

Evaluation of the Impact of Renal Failure on Correlation and Concordance Between 2 Free Light Chain Assays

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Abstract

Measurement of serum free light chains (FLCs) is recommended for diagnosis of monoclonal gammopathies. FLC measurements with Freelite (Binding Site) and N Latex FLC (Siemens) assays were performed on 1215 fresh sera samples from patients with or without monoclonal gammopathy and renal failure. A good correlation was demonstrated between both assays, but it remained 7.6% to 20.8% discordances between the methods related to the FLC ratio interpretation. In patient follow-up, few discrepancies were observed. Neither of the assays performed better than the other: they provide comparable but not equivalent results, and discrepancies are not linked to renal failure stage. Interpretation must take into account clinical data and the same assay must be used for patient follow-up.

Background: Free light chain (FLC) assays are essential for diagnosis and follow-up of plasma cell dyscrasia. Two assays are available: Freelite (Binding Site) and N Latex FLC (Siemens). The aim of our study was to evaluate the impact of renal failure on concordance and correlation between the 2 FLC assays. **Methods:** FLC measurements using both assays were performed on 1215 fresh serum samples from patients with or without monoclonal gammopathy and renal failure. Concordance and correlation were evaluated using Passing-Bablok regression, Pearson correlation coefficient, and the Cohen kappa coefficient, taking into account the renal failure stage (evaluated with Chronic Kidney Disease–Epidemiology Collaboration formulae) and evaluation of treatment response in patients' follow-up. **Results:** A good correlation was demonstrated between both assays, irrespective of the renal failure stage (Pearson correlation coefficient > 0.90). For FLC ratio interpretation, there remained 7.6% to 20.8% discordances between the 2 methods throughout the whole range of renal impairment. To evaluate FLC evolution in patient follow-up, 41 patients were selected with at least 6 consecutive serum samples being collected during the study period: we observed a concordant evolution of FLC concentrations between both assays. However, few discrepancies were observed with 4 patients. **Conclusions:** Despite adjusted reference ranges for Freelite FLC ratio, there are approximately 12.5% discrepancies in interpretation of FLC ratio between the 2 available assays. They are not linked to renal failure stage and neither of the assays performed better than the other: results must be interpreted taking into account clinical data and the same assay must be used for patient follow-up.

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Introduction

Monoclonal gammopathy (MG) constitutes a heterogeneous family of pathologies, including MG of undetermined significance (MGUS), multiple myeloma (MM), smoldering MM (SMM), and

Waldenström macroglobulinemia. MGUS and SMM are asymptomatic, premalignant stages of MM; however, SMM is at an intermediary clinical stage between MGUS and MM, with a higher risk of progression to malignant disease.^{1,2} To diagnose and monitor

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patients with MG, protein electrophoresis, immunofixation, and kappa (κ) and lambda (λ) free light chains (FLCs) measurement in serum are the gold standard. The FLC ratio (ratio of the concentration of κ -FLC to λ -FLC) reflects the FLC type distribution. Since 2006, normalization of this ratio has been considered to be a marker for treatment response in recommendations.³ Since 2011, the FLC ratio has become the definition of complete response for light chain MM and nonsecreting myelomas. Furthermore, the FLC ratio can be applied for prognostication.⁴ At diagnosis, it represents an independent risk factor for progression in MGUS, SMM, and MM, as well as in solitary plasmacytoma.^{5,6} MM diagnosis criteria were updated in 2014 by a consensus of the International Myeloma Working Group (IMWG) to integrate biological markers of malignancy.⁷ In particular, these new criteria include a serum FLC ratio of involved/uninvolved FLCs of 100 or greater, provided the absolute range of the involved FLC is at least 100 mg/L. In this context, developing a method for FLC quantification is of special interest because it represents the tumor marker of choice directly connected to physiopathology. The first available method for FLC measurement was developed in 2001 by The Binding Site Company (Birmingham, UK).⁸ Freelite assay is an immunonephelometric or immunoturbidimetric method using polyclonal antibodies directed against the hidden epitopes of the FLC, and it recognizes only FLCs that are untied to heavy chain. In 2011, another N Latex FLC method using monoclonal antibodies was developed by Siemens (Marburg, Germany).⁹ These 2 assays are not entirely equivalent and seem to have limited clinical utility in detecting MG in some clinical situations, and care should be taken for interpretation if assays are switched.¹⁰⁻¹⁷

Plasma cells normally produce light chains in excess that do not bind to heavy chains to form complete immunoglobulin molecules and instead enter the bloodstream as FLCs. The excess of polyclonal FLCs is rapidly eliminated via glomerular filtration, followed by tubular reabsorption and degradation. In patients with chronic kidney disease (CKD) having a reduced glomerular filtration rate (GFR), the renal clearance of polyclonal FLCs decreases and serum concentrations rise. In 2008, Hutchison et al¹⁸ described an increase in the FLC κ/λ ratio using the Freelite method in patients with CKD (estimated GFR [eGFR] < 60 mL/min/1.73 m²) and established an extended FLC ratio reference range for these patients. Similar work on the N Latex FLC method was performed by Jacobs et al¹⁹ in 2014, who reported that reference values in the FLC κ/λ ratio did not differ in patients with CKD compared with healthy subjects. They concluded that N Latex FLC ratio in patients with CKD without MG was eGFR independent, with parallel increase of κ and λ FLCs with decreasing eGFR. They also demonstrated that N Latex κ -FLC and Freelite κ -FLC are similar in patients with CKD, whereas N Latex λ -FLCs are higher than Freelite λ -FLCs in patients with CKD.¹⁹ As 20% to 40% of patients with newly diagnosed MM present renal impairment, and as renal impairment is a common complication of MM,²⁰ it is important to understand the impact of renal function on the existing diagnostic methods. Do these 2 assays allow patients with monoclonal FLC to be similarly identified? To address this issue, the aim of our study was to evaluate the concordance and the correlation between the 2 FLC assays in 1215 serum samples from patients with or without gammopathy.

Materials and Methods

Samples

This was a monocentric prospective study performed in the biochemistry laboratory of Rennes University Hospital. Consecutive sera of patients screened or followed-up for MG were collected between July 2012 and December 2012.

FLC Quantification

FLC measurements were performed on fresh serum, within 5 days after blood sample, on a BN Prospec (immunonephelometer; Siemens) using the Freelite commercial kit (The Binding Site Ltd) based on polyclonal antibodies from sheep recognizing only FLCs that are not bound to heavy chains,⁸ and the N Latex FLC commercial kit (Siemens) using a cocktail of monoclonal antibodies of murine origin.⁹ Both kits were used according to the manufacturer's instructions.

FLC Interpretation

We chose to use the reference ranges proposed in the literature^{8,9,18}: Freelite κ -FLC 3.30 to 19.40 mg/L, Freelite λ -FLC 5.71 to 26.30 mg/L, Freelite FLC κ/λ ratio 0.26 to 1.65 or 0.37 to 3.10 if renal insufficiency (eGFR < 60 mL/min/1.73 m²); N Latex κ -FLC 6.7 to 22.4 mg/L, N Latex λ -FLC 8.3 to 27.0 mg/L and N Latex FLC κ/λ ratio 0.31 to 1.56 without specific range required for patients with CKD.

