Clinical comparison of new monoclonal antibody-based nephelometric assays for free light chain kappa and lambda to polyclonal antibody-based assays and immunofixation electrophoresis

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Abstract

Background: New monoclonal antibody-based assays for serum-free light chains (FLC) have become available.

Methods: In a clinical study with 541 patients, the new N Latex FLC assays were compared with the Freelite™ FLC assays and immunofixation electrophoresis (IF).

Results: Comparison of the different FLC kappa (κ) assays showed a slope of 0.99 with a deviation of 5.0%, r_s=0.92, for FLC lambda (λ) a slope of 1.22, deviation 13.8%, r_s=0.90 and for the κ/λ ratio a slope of 0.72, deviation –4.6%, r_s=0.72. The concordance for the FLC κ assays was 91%, for FLC λ 85% and κ/λ ratio 95%. The clinical sensitivity and specificity of the κ/λ ratios in the study were comparable: 60% and 99% for the N Latex FLC assay and 61% and 97% for the Freelite™ assay. In IF-FLC positive samples, the N Latex FLC κ/λ ratio scored 20/23 (87%) samples outside the reference range and Freelite™ 21/23 (91%). For IF-FLC negative samples, N Latex FLC assay κ/λ ratio scored 338/350 (97%) within the reference range and Freelite™ scored 332/350 (95%).

Conclusions: The concordance scores and the clinical sensitivity and specificity of the new N Latex FLC assays and Freelite™ assays appeared comparable, but there are some differences in measurement of concentrations between the methods.

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Introduction

The detection of free light chains kappa (κ) and lambda (λ) in the serum of patients with monoclonal plasmaproliferative disorders has been proven to be a valuable tool for screening, monitoring and prognosis (1–5). The International Working Group for Multiple Myeloma incorporated the measurement of serum FLC into the guidelines for detection of multiple myeloma and related disorders and defined response criteria during treatment (6). In particular, the κ/λ ratio discriminated patients with plasma cell disorders from polyclonal increases and/or renal failure. Together with serum protein electrophoresis (SPE) and/or serum immunofixation electrophoresis (IF) near to 100% of the patients with plasma cell disorders were recognized during screening (1, 6, 7).

Despite the ground-breaking work in hematology and several advantages over the laborious SPE and IF, several technical drawbacks have been reported for the Freelite™ assays (8, 9). Recently, new monoclonal antibody-based nephelometric N Latex FLC assays for the Siemens BN™ systems were developed with good performance characteristics (10). The N Latex FLC assays showed good batch-to-batch reproducibility, antigen excess security and high precision.

The aim of this study was to compare the performance of the new N Latex FLC assays to the Freelite™ FLC assays in a clinical study consisting of 541 consecutive patients at a regional hospital. Patients with well-defined diagnosis were compared for clinical sensitivity and specificity. The clinical sensitivity and specificity were further analyzed by comparison to serum immunofixation electrophoresis.

Materials and methods

Free light chain assays

New monoclonal antibody-based nephelometric N Latex FLC assays for FLC κ and λ in serum and plasma for the BN™ nephelometric systems of Siemens were developed (Siemens Healthcare Diagnostics GmbH, Marburg, Germany) as described before (10). In brief, specific monoclonal antibodies against FLC κ and FLC λ

were covalently bound to polystyrene beads. The reference ranges are comparable to the ranges of the Freelite™ assays of The Binding Site (The Binding Site Ltd, Birmingham, UK): N Latex FLC κ 6.7–22.4 mg/L, Freelite™ κ 3.3–19.4 mg/L; N Latex FLC λ 8.3–27 mg/L, Freelite™ λ 5.7–26.3 mg/L; κ/λ N Latex FLC ratio 0.31–1.56, Freelite™ ratio 0.26–1.65. The Freelite™ assays were performed on the BN ProSpec®. The N Latex FLC assays were performed on BN™II and BN ProSpec®. There is no difference between the BN™II and BN ProSpec® for the N Latex FLC assays (10). We performed the Freelite™ assays according to information provided by The Binding Site in the insert.

