N Latex FLC – new monoclonal high-performance assays for the determination of free light chain kappa and lambda

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Abstract

Background: High serum concentrations of monoclonal free light chain (FLC) kappa or lambda are markers of plasma cell dyscrasia.

Methods: We developed new, latex-enhanced, specific nephelometric assays based on monoclonal antibodies for the determination of FLC kappa and lambda in serum, EDTA plasma and Li-heparin plasma for use on the Siemens BN™ systems.

Results: Reference ranges were determined from 369 samples: FLC kappa 6.7–22.4 mg/L, FLC lambda 8.3–27.0 mg/L and kappa/lambda ratio 0.31–1.56. Protection from falsely low results due to antigen excess is obtained with a built-in pre-reaction in the assay protocols. Lot-to-lot consistency between three different lots of reagent, calibrators and supplementary reagent lots showed normalized differences <7.5%. The reproducibility of serum samples varied between 4% and 7%. The method comparison with Freelite™ assays showed normalized differences of 19.7%, 32.7% and 21.7%, respectively, for FLC kappa, lambda and ratio, correlations of 0.94, 0.77 and 0.73, and concordance rates of 99.2%, 94.2% and 95%.

Conclusions: N Latex FLC demonstrates high precision, good lot-to-lot consistency and freedom from a high-dose hook effect. The method comparison between Freelite™ and the N Latex FLC assays showed good clinical concordance. Further studies need to reveal the clinical value of the new FLC assays.

Introduction

For many decades, monoclonal gammopathies have been diagnosed by the detection of M-proteins in serum and urine. Besides the release of complete immunoglobulins, plasma cells produce an excess of 40% of light chains (1), which results in the presence of free light chains (FLC) in plasma. Although light chains are cleared much faster than immunoglobulins, detectable concentrations in the range of 5–25 mg/L can be measured in plasma of healthy donors (2). The increased production and release of FLC into plasma is a valuable diagnostic marker of plasma cell disorders. For this purpose, several groups have set up FLC specific assays in nephelometric or ELISA formats (2–6). Use of specific antibodies that recognize epitopes expressed on FLC, which are normally hidden in the complex with heavy chains, are the key to obtaining the analytical specificity for detection of free, but not bound, light chains. The development of assays specific for FLC is complicated by the fact that only a few constant domain epitopes are available for specific recognition of FLC; the formation of multimers, especially for FLC lambda, further reduces the number of eligible binding sites (7, 8). In plasma and urine, FLC lambda is present primarily as a homo-dimer, but larger complexes have also been described consisting of exclusively FLC lambda or FLC kappa (7).

Here, we present data on the development of N Latex FLC, a new immuno-nephelometric assay for the determination of FLC kappa and lambda for use on the Siemens BN™ systems, which are widely available in routine clinical laboratories. The use of the built-in pre-reaction protocol allows detection of a high-dose hook effect before starting the reaction; this protection from antigen excess is especially important for analytes like FLC, where the concentration can vary over a wide range.

Materials and methods

Development and selection of monoclonal antibodies

We raised monoclonal antibodies against FLC kappa and lambda using Bence Jones proteins purified from urine obtained from patients with multiple myeloma. BALB/c mice were immunized with purified protein in complete Freund’s adjuvant. A booster with an emulsion prepared in incomplete adjuvant was given after 4 weeks, and another booster without adjuvant was administered after 8 weeks. During the final 3 days before fusion, the mice received daily intravenous boosters. The study was performed in accordance with the guidelines of Helsinki.

After the mice were sacrificed, the spleens were removed and the B-cells fused with SP2/0 myeloma cells. Single hybrid cells that
produced antibodies against FLC kappa or FLC lambda were cloned, and appropriate clones were expanded. After removing the cells, the solution was concentrated and the antibodies purified using protein A sepharose fast flow chromatography (GE Healthcare/Amersham Biosciences).

The specificity of the selected monoclonal antibodies was analyzed by using an ELISA assay. ELISA plates (Nunc maxisorb, Roskilde, Denmark) were coated with 3 μg/mL rat-anti mouse kappa monoclonal antibody (RM19, Sanquin Reagents, Amsterdam, The Netherlands). RM19 was incubated in the plates in antibody-phosphate-buffered saline (PBS) overnight. Only mouse kappa antibodies were bound by the RM19 antibody, because it does not react with human kappa and also does not interfere with the antigen binding to the monoclonal antibody. After washing five times with PBS and 0.1% (V/V) Tween 20 (Sigma-Aldrich, St-Louis, USA), anti-FLC mouse monoclonal antibodies were added to the plates in serial dilutions of 12-steps starting with 1 μg/mL in PTG [10 mM PBS (NPBI Int bv, Emmer-Compascuum, The Netherlands) pH 7.4 with 0.05% Tween 20 and 0.1% gelatine (Merck, Darmstadt, Germany)]. After washing, biotinylated, purified FLC kappa, FLC lambda, IgG-kappa or IgG-lambda in PTG were added to the plates. Poly-streptavidin horseradish peroxidase (poly-HRP, Sanquin Reagents) was then added in PBS with 2% bovine milk and color formation was performed with tetramethylbenzidine (TMB, Merck, Darmstadt, Germany) in the presence of 0.03% (V/V) hydrogen peroxide (Merck). The reaction was stopped with 2 M H₂SO₄ (Merck).