Renal function was assessed on the basis of serum creatinine levels and GFR was estimated using the Chronic Kidney Disease—Epidemiology Collaboration formula.²¹ The GFR was defined as normal or slightly decreased when eGFR is \geq 60 mL/min/1.73 m² (designated CKD _{\geq 60}). We consider a moderate renal insufficiency as eGFR of 31 to 60 mL/min/1.73 m² (designated CKD₃₁₋₆₀), severe renal insufficiency as eGFR 15 to 30 mL/min/1.73 m² (designated CKD₁₅₋₃₀), and renal failure as eGFR < 15 mL/min/1.73 m² (designated CKD_{<15}).

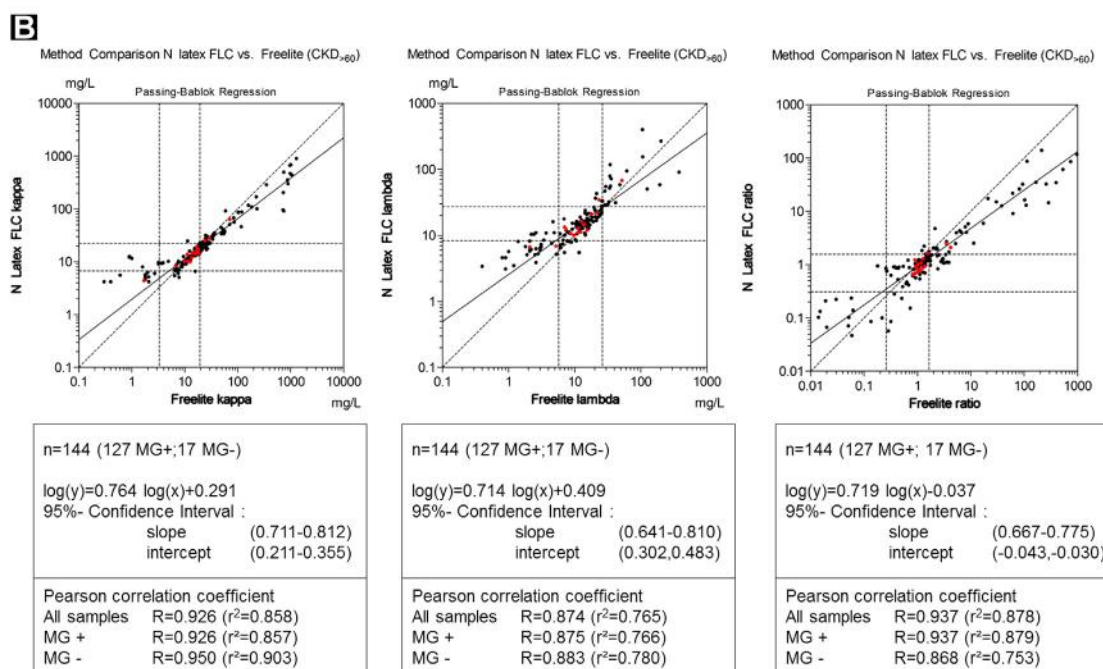
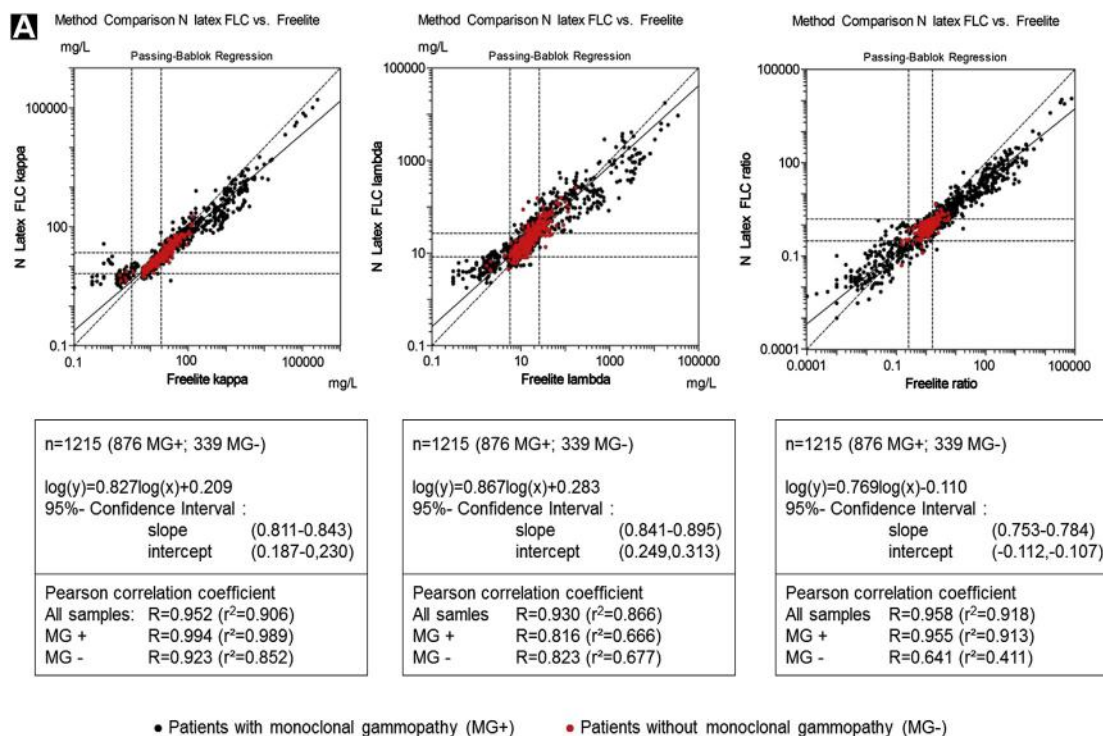
The purpose of FLC serum assay is to detect monoclonal components in the distribution of FLCs. The presence of a monoclonal component leads to an increase in the FLC κ/λ ratio with a monoclonal κ -FLC and to a decrease in the FLC ratio with a monoclonal λ -FLC. We considered concordant results to be those with the same clinical interpretation of FLC ratio (normal or suspected monoclonality) and discordant to be those with a divergent clinical interpretation of FLC ratio with each assay. In patients with renal impairment, the Freelite FLC ratio reference ranges were adjusted to avoid monoclonality misinterpretations.¹⁸

To evaluate the correlation of FLC evolution during follow-up with both assays, patients with at least 6 consecutive samples were selected. We focused on a patient's response to therapy. FLC response was examined even if the patients had measurable disease in serum and/or urine protein electrophoresis. Partial response (PR) and very good PR were defined as \geq 50% and \geq 90% decrease, respectively, in the difference between involved and uninvolved FLC levels.³