Clinical study and patient samples

During a 5-month period, 647 samples of 541 patients presented to the hospital for laboratory screening or follow-up of monoclonal gammopathy were included in this study. Only the first sample from each patient was included in this study. Patients were informed about the possibility of additional laboratory testing for method comparison and validation studies. For this study, serum samples from each patient were taken and analyzed according to the national guidelines (Centraal Begeleidings Orgaan CBO 2001) for analysis of patients with monoclonal gammopathy. This analysis involved SPE and/or urine protein electrophoresis with additional serum and/or urine IF. On the day of withdrawal, an aliquot of the serum sample was frozen at –20°C for further analysis. Clinical diagnoses of the patients were determined by the local physicians. Samples included were either from patients with diagnosed monoclonal gammopathy [multiple myeloma, AL amyloidosis, monoclonal gammopathy of undetermined significance (MGUS) and Waldenström's macroglobulinemia] or from patients without signs of a monoclonal gammopathy, including patients with a polyclonal increase of immunoglobins (increase of IgG, A or M), patients with renal impairment [Modification of Diet in Renal Disease (MDRD) <60 mL/min/1.73 m^2] and patients without a specific diagnosis ("monoclonal protein not detected").

Method comparison

For the method comparison Passing-Bablok regression, median normalized differences between the methods and Spearman's rank correlation coefficients were determined. Concordance analysis was performed on the patient groups for both the N Latex FLC assays and the Freelite™ assays and the κ/λ ratios. The samples were divided into below reference range (abnormal low), within the reference range (normal) and above reference range (abnormal high). The evaluations described above were applied to the total patient group as well as different sub-groups with monoclonal gammopathy or without monoclonal gammopathy (11).

Immunofixation electrophoresis

IF on 373 samples (both serum and urine) was performed using the Hydragel 12 IF penta gels on the Hydrasys Focussing sysem of Sebia (Evry, France). FLC κ and λ were stained using FLC κ and λ polyclonal antibodies from Sebia. The analysis was performed with the Bence Jones program and the precipitates were immune fixed with acid violet. The detection limit for IF in serum is around 150 mg/L. All urine samples were concentrated by freeze drying (100× or 40 g/L) before analysis on immunofixation electrophoresis. The gels were evaluated by three independent readers. The evaluation was performed without knowledge of the outcome from the nephelometric assays.

Clinical sensitivity and specificity

We calculated the clinical sensitivity (true positive) and specificity (true negative) of the κ/λ ratios of the N Latex FLC and Freelite™ FLC assays vs. the clinical diagnosis and vs. IF analysis. To calculate clinical sensitivity, patients with monoclonal gammopathy (multiple myeloma, AL amyloidosis, MGUS and Waldenström's macroglobulinemia) were scored for the κ/λ ratios for values outside the reference ranges for both the N Latex FLC assays and the Freelite™ assays. For calculation of the "clinical" specificity the patient groups without monoclonal gammopathy [polyclonal increase (elevated concentrations of IgG, A or M), renal impairment and "monoclonal protein not detected"] were scored for the κ/λ ratios within the reference ranges for both tests.

Statistics

All statistics were performed using Microsoft Excel (Microsoft Corporation) with add-in software Analyse-it® (Analyse-it® software v2.03, Ltd, Leeds, UK: www.analyse-it.com). Normalized median difference or deviation between groups is the median of all differences between sample outcome of the two methods as calculated in % by [(Y–X)/(X+Y)]/2.

Results

We performed a clinical study on 541 patients admitted to the Jeroen Bosch Hospital for screening or follow-up of monoclonal gammopathy between April and September 2008. The local physicians diagnosed the patients into 10 categories based on their clinical diagnosis (Table 1).

In this study 54/164 of patients with a diagnosis of monoclonal gammopathy received treatment for their disease

Table 1   Study group of 541 consecutive patients.

Diagnosis	Number of patients	Complete panel
AL amyloidosis	2	1
Multiple myeloma (MM)	63	20
κ-Light chain MM	3	1
λ-Light chain MM	6	3
Monoclonal gammopathy of undetermined significance (MGUS)	72	24
Waldenström's macroglobulinemia	18	1
Renal impairment	145	125
Reactive (polyclonal immunoglobulin)	56	47
Monoclonal protein not detected	165	148
Miscellaneous[a]	11	3
Total	541	373

In 373 patients, the complete panel of results were available for N Latex FLC assays, Freelite™ FLC assays, serum and urine protein electrophoresis, serum and urine immunofixation electrophoresis and urine total protein.