**Latex-based assays for FLC kappa and lambda for the BN™ nephelometers**

Mouse monoclonal antibodies were covalently coupled to polystyrene particles. Each monoclonal was coupled in a separate batch. Thereafter the batches of coupled monoclonal antibodies were mixed together to obtain the final reagent. An assay-specific supplementary reagent was developed containing mouse immunoglobulins in a Tris-HCl buffer pH 7.4 with a strong detergent to reduce non-specific reactions and enhance antigen recognition in the reaction cuvette. The exact constitution of the supplementary reagents is the proprietary information of the manufacturer. In the sequence of events in the reaction cuvette in the BN™ systems, the supplementary reagent is first mixed with the antibody-polystyrene particle reagent. For the pre-reaction, 8 μL of sample is added for FLC kappa and 7 μL for the FLC lambda assay. After a pre-reaction of 2 min, the rest of the sample (75 μL for FLC kappa and 55 μL for FLC lambda) is added for the primary reaction. If the signal at the end of the pre-reaction is above a certain threshold value, the BN™ system will start a new analysis of the sample using the next higher dilution.

An international reference material is not available for FLC kappa and lambda. A single calibrator solution containing purified polyclonal FLC kappa and lambda in PBS containing 1% human serum albumin (Sanquin Pharmaceuticals, Amsterdam, The Netherlands) was used to calibrate the assay to give results comparable to the Freelite™ assays of The Binding Site (The Binding Site, Birmingham, UK). Achieving similar results with the Freelite™ and N Latex FLC assays would allow clinicians to use the guidelines of the International Working Group on Multiple Myeloma (9). The master standards were calibrated against the Freelite™ assays. Further calibration lots were calibrated against the Master calibrator lots only.

**Reference ranges and sample type selection**

Fresh serum, EDTA plasma and Li-heparin plasma were simultaneously collected from 54 healthy lab donors for sample-type comparison using Passing and Bablok regression analysis, normalized difference analysis and Spearman rank correlation analysis. Fresh serum samples and EDTA plasma samples from healthy blood bank donors and healthy lab donors were used to determine reference ranges. The blood bank donors were physically healthy as determined by the physician on-site. All donors gave informed consent. The panel consisted of 116 serum and 253 EDTA plasma samples. For FLC kappa and lambda the 2.5th–97.5th percentile range was used, and for the ratio, the minimum to maximum range.

**Method imprecision**

The precision study was performed according to the CLSI EP5-A2 guideline. Serum and EDTA plasma pools were made by selection of samples with low and normal FLC concentrations from healthy donors. The high pools were spiked with no more than 20% serum from donors with high FLC kappa or lambda from Sanquin Diagnostics Services.

**Linearity**

Linearity was determined according to CLSI guideline EP6A using serum samples with FLC concentrations just above the initial measuring range, i.e., above 112 mg/L for FLC kappa and above 60 mg/L for FLC lambda. Samples with monoclonal FLC were selected for this analysis. At least 12 steps of dilutions of 90%–2.5% were measured on both the BN™II and BN ProSpec®. Five replicates for each dilution were measured and the mean result was analyzed by linear and non-linear (cubic) analysis. The analysis was performed with at least six samples. The fits were evaluated using the Microsoft Excel add-in Analyse-it®.

**Cross-reactivity and interference testing**

To assess cross-reactivity, immunoglobulins were purified from urine obtained from multiple myeloma patients using ion-exchange chromatography and affinity chromatography on protein A sepharose (Amersham Pharmacien Biotech AB, Uppsala, Sweden). We identified more than 90% purity on SDS PAGE (Fast system, Pharamcia Biotech AB, Uppsala, Sweden). A pool of sera from healthy donors with a FLC kappa concentration of 14.7 mg/L (pool A) and a FLC lambda concentration of 15.7 mg/L (pool B) was spiked with the purified immunoglobulins. Purified FLC lambda (200 mg/L), IgG-kappa (10 g/L), IgA-kappa (6.4 g/L), IgM-kappa (5.2 g/L) were added to serum pool A and purified FLC kappa (200 mg/L), IgG-lambda (8 g/L), IgA-lambda (2.6 g/L) and IgM-lambda (3.9 g/L) were added to serum pool B. The spiked samples were measured in triplicate and the mean difference to the unspiked reference samples were recorded. We evaluated potentially interfering endogenous substances according to CLSI Guideline EP7-A2. All concentrations of interfering substances were measured according to the suggested concentrations in the guideline.