Statistical Analysis

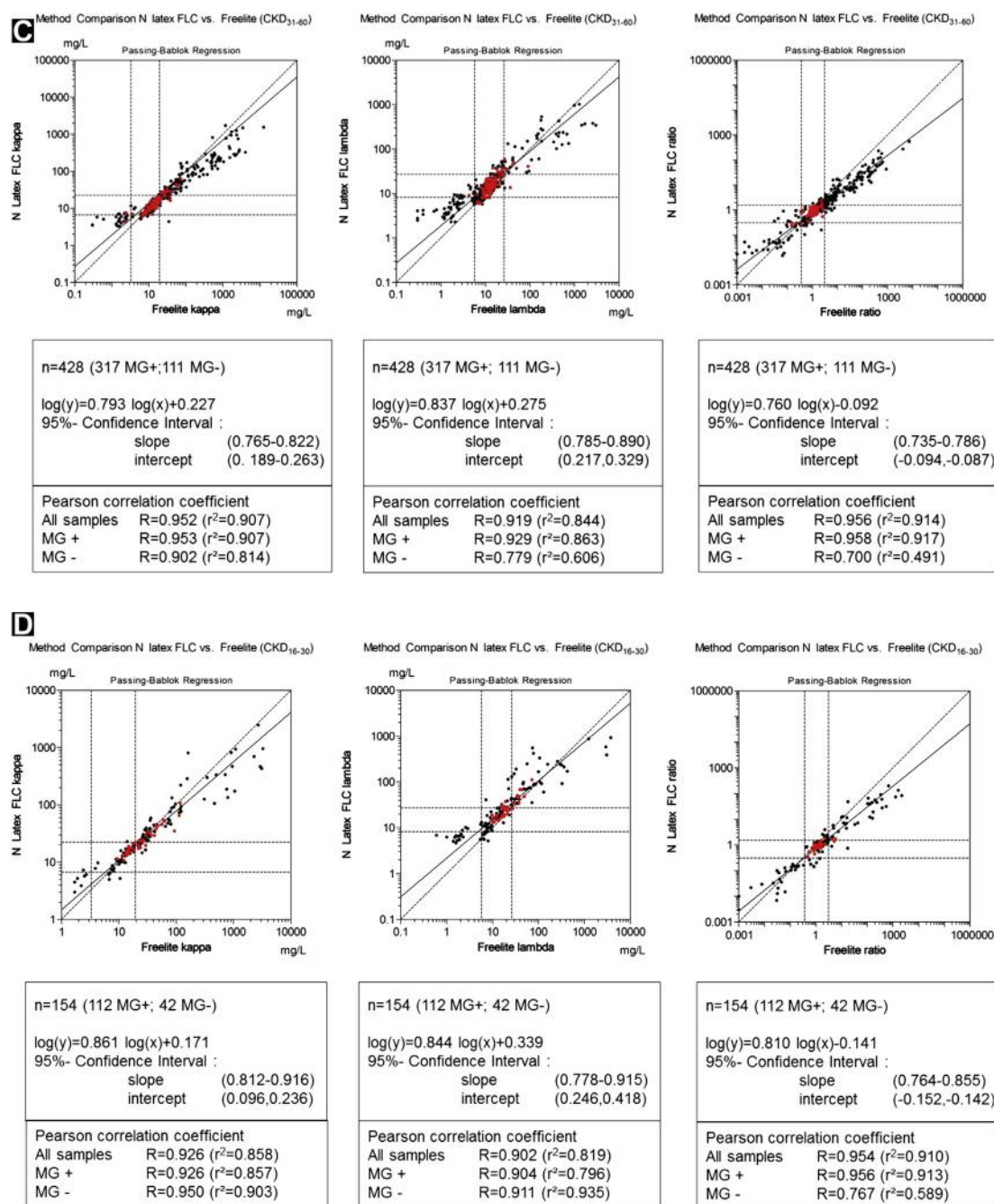
To evaluate the correlation between the 2 assays, analysis of the quantitative results of κ -FLC, λ -FLC, and FLC κ/λ ratio was

Figure 1 Freelite Assay Versus N Latex Assay for the Comparison of κ -FLC, λ -FLC, and FLC Ratio in 1215 Samples (A) and in Patients With Chronic Kidney Disease (CKD) (B: Patients With CKD > 60 mL/min/1.73 m² [n = 144]; C: Patients With $31 < \text{CKD} < 60$ mL/min/1.73 m² [n = 428]; D: Patients With $15 < \text{CKD} < 30$ mL/min/1.73 m² [n = 154]; E: Patients With CKD < 15 mL/min/1.73 m² [n = 92]). Parallel Dotted Lines Indicate the Reference Ranges for the Specific Assays. The Solid Line Indicates the Passing-Bablok Regression Equation and the Dotted Line Indicates the $y = x$ Axes. Black Plots Represent Patients With Monoclonal Gammopathy (MG+) and Red Plots Those Without Monoclonal Gammopathy (MG-). Under Each Figure Is Shown the Passing-Bablok Regression and Pearson Correlation Factor for All Samples and Samples With (MG+) or Without (MG-) Monoclonal Gammopathy



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Figure 1 continued



performed by using Passing-Bablok regression and Pearson correlation coefficient r .

To evaluate the qualitative concordance of the 2 tests, the Cohen kappa (κ) coefficient was calculated. We concluded a complete agreement was when $\kappa = 1$, a high agreement when $0.81 \leq \kappa$ coefficient < 1 and a good agreement when $0.61 \leq \kappa$ coefficient < 0.80 . Statistical analyses were performed by using SAS software version 9.1 (SAS Institute, Cary, NC).

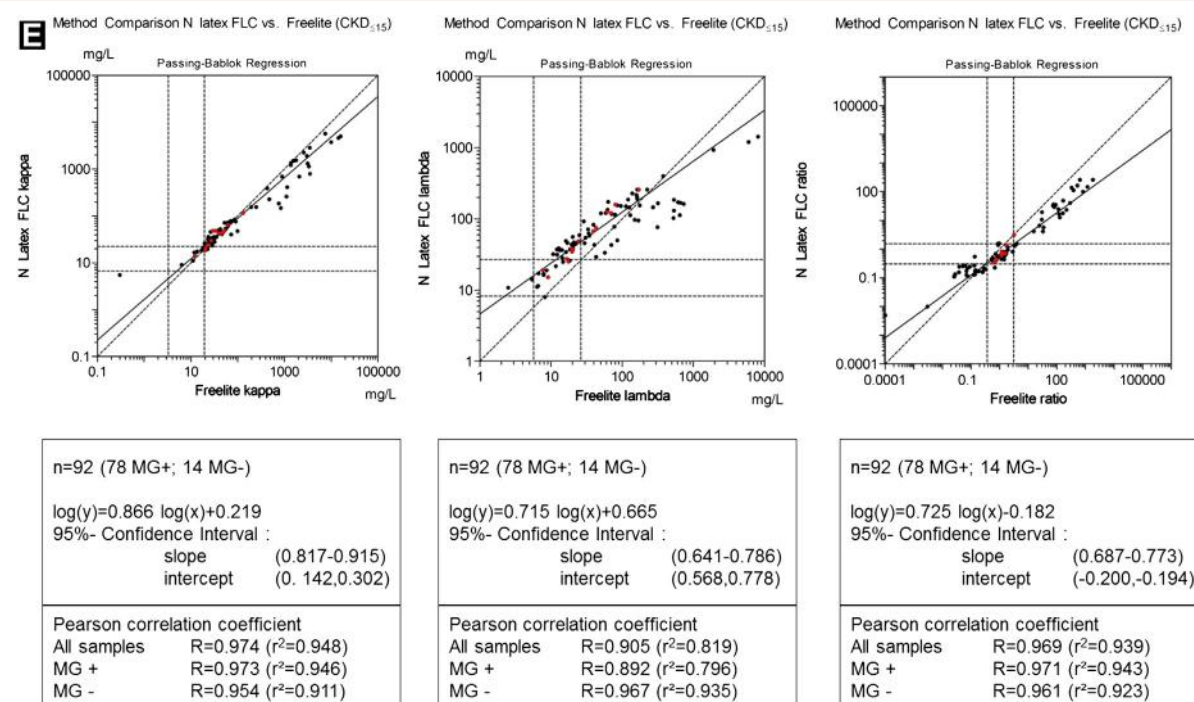
Because of the low number of patients having serial samples, we did not perform statistical analysis for correlation during follow-up.