[a]The miscellaneous group contains six non-Hodgkin's lymphoma patients, one patient suspected of non-Hodgkin's lymphoma, one κ-light chain cryoglobulin patient, one patient with myelodyplastic syndrome, one patient with polyneuropathy and one patient with acute lymphatic leukemia.

at the time of collection of the sample (i.e., 51/72 multiple myeloma/LCMM patients, 3/18 Waldenström macroglobulinemia patients).

The published reference ranges for Freelite™ assay and the N Latex FLC assay were used for the analysis (10, 12). The concentration of FLC κ vs. FLC λ was plotted for both assays (Figure 1). The FLC κ vs. FLC λ plots and the detection range analysis in Table 2 showed that with the N Latex FLC assays the detection ranges were narrower for FLC κ, FLC λ and κ/λ ratio than for the Freelite™ assays. The method comparison for κ, λ and κ/λ ratio showed that the Spearman's rank correlations between the methods were 0.92 for κ, 0.90 for λ and 0.72 for the κ/λ ratio (Table 2).

The comparison between the methods for FLC κ appeared good for the total patient group and for the patient groups with or without monoclonal gammopathy (Table 2). The slope for the Passing-Bablok regression analysis for the FLC κ comparison was 0.99 with a normalized difference of 5.0%. The concordance for FLC κ between both methods was 91% (Figure 2). The differences between the methods were mainly visible at the high end of the concentration range (Figure 3). Especially at the high end of the FLC λ concentration range, the Freelite™ assays concentrations appeared a factor 5–10 higher, which resulted in a normalized difference of 13.8% and a slope of 1.22 in the Passing-Bablok regression analysis for the FLC λ comparison (Table 2). The method comparison for FLC λ showed a moderate correlation, with a concordance of 85% for FLC λ (Figure 2). The method comparison for the κ/λ ratio showed a good concordance between the two methods of 95%. Discordant results for the κ/λ ratio were observed in 18/164 patients with monoclonal gammopathy, i.e., 5/18 Waldenström's macroglobulinemia patients, 7/63 multiple

myeloma patients, 1/9 LC-multiple myeloma patients and 5/72 MGUS patients. The discordance was mainly observed in patients with low levels of FLC (<50 mg/L) and in patients with renal damage. In this latter group the κ/λ ratio was influenced by increased concentrations of the corresponding FLC possibly due to the decreased clearance of the FLC.

A significant difference between the methods was observed for FLC λ in patients without monoclonal gammopathy. In 38/366 (10%) patients the FLC λ concentration was above the limit for the reference value of 27 mg/L with the N Latex FLC λ assay, whereas in the Freelite™ λ assay the levels were within the reference range (Figure 2). In these 38 patients, the FLC λ was between 27 and 79 mg/L with the N Latex FLC λ assay. The slight increase of polyclonal FLC λ did not result in an abnormal κ/λ ratio because in all patients FLC κ was also increased. All 38 patients showed renal impairment with MDRD <60 mL/min/1.73 m².

The clinical sensitivity and specificity were calculated from the clinical diagnosis. For the clinical sensitivity, we combined the samples of patients with monoclonal gammopathy (i.e., multiple myeloma, Waldenström macroglobulinemia, AL amyloidosis and MGUS) from the clinical study (n=164) and found 98/164 (60%) positive (abnormal) ratios for the N Latex FLC assays and 100/164 (61%) for the Freelite™ assays.

