpretation or longitudinal monitoring,

and new patient baselines may be needed after platform transition.

Ethical approval

This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Research Ethics Committee of the Faculty of Medicine, Chiang Mai University (Study Code: PAT-2568-0329).

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Conflict of interest

The authors declare no conflict of interest.

CRedit authorship contribution statement

Dararat Dankai: conceptualization, investigation, methodology, validation, data curation, writing: original draft; **Phichayut Phinyo:** methodology, formal analysis, software, visualization, writing: review and edit; **Wiyada Dankai:** conceptualization, methodology, supervision, project administration, resources, validation, writing: review and edit. All authors approved the final version of the manuscript.

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