Results

Quantitative Analysis: FLC Absolute Value Comparison

In this cohort study, 1215 sera were measured for FLC with Freelite and N Latex assays. Among these 1215 sera, 818 samples corresponding to 455 patients were assayed for FLC measurement with evaluated renal

Figure 1 continued



Abbreviation: FLC = free light chain.

function: 144 samples were obtained from patients with normal renal function, 428 samples with moderate renal insufficiency, 154 samples with severe renal insufficiency, and 92 samples with renal failure.

In the whole cohort, 368 patients suffered from MG, which represented 876 samples.

Figure 1 illustrates Passing-Bablok regressions for κ -FLC, λ -FLC, and κ/λ -FLC ratio for the 1215 sera samples and according to renal impairment.

As expected, MG- samples are essentially located within normal values.

With regard to the complete cohort of 1215 samples, a good correlation was observed between both assays with a Pearson correlation coefficient > 0.93 (Figure 1A). Passing-Bablok regressions for κ -FLC, λ -FLC, and κ/λ -FLC resulted in the conclusion that the bias between the 2 assays is neither constant nor proportional. In the low range for κ and λ FLC, higher results were observed with N Latex assay compared with Freelite assay. For the κ -FLC and λ -FLC below 7 mg/L (0.85 logged), an almost systematic negative bias was observed, which surpassed the limits of agreement below 4.5 mg/L (0.65 logged). A bias was noted in higher values for κ -FLC (from 155 mg/L), λ -FLC (from 49.4 mg/L), and the FLC κ/λ ratio (from 11.6). We observed that concentrations obtained with the Freelite method were more elevated than those with N Latex FLC assay. Nevertheless, contrary to low values of FLC, the plots were not distributed exclusively, but only preferentially, on one side of the $y = x$ -axis.

Results of the 2 assays according to renal failure stages were then compared (CKD_{>60}, Figure 1B; CKD₃₁₋₆₀, Figure 1C; CKD₁₅₋₃₀,

Figure 1D; and CKD_{<15}, Figure 1E). A good correlation between the 2 assays was demonstrated, irrespective of the renal failure stage (Pearson correlation coefficient > 0.90 , except for comparison of λ -FLC for CKD > 60 mL/min/1.73 m², Pearson correlation coefficient 0.874). As previously described in the whole cohort, the N Latex assay provides higher results in the low range and lower results in the high range, when compared with the Freelite method.

This observation concerning low values of κ -FLC and λ -FLC could be explained by the inability of Freelite assay to reliably detect very low concentrations of FLC. Indeed, reproducibility studies performed in our laboratory for κ -FLC and λ -FLC in sera highlighted that the variation coefficient could rise up to 11.2% for low values of λ -FLC (data not shown). Of note, Pretorius et al²² evaluated a variation coefficient up to 20% for this test in low values of λ -FLC. We could therefore conclude that the 2 assays are in good correlation in absolute values, irrespective of the renal failure stage.

When examining the results obtained in patients with or without gammopathy, it seems that patients with MG are not distributed differently across the different panels of renal failure stages. Moreover, we found a good correlation between both assays for κ -FLC in MG+ and MG- when eGFR > 60 mL/min/1.73 m². However, in this stage, the Pearson correlation coefficient was always lower than 0.90 for λ -FLC (Figure 1B).

As concerns MG+ samples, we observed a good correlation ($R > 0.90$) between both assays for κ -FLC and FLC ratio, irrespective of the renal failure stage. For λ -FLC, the correlation between the 2 assays is less good with $R < 0.90$ when eGFR

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was $< 15 \text{ mL/min/1.73 m}^2$. Irrespective of the renal failure stage, concerning FLC ratio, Pearson correlation coefficient was > 0.93 in MG+ samples.

With regard to MG- samples, we observed a poor correlation between assays for FLC ratio when eGFR was $< 60 \text{ mL/min/1.73 m}^2$ (except for eGFR $< 15 \text{ mL/min/1.73 m}^2$, probably due to the low effective $n = 14$ MG-).

It seems that in patients with renal failure, the correlation between the 2 assays is better in patients with MG than in patients without MG.

Qualitative Analysis: Clinical Interpretation of FLC Ratio

The next step of our study was to evaluate the concordance of the clinical interpretation of FLC ratio.

For patients without renal impairment (eGFR $> 60 \text{ mL/min/1.73 m}^2$), 79.2% (114/144) of results were concordant (Table 1A). For patients with renal failure (eGFR $< 60 \text{ mL/min/1.73 m}^2$), the rate of concordant results rose up to 92% (88.8 % for CKD₃₁₋₆₀, 89.0% for CKD₁₅₋₃₀, 92.4% for CKD_{<15}) (Table 1B-D). For patients with eGFR $< 30 \text{ mL/min/1.73 m}^2$, we found a high agreement (κ coefficient > 0.81) and for patients with eGFR $> 31 \text{ mL/min/1.73 m}^2$, we found a good agreement ($0.61 \leq \kappa$ coefficient < 0.80). These results confirmed that CKD was not linked to a higher rate of discordances when applying a specific reference range for Freelite FLC ratio. Moreover, there remained 7.6% to 20.8% of discordances between the 2 methods throughout the whole range of renal impairment. In the whole cohort, 12.5% (102/818) of discordant results were observed, corresponding to 40 patients. Regarding the clinical data, these discrepancies are not exclusively in favor of either of the 2 assays, and results should be interpreted on a case-by-case basis. In a few samples, these observations suggested that follow-up should be done with both assays to enable the clinician to interpret FLC results according to the patient's clinical state. These discrepancies are currently under further evaluation.

These statistical tests were repeated, including only the first sample of each patient to determine whether successive samples from the same patients have any influence on the study's consistency. Considering only the first sample of each patient ($n = 457$), it did not change our findings (data not shown).