The clinical specificity was calculated from the groups without monoclonal gammopathy (i.e., renal impairment, reactive immune stimulation and "monoclonal protein not detected"). We found similar clinical specificities with the N Latex FLC assays compared to the Freelite™ ratio: 362/366 (99%) vs. 356/366 (97%), respectively. The 11 patients in the miscellaneous group were not incorporated in the groups with or without monoclonal gammopathy.
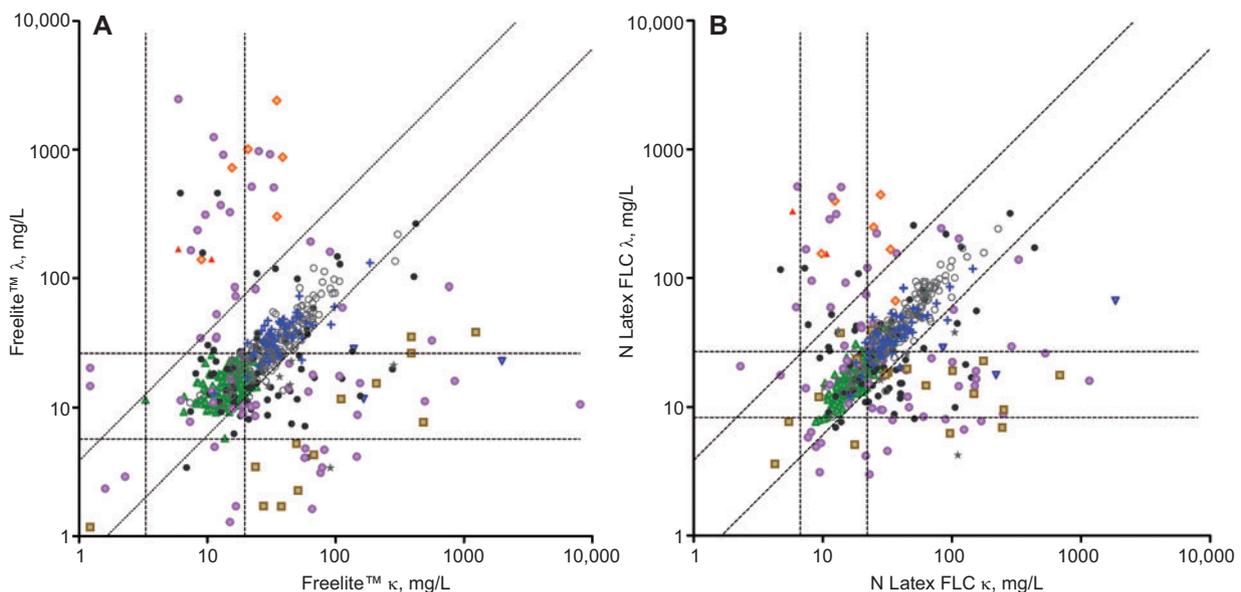
Figure 1   FLC κ vs. FLC λ for Freelite™ assays (A) and N Latex FLC assays (B) in 541 samples.
Patient groups: ▲="monoclonal protein not detected", ▼=κ-Light chain multiple myeloma, ◆=λ-Light chain multiple myeloma, ●=multiple myeloma, ●=MGUS, ▲=AL amyloidosis, +=polyclonal increase (without renal damage), ○=renal damage, ■=Waldenström's macroglobulinemia, ★=miscellaneous. Dotted lines indicate the reference ranges for the specific assays.

Table 2 Method comparison between the new N Latex FLC assays and the Freelite™ assays for all patients and patients with or without diagnosis of monoclonal gammopathy.

All patients	n	Range N Latex FLC, mg/L	Range Freelite™, mg/L	Slope Passing-Bablok, 95% CI	Spearman's correlation	Normalized difference, %
FLC κ	541	2–1850	1.2–8060	0.99 (0.95–1.04)	0.92	5.0%
FLC λ	541	3.0–513	1.3–2480	1.22 (1.16–1.28)	0.90	13.8%
κ/λ ratio	541	0.012–73	0.002–760	0.72 (0.67–0.77)	0.72	−4.6%
Monoclonal gammopathy[a]						
FLC κ	164	2–1850	1.2–8060	0.91 (0.83–0.98)	0.93	2.1%
FLC λ	164	3.0–513	1.3–2480	1.00 (0.88–1.09)	0.92	13.7%
κ/λ ratio	164	0.012–73	0.002–760	0.68 (0.62–0.75)	0.94	−9.1%
No monoclonal gammopathy[b]						
FLC κ	366	8.8–228	3.3–304	1.02 (0.98–1.07)	0.93	8.4%
FLC λ	366	7.7–241	5.8–220	1.38 (1.29–1.46)	0.89	13.8%
κ/λ ratio	366	0.49–2.29	0.29–2.69	0.71 (0.62–0.80)	0.46	−3.6%

[a]The group of monoclonal gammopathy consisted of patients diagnosed with multiple myeloma (including light chain MM), AL amyloidosis, MGUS and Waldenström's macroglobulinemia. [b]The group without monoclonal gammopathy consisted of patients with immune stimulation, renal impairment and patients with "monoclonal protein not detected". 95% CI, 95% confidence interval.