We also studied the clinical interpretation in FLC ratio in patients with or without MG and with (CKD $< 60 \text{ mL/min/1.73 m}^2$) or without (CKD $> 60 \text{ mL/min/1.73 m}^2$) CKD (Table 2). Our results showed a high agreement between the 2 assays in patients without MG and with renal failure (Table 2D) with a κ -coefficient > 0.96 . These results could be explained because normal values of FLC ratio for Freelite were established in patients with CKD without MG.¹⁸ In contrast, for patients without CKD and without MG, our results provided a good to low agreement with κ coefficient = 0.61 (Table 2A). It would be probably necessary to divide this group into 2 subgroups: CKD 60-89 mL/min/1.73 m² and CKD $\geq 90 \text{ mL/min/1.73 m}^2$ but only 8 have CKD $\geq 90 \text{ mL/min/1.73 m}^2$.

To confirm these results, we also evaluated the correlation between both assays with analysis of the quantitative results of κ -FLC, λ -FLC, and FLC κ/λ ratio (using Passing-Bablok regression and Pearson correlation coefficient r) when glomerular renal function

Table 1A Concordance of FLC Ratio Clinical Interpretation Between Freelite and N Latex FLC Assays

CKD $> 60 \text{ mL/min/1.73 m}^2$ ($n = 144$)			
N Latex FLC ratio	Freelite FLC Ratio		
	< 0.26	0.26-1.65	> 1.65
	n	n	n
< 0.31	12	5	0
0.31-1.56	2	66	18
> 1.56	0	5	36

Cohen kappa coefficient 0.6323.

Table 1B

CKD $31-60 \text{ mL/min/1.73 m}^2$ ($n = 428$)			
N Latex FLC ratio	Freelite FLC Ratio		
	< 0.26	0.37-3.1	> 1.65
	n	n	n
< 0.31	40	7	0
0.31-1.56	11	217	7
> 1.56	0	23	123

Cohen kappa coefficient 0.802.

Table 1C

CKD $15-30 \text{ mL/min/1.73 m}^2$ ($n = 154$)			
N Latex FLC ratio	Freelite FLC Ratio		
	< 0.26	0.37-3.1	> 1.65
	n	n	n
< 0.31	25	6	0
0.31-1.56	0	78	3
> 1.56	0	8	34

Cohen kappa coefficient 0.8121.

Table 1D

CKD $< 15 \text{ mL/min/1.73 m}^2$ ($n = 92$)			
N Latex FLC ratio	Freelite FLC Ratio		
	< 0.26	0.37-3.1	> 1.65
	n	n	n
< 0.31	26	1	0
0.31-1.56	1	33	2
> 1.56	0	3	26

Cohen kappa coefficient 0.8848.

Results are presented according to the reference range proposed by manufacturer. Abbreviations: CKD = chronic kidney disease; FLC = free light chain.

was evaluated with MDRD (Modification of the Diet in Renal Disease) formulae. We then evaluated the qualitative concordance of the 2 tests (Cohen kappa coefficient) when GFR was evaluated with MDRD formulae. Results are comparable to those presented previously (data not shown).

FLC Evolution and Patient Follow-Up

To evaluate FLC evolution measured with both assays in patient follow-up, 41 patients were selected with at least 6 consecutive serum samples collected during the study period. Of these 41 patients, 19 had normal FLC ratio for both assays during follow-up and 22 presented an abnormal FLC ratio with Freelite and N Latex with an objective monoclonality, corresponding to 155 samples. Seven patients had monoclonal λ -FLC (P6, P9, P12, P15, P16, P18, and P19) and 15 patients had monoclonal κ -FLC. Of these 22 patients, 14 had no response or progressive disease, 5 presented PR, and 3 presented VGPR, with the 2 assays. Thus, in patient follow-up, both assays allowed similar classification of the patient's response to treatment. Figure 2 shows the trend of κ/λ FLC ratio with Freelite assay (gray line, left axis) and N Latex assay (black line, right axis) for 22 patients who were sampled at least 6 times. It can be seen that, for 11 patients, Freelite FLC ratio and N Latex FLC ratio showed a parallel evolution (P1, P4, P5, P7, P8, P9, P10, P11, P12, P13, and P21). For 5 patients (P3, P14, P17, P20, and P22), few discrepancies were observed with no clinical impact. These discrepancies are linked to the very low concentration of uninvolved FLC, which was systematically λ -FLC. Of note, as previously described, the variation coefficient, which could increase to 11.2% for low values of λ -FLC, is responsible for huge variations, explaining the variations in the ratios. For patient 6 (P6) we observed a discrepancy in the FLC ratios; there were no discordances in the κ -FLC and λ -FLC absolute values. However, a few significant discrepancies were observed with 4 patients: no response or progressive disease with Freelite and PR with N Latex (3 cases, P15-P18-P19), no response or progressive disease with N Latex and PR with Freelite (1 case, P2) (Figure 2). As a result of repeated serum samples, however, these discrepancies did not influence evaluation of patients' response. Patient 16 (P16) was particularly interesting because during follow-up, Freelite FLC ratio was within the normal range, whereas N Latex FLC ratio and λ -FLC measured with N Latex suggested a monoclonal λ -FLC (Figure 2). He was diagnosed with an immunoglobulin G lambda MM in January 2012.

These differences in absolute concentrations illustrated that this evaluation (especially with FLC assays) needs at least 2 consecutive analyses to be interpreted. For example, Figure 3 illustrates 1 patient's follow-up (P18): between June 26, 2012, and July 24, 2012, λ -FLC measured with Freelite assay increased from 260 to 272 mg/L (+4.6%) while λ -FLC measured with N Latex assay decreased from 282 to 146 mg/L (-48.2%) (Figure 3). When monitoring the disease with dFLC (dFLC = involved FLC - uninvolved FLC), these results indicated a stable condition with Freelite (slight increase of 1.9%) and a PR (-53.1%) with N Latex FLC. This PR was also found with Freelite in the next sample.

Discussion

Serum protein electrophoresis, immunofixation, and FLC measurement are now the gold standard for diagnosis and monitoring of

Table 2A Concordance of FLC Ratio Clinical Interpretation Between Freelite and N Latex FLC Assays Considering the Production of a Monoclonal Compound by the Patient

CKD >60mL/min/1.73 m ² With Monoclonal Gammopathy (n = 127)			
N Latex FLC ratio	Freelite FLC Ratio		
	<0.26	0.26-1.65	>1.65
	n	n	n
<0.31	12	5	0
0.31-1.56	2	52	18
>1.56	0	4	34

Cohen kappa coefficient 0.6133.

Table 2B

CKD >60mL/min/1.73 m ² Without Monoclonal Gammopathy (n = 17)			
N Latex FLC ratio	Freelite FLC Ratio		
	<0.26	0.26-1.65	>1.65
	n	n	n
<0.31	0	0	0
0.31-1.56	0	14	0
>1.56	0	1	2

Cohen kappa coefficient 0.7671.

Table 2C

CKD ≤60mL/min/1.73 m ² With Monoclonal Gammopathy (n = 507)			
N Latex FLC ratio	Freelite FLC Ratio		
	<0.37	0.37-3.1	>3.1
	n	n	n
<0.31	87	14	0
0.31-1.56	11	173	11
>1.56	0	30	181

Cohen kappa coefficient 0.7964.

Table 2D

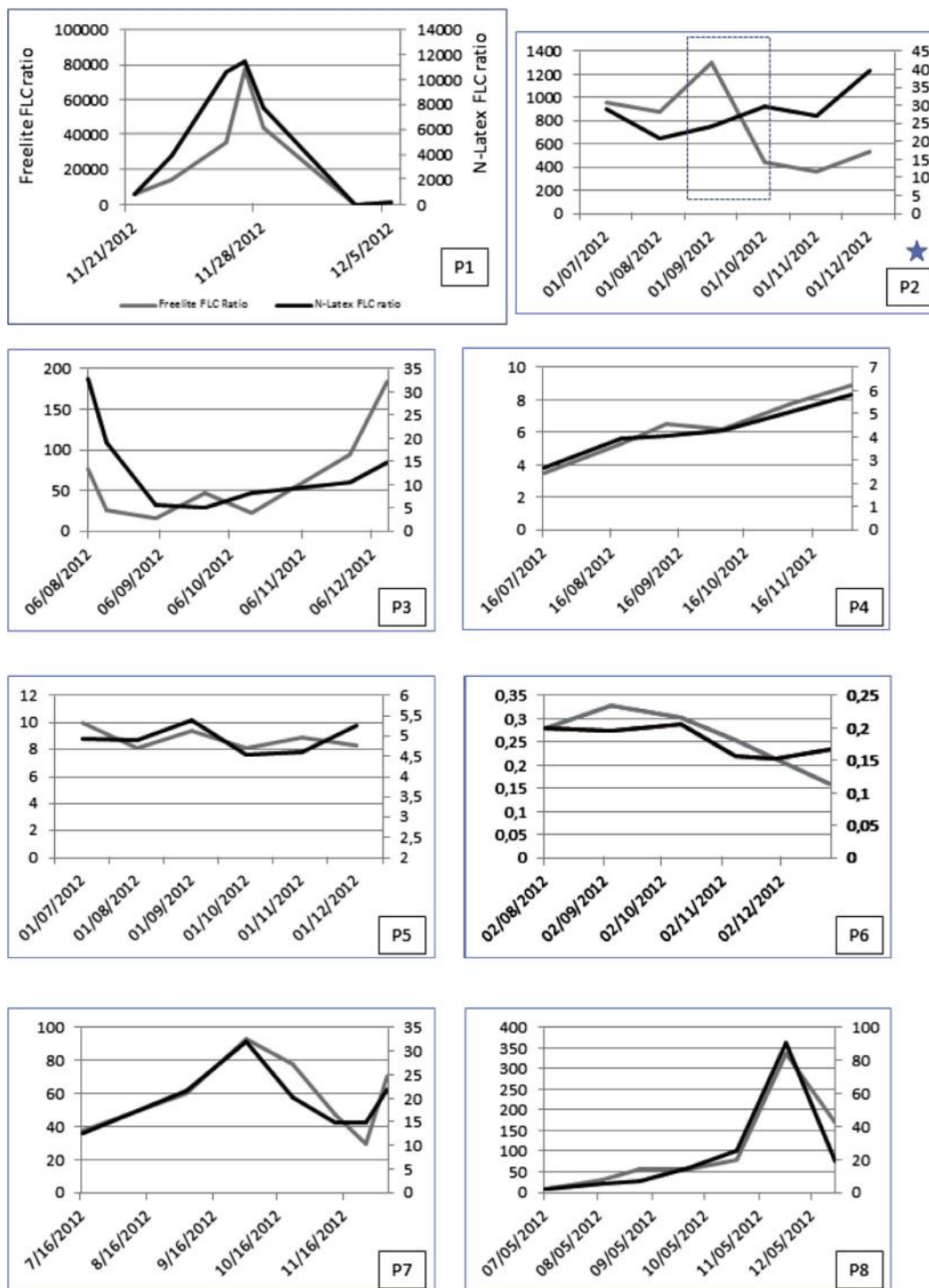
CKD ≤60mL/min/1.73 m ² Without Monoclonal Gammopathy (n = 167)			
N Latex FLC ratio	Freelite FLC Ratio		
	<0.37	0.37-3.1	>3.1
	n	n	n
<0.31	4	0	0
0.31-1.56	1	155	1
>1.56	0	4	2

Cohen kappa coefficient 0.9626.

Results are presented according to the reference range proposed by manufacturer. Abbreviations: CKD = chronic kidney disease; FLC = free light chain.

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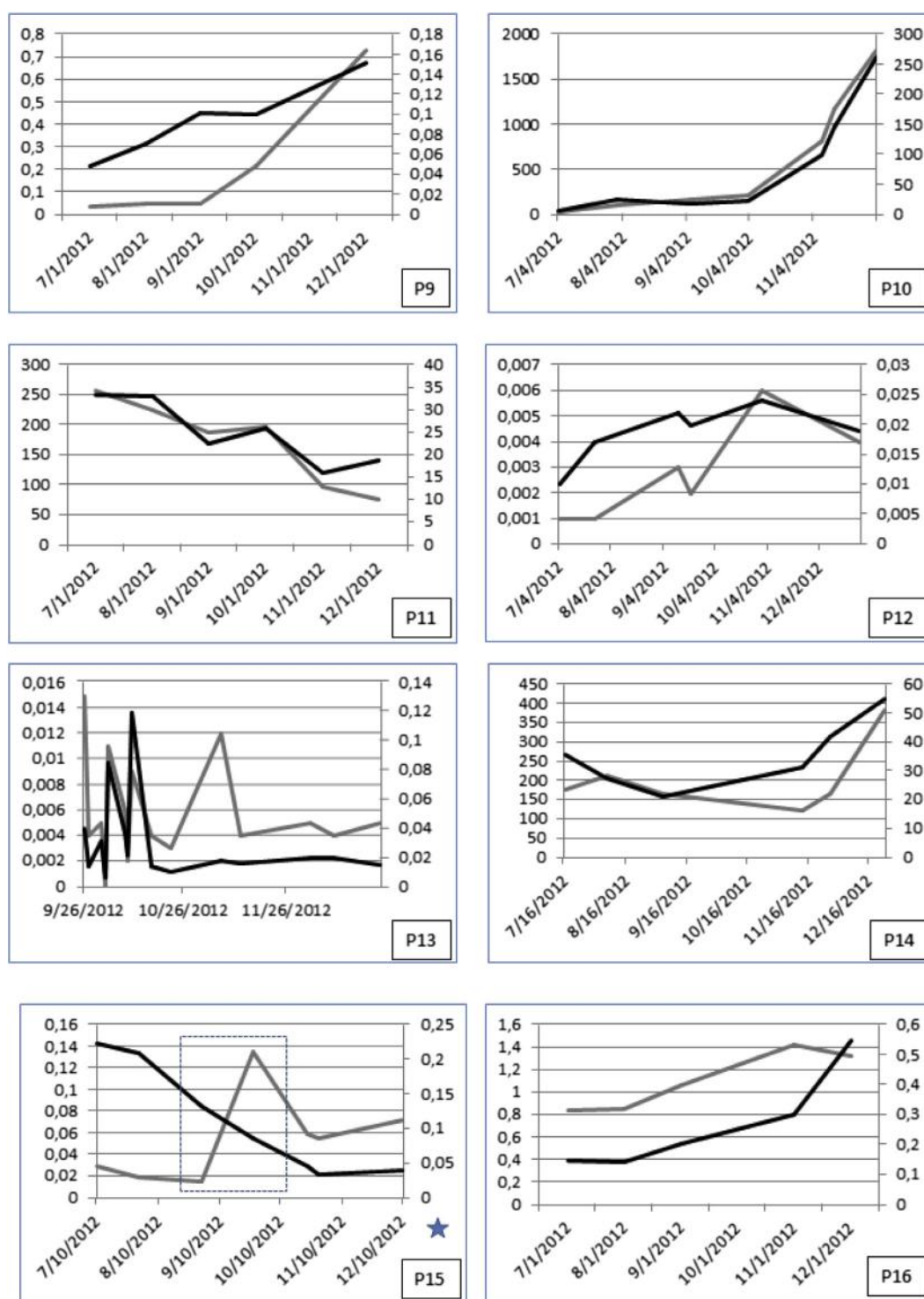
Figure 2 Follow-Up of the Freelite FLC Ratio and N Latex FLC of the 22 Patients With an Abnormal FLC. Graphs Represent the Trend of κ/λ FLC Ratio With Freelite Assay (Gray Line, Left Axis) and N Latex Assay (Black Line, Right Axis) for 22 Patients Who Were Sampled at Least 6 Times. Patients With a Star Are Those Having a Significant Clinical Discrepancy in Their Care, Which Is Framed on the Graph