Comparison of the N Latex FLC and Freelite™ FLC test with IF analysis was performed on all samples where both urine and serum were available (n=373). The clinical sensitivity was calculated from all IF-FLC positive samples (serum and/or urine) (n=23) and compared to a κ/λ ratio outside the reference range. The clinical sensitivity for the N Latex FLC ratio was 20/23=87% and for the Freelite™ ratio 21/23=91%. The clinical specificity was calculated from all IF-FLC negative samples (n=350) and compared to a κ/λ ratio within the reference range. The clinical specificity for the N Latex FLC ratio was 338/350=97% and for the Freelite™ ratio was 332/350=95%.

Discussion

The N Latex FLC assays were compared to the Freelite™ assays on samples of 541 patients. The patients were diagnosed by the local physicians and divided into 10 different groups. We further divided the groups into patients with or without monoclonal gammopathy. A good correlation was observed in the method comparison for the FLC κ assay and moderate correlations for FLC λ and κ/λ ratio. However, high concordance scores between the methods could be demonstrated for FLC κ (91%), FLC λ (85%) and κ/λ ratio (95%). Although there seemed to be a numeral difference between N Latex FLC assays and the Freelite™ assays, the results on interpretation were highly comparable.

When we compared the results of both methods in our clinical study, we observed some clear differences. Just like the reference ranges, the new N Latex FLC assays showed result ranges which were more narrow than the results for the Freelite™ assays. We found a 5–10-fold higher concentration of FLC in the Freelite™ assays compared to the N Latex FLC assays in samples with very high concentrations of FLC. Why these assays detect FLC so differently at very high concentrations remains unclear. Several reports described an overestimation of Freelite™ FLC in patients with high serum or urine levels of FLC (6, 13). Whether the monoclonal based N Latex FLC assays are less sensitive to detection of FLC aggregates in the nephelometer, as proposed by Mead and Carr-Smith (14) for the Freelite™ assays, needs to be determined. The N Latex FLC assays may be more linear in measurement of monoclonal free light chains which may result in less over-estimation. Further studies must elucidate the exact nature of the differences observed between the two methods.

For the FLC λ and the κ/λ ratio moderate correlations were observed. This moderate correlation was mainly observed in patients without monoclonal gammopathy with increased concentrations of polyclonal FLC caused by renal impairment. In 10% of the patients without monoclonal gammopathy, the N Latex FLC λ assay detected FLC λ just above the reference range, whereas with the Freelite™ λ assay levels were within the reference range. The slight increase in FLC λ up to 70–80 mg/L corresponded with increases in FLC κ levels, which was reflected in κ/λ ratios within the reference ranges. For the Freelite™ assays it was proposed to use special reference ranges for the κ/λ ratio in patients with renal damage of 0.37–3.1 (15). It would be interesting to see whether the adjusted ratio is also needed for the N Latex FLC assays with the higher detection of polyclonal FLC λ in patients with renal damage.

In this study, the results of κ/λ ratios of both methods were highly comparable (95%). The discordant results were either observed in patients without monoclonal gammopathy with only minor deviations between the κ/λ ratios of both methods (Figure 3C), or patients with monoclonal gammopathy receiving treatment with reduced levels of the monoclonal FLC (<50 mg/L).