patients with MG. In 2001, the Binding Site Company developed the first quantitative assay (Freelite assay), which is an adaptable turbidity measurement and nephelometry method based on

polyclonal antibodies.⁸ This assay was the only commercially available assay when recommendations for evaluation and management of MM by the IMWG were established. A second assay,

Figure 2 continued



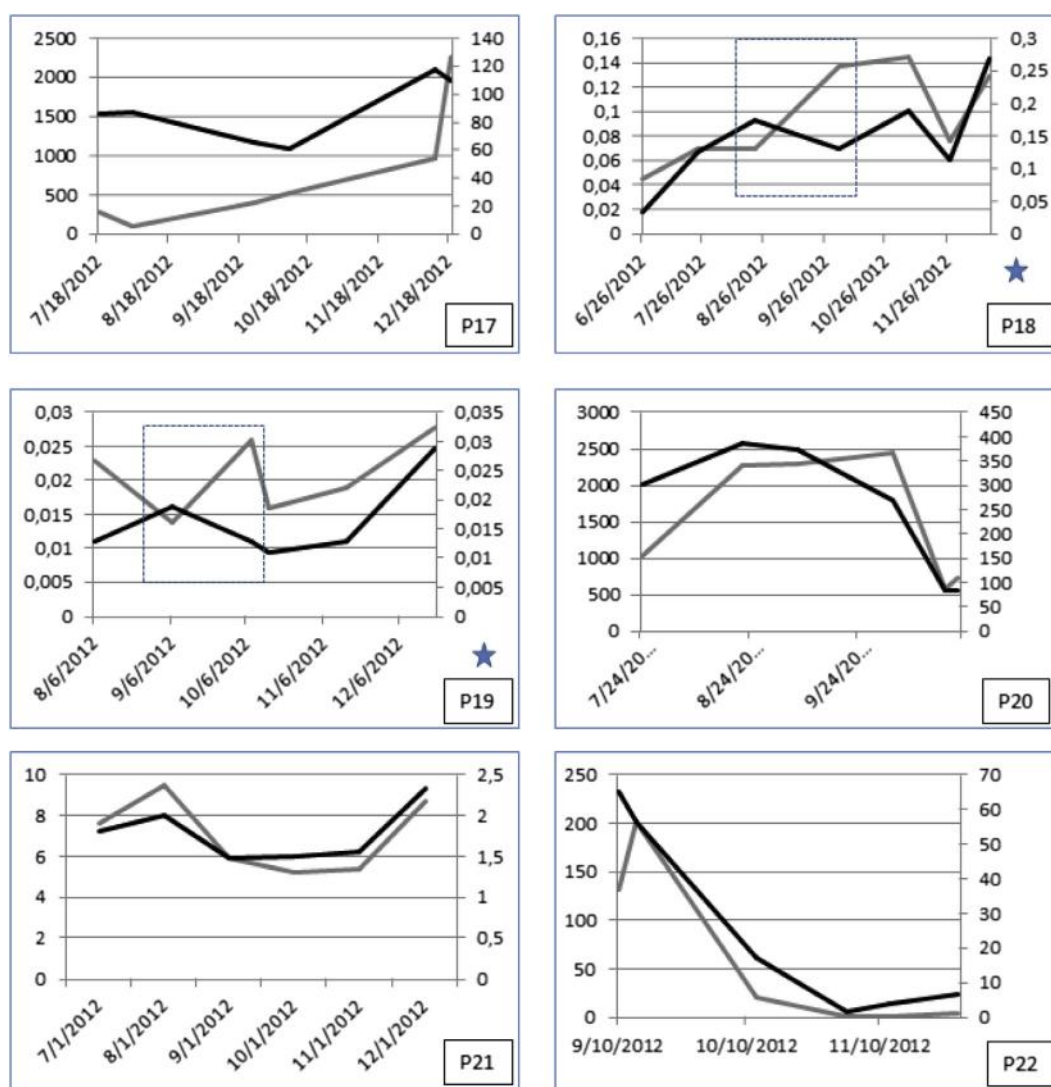
N Latex, developed by Siemens in 2011, uses only nephelometry and uses a mixture of monoclonal antibodies. More recently, the Seralite dual κ and λ assay was developed by Abingdon Health (York, UK): this is a rapid and portable diagnostic device enabling

the simultaneous quantification measurement of serum κ and λ FLC, but a very few data are available concerning this assay.¹⁷

Many studies have been carried out to compare results of Freelite and N Latex assays, and they have concluded that, besides analytical