The N Latex FLC assays are monoclonal antibody-based assays, whereas the Freelite™ assays are polyclonal antibody-based assays (5). In both methods, the antibodies are immobilized on latex to obtain high sensitivity. The difference in detection therefore might relate to the specificities and affinities of the antibodies. Finding monoclonal antibodies with the
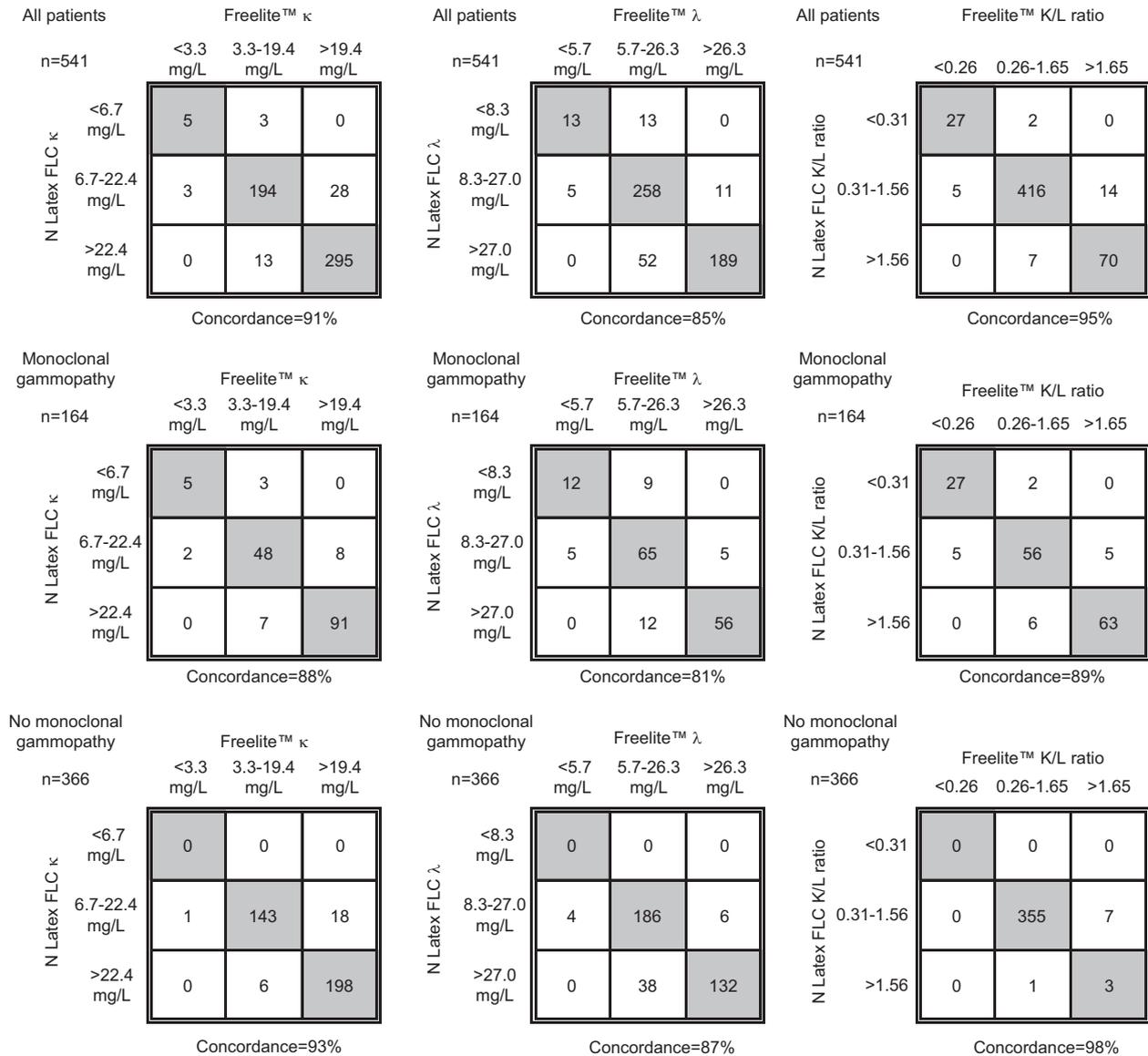
Figure 2 Concordance analysis for FLC κ, FLC λ and κ/λ ratio of the N Latex FLC assays and the Freelite™ assays.
The gray area indicates the number of samples which were either below, within or above the reference ranges of the specific assays. The group of monoclonal gammopathy consisted of patients diagnosed with multiple myeloma (including light chain MM), AL amyloidosis, MGUS and Waldenström's macroglobulinemia. The group without monoclonal gammopathy consisted of patients with immune stimulation, renal impairment and patients with "monoclonal protein not detected".

correct specificities, especially FLC λ, has been a problem for many groups, but the FLC assays appear to have detected all variants of the FLC molecules in the different monoclonal light chain disease reported in this study (16–18). More studies are needed to incorporate more clinical samples of patients with monoclonal gammopathy and especially patients with rare diseases with just small amounts of monoclonal FLC like non-secretory myeloma and AL amyloidosis.