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Figure 2 continued

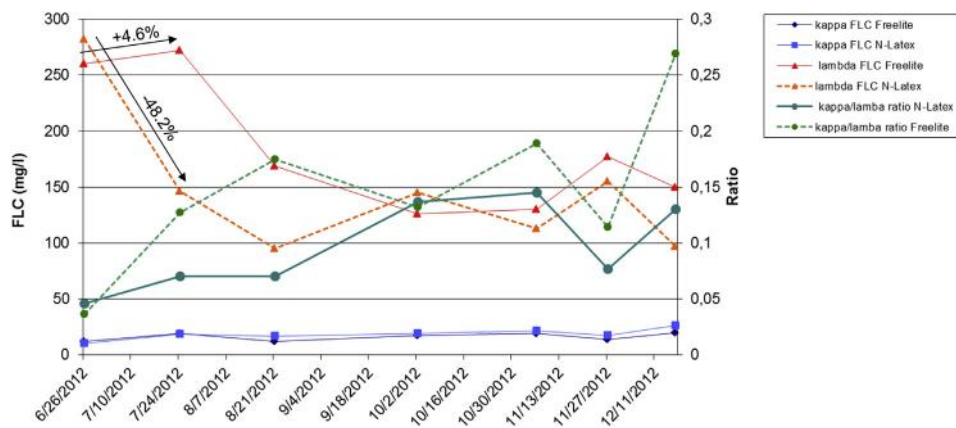


limitations, numerical results are not equivalent but overall show a good match, with slightly lower correlations for λ -FLC and FLC κ/λ ratio.^{10-14,17,23-25} As FLCs are cleared via glomerular filtration, FLC levels increase with decreasing GFR. Recently, Jacobs et al¹⁹ demonstrated that N Latex FLC ratio in patients with CKD without gammopathy seem to be eGFR independent, with a parallel increase of κ -FLC and λ -FLC with decreasing GFR. In this study, N Latex κ -FLCs and Freelite κ -FLCs were similar in patients with CKD, whereas N Latex λ -FLCs provided higher results than Freelite λ -FLCs. As a result, FLC κ/λ ratio increases with decreasing GFR with Freelite, which explains the necessity of a specific reference range for patients with CKD with Freelite. In contrast, with N Latex, the FLC ratio reference range remains unaffected over all stages of CKD. Moreover, Kennard et al²⁵ demonstrated in a cohort of 112 patients receiving hemodialysis with polyclonal FLC, that λ -FLC levels were higher with the N Latex assay than with Freelite

assay and this excess of λ -FLC persisted after dialysis but slightly attenuated. However, all these results were obtained in patients with CKD without MG. In this context, we evaluated the concordance and the correlation between both FLC assays in patients with or without gammopathy taking into account the impact of kidney disease. Our results showed a good correlation between the 2 assays ($R > 0.90$), when considering absolute values of FLC and FLC ratio from the whole cohort of 1215 samples. In samples with CKD and without MG ($n = 167$), contrary to Jacobs et al¹⁹ and Kennard et al,²⁵ we observed a significant difference between the 2 assays for κ -FLC ($P = .006$, Student test), λ -FLC ($P < .0001$, Student test), and FLC κ/λ ratio ($P < .0001$, Student test).

To date, our results allowed the conclusion that both FLC assays presented a good correlation, especially when values are in the reference range of each method. Nevertheless, 2 other population groups became apparent: a group of values with low concentrations

Figure 3 Example of 1 Follow-Up for Whom Neither Assay Allowed Similar Classification of This Patient's Treatment Response. The Graph Represents the Trend of κ -FLC, λ -FLC, and κ/λ FLC Ratio Measured With Freelite and N Latex FLC Assays.



of FLCs (1-7 mg/L) for which N Latex FLC assay found higher results than the Freelite assay. In contrast, we observed a group of values with high FLC concentrations (> 100 mg/L) for which Freelite assay resulted in slightly higher results than the N Latex FLC. This latter group could be a progressive deviation from the difference between the 2 assays. This mismatch of assays in low values does not necessarily have a clinical impact on the detection of monoclonal components because values are then sufficiently low to exclude the presence of a monoclonal FLC or sufficiently high to confirm their presence. To explain these results, hypotheses regarding the formation of FLC polymers in certain cases of monoclonal protein synthesis already have been formulated. Recently, Di Noto et al¹⁰ argued that FLC polymers influence the immunometric quantification. They highlighted that the 2 methods behave differently in monoclonal samples, particularly at higher concentrations. This confirms that a monoclonal protein, depending on its biochemical/biophysical characteristics, could present different stoichiometric interactions with different test antibodies.¹⁰ This observation is usually related to the presence of FLC dimers (and sometimes multimers) in mismatching sera that present a probably modified reactivity with antibody detection and light diffraction properties.^{16,26}

Nevertheless, to date no direct relation has been found and this hypothesis of overestimation for high concentrations does not explain the mismatch observed at low concentrations. A possible relation between the nature of antibodies and implicated epitopes could be formulated. Thus, the monoclonal antibodies applied in N Latex FLC assay present better specificity for polyclonal FLC detection because they target an epitope found in all normal FLCs with a strong affinity.

When considering renal function, our results allowed the conclusion that there was good correlation between both assays, irrespective of the renal impairment stage. In our study, the N Latex FLC method detected higher concentration in λ -FLCs and κ -FLCs, contrary to Jacobs et al¹⁹ who found no difference in κ -FLC

concentrations between the 2 assays. Our results could be explained by the studied population, mainly consisting of patients with gammopathy (368 patients/455, 81% and 876 samples/1215, 72%). When we interpreted independently patients with or without gammopathy, we observed that patients with MG are not distributed differently across the different panels of renal failure stages: the correlation between both assays seems to be better in patients with MG than in patients without MG.

When comparing the interpretation of the FLC ratio, 12.5% discrepancies remained between the 2 assays and the rate was independent of the renal failure stage. In MG samples, we found 15% discordant results: 23% in $eGFR > 60$ mL/min/1.73 m² and 12% in $eGFR < 60$ mL/min/1.73 m². For these discordant results, neither assay was better than the other and results should be interpreted taking into account clinical data. To date, the 2 tests could be used in patients with renal impairment, because correlation between both assays is good even in patients with severe renal failure.

For follow-up patients, our study demonstrated generally a good correlation between the 2 assays by indicating an identical response to treatment. However, it is necessary to interpret results taking into account absolute FLC values, FLC ratio, and dFLC when applicable. Nevertheless, few discrepancies remained between both tests and the reason for these differences is still unknown. We confirmed that quantitative discrepancies remain between the 2 assays and the methods are not interchangeable in the follow-up of MG.^{12,16,23}

Conclusion

To conclude, in view of currently published data and results of this study, we can confirm a good correlation between the 2 FLC assays, even in patients with renal failure.^{15,19,25} However, results in absolute values of both assays remain comparable but not numerically identical, and the results of one assay cannot be considered to be better than the other. Because of the differences observed

Two FLC Assays: 1215 Interpretations

between the 2 assays, they cannot be used indiscriminately. When a laboratory wants to change from one test to another, it is necessary to use both tests for an approximately 6-month period of transition to avoid misinterpretation of results.

Clinical Practice Points

- FLC assays play an important role in diagnosis and follow-up of plasma cell dyscrasia.
- Two assays are available: Freelite(Binding Site) and N Latex FLC (Siemens).
- An extended Freelite FLC ratio reference range has been defined for patients with CKD, whereas N Latex FLC ratio was described as being eGFR independent.
- We evaluated concordance and correlation between the 2 assays according to renal failure stage and for evaluation of response.
- Our results demonstrated a good correlation between both assays, irrespective of the renal failure stage (Pearson correlation coefficient > 0.90); however, there remained 12.5% of discrepancy in the interpretation of FLC ratio between the 2 assays. The rate of discrepancies is independent of renal failure stage.
- Our results suggest that the 2 assays allow similar classification of the patient's response to treatment.
- Neither of the assays is better than the other and results must be interpreted by taking clinical data into consideration.
- The 2 assays cannot be interchanged. When a laboratory wants to change from one test to the other, we suggest using both tests for an approximately 6-month transition period to avoid misinterpretation of results.

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Disclosure

The authors declare no conflicts of interest; however, Siemens Healthcare Diagnosis provided reagents for the present study.

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