The clinical specificity of the N Latex FLC assays was comparable to the Freelite™ assays. Both assays showed a very high accuracy. In a large group of patients without monoclonal gammopathy, the N Latex FLC assays detected FLC κ, λ or κ/λ ratio in 362/366 within the reference ranges,

whereas with Freelite™ assays this was 356/366. Also, the clinical sensitivity of the N Latex FLC assays in patients with monoclonal gammopathy appeared highly comparable with Freelite™ assays. In this group the number of patients with κ/λ ratio outside the reference range was 60% for N Latex FLC assay and 62% for Freelite™ FLC assay. In this study, a high number of patients received treatment for their disease at the time of collection of the sample, resulting in a higher number of patients with normalized or suppressed levels of FLC, compared to studies with patient samples collected at diagnosis. Furthermore, this study contains a high number of MGUS patients (72/164), also resulting in a number of patients with normal FLC concentrations and κ/λ ratios.
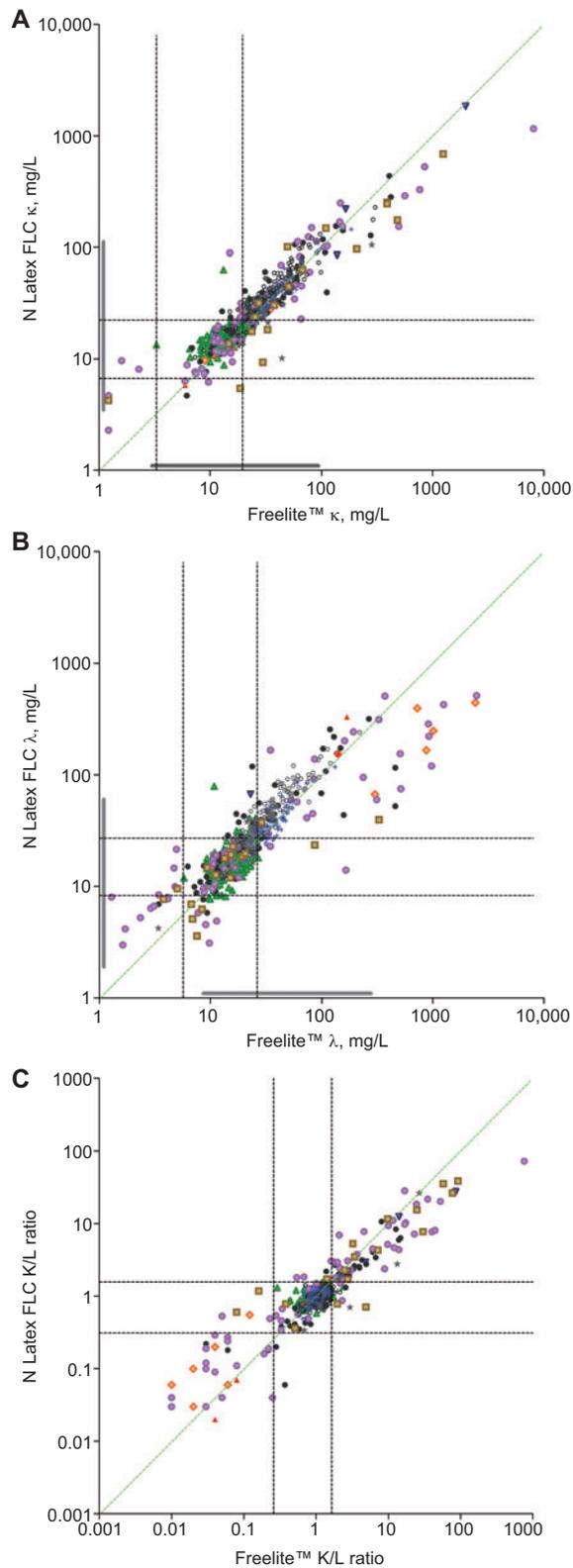
Figure 3 Method comparison N Latex FLC assays vs. Freelite™ assays for FLC κ (A), FLC λ (B) and κ/λ ratio (C) for 541 samples. Patient groups: see Figure 1. The gray bars indicate the initial dilution of the standard curve. Dotted lines indicate the reference ranges for the specific assays and the green line indicates the y=x axes.

We performed IF analysis on 373 samples, both for serum and urine. Positive identification of FLC on immunofixation electrophoresis showed comparable results for κ/λ ratios outside the normal range for the Freelite™ and N Latex FLC test (91% and 87%, respectively). With negative immunofixation electrophoresis samples, comparable results were observed for the Freelite™ and N Latex κ/λ ratio within the normal range (95% and 97%, respectively).

Our clinical study in 541 consecutive patients demonstrated that there is a high concordance of the N Latex FLC assays and the Freelite™ assays in clinical practice. Although differences in serum concentrations were observed in some patients, we conclude that both methods were highly comparable for the result interpretation.

Conflict of interest statement

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