**High-dose hook effect**

Over 2000 serum samples were screened for FLC kappa and FLC lambda concentration using the N Latex FLC assays; the two samples with the highest concentration of FLC kappa (23,000 mg/L; Freelite™ 37,000 mg/L) and lambda (57,000 mg/L; Freelite™ 54,000 mg/L) were used for the high-dose hook analysis. Serial 1:2 dilutions of these samples in at least 10 steps were tested at the initial dilutions of 1:100 and 1:20 for FLC kappa and FLC lambda, respectively. The raw data measurements (signal in bits) of the pre-
reaction and the main reaction were recorded and plotted against the calculated concentrations to check for high-dose hook effects.

**BN™ II and BN ProSpec® comparison**

We tested 120 serum and 40 EDTA plasma samples on each BN™ system; data were analyzed using Passing and Bablok regression analysis, normalized difference analysis and Spearman correlation analysis. Outliers were discarded prior to statistical analysis.

**Lot-to-lot consistency**

Three consecutive lots of FLC kappa, lambda, supplementary reagents and calibrators and controls were produced. We tested 100 samples on each lot and analyzed the data using Passing and Bablok regression analysis and Spearman correlation analysis. We calculated the median normalized difference between the reagent lots, calibrators and the supplementary reagent lots. The samples covered the initial measuring range of the assays; >20% of the samples were above the reference range of each of the assays.

**Method comparison vs. Freeelite™ assays**

The comparison between the new N Latex FLC assays and the Freeelite™ assays was performed according to the CLSI EP9A2 guideline, including the outlier analysis. One hundred and twenty serum samples that included the entire measurement range of the N Latex FLC assays were used in this analysis. Samples were obtained from Sanquin Diagnostics Services or from healthy lab donors. Samples were either fresh or stored at -20°C and thawed on the day of analysis. Both methods were run in parallel on the BN™II system. No clinical information is available for these donors.

**Statistics**

Passing and Bablok regression, Spearman rank testing and linearity analysis of the data were performed using the Microsoft Excel add-in Analyse-it® v2.03. Method Evaluation, www.analyse-it.com. Normalized differences were defined as the median value of all differences [(y–x)/(y + x)]/2 between samples. For the outlier analysis, the normalized difference was defined as the difference of two duplicates divided by the mean of the duplicates. Samples were identified as an outlier when this difference was more than four times the mean normalized difference of all samples, and outliers were discarded prior to statistical analysis.

**Results**

**Specificity of monoclonal antibodies and assay characteristics**

A panel of monoclonal antibodies was selected for FLC kappa and FLC lambda to react with purified monoclonal FLC kappa and lambda proteins, but not with light chains bound to heavy chains (Figure 1). The ELISA results demonstrated the high specificity of the selected monoclonal antibodies used in the final assay. In the ELISA assays, almost no crossover was detected for the selected antibodies.

The optimized assay protocols for BN™II and BNProSpec® systems for both FLC kappa and lambda include a 2-min pre-reaction and 10-min main reaction. The initial dilution chosen for the FLC kappa assay is 1:100 (range 3.5–112 mg/L), for the FLC lambda assay 1:20 (range 2–60 mg/L) with concentrations of FLC kappa of approximately 22 mg/L and 30 mg/L for FLC lambda in the single calibrator for both assays.

**Sample-type selection and reference ranges**

Serum, EDTA and Li-heparin plasma samples were collected in parallel from 54 healthy donors. When compared to serum, EDTA plasma gave comparable results for FLC kappa (normalized difference –3.2%), FLC lambda (0.1%) and Kappa/lambda ratio (–2.2%). Li-heparin plasma also gave comparable results to serum and EDTA plasma for FLC kappa (1.2%), but a larger normalized difference of 10% was observed for FLC lambda and 8% for the K/L ratio compared to serum and EDTA plasma.

The reference ranges obtained for N Latex FLC (Table 1) are very close to those provided for the Freeelite™ assays: 6.7–22.4 mg/L FLC kappa (Freeelite™ kappa 3.3–19.4 mg/L); 8.3–27 mg/L FLC lambda (Freeelite™ lambda 5.7–26.3 mg/L); and 0.31–1.56 for FLC ratio kappa/lambda (Freeelite™ ratio 0.26–1.65). Samples for the reference range analysis were obtained from healthy blood bank donors (age: range 18–70 years). The gender and race distribution of the donor population is assumed to be representative of the Dutch population.

**Method imprecision**

The precision data provided in Table 2 demonstrated the high precision of the N Latex FLC kappa and lambda on BN™II and BN ProSpec® systems, with %CVs of 2%–6% for low, medium and high serum and EDTA plasma pools. The maximum within device imprecision observed on BN™II and BN ProSpec® system were 6.3% and 5.8%, respectively, in the low range of N Latex FLC lambda.

**Linearity**

For testing linearity, serial dilutions of samples with a concentration just above the initial measuring range were prepared. The results of two representative samples run on the BN ProSpec® system are shown in Figure 2. The differences between linear and cubic fit were calculated, and at all dilutions the maximal absolute difference between the samples was 14.7% for FLC kappa and 17.9% for FLC lambda. For N Latex FLC kappa, linearity was confirmed between 2.1 and 122 mg/L, for N Latex FLC lambda linearity was between 0.8 and 66.2 mg/L.

**Cross-reactivity and interference testing**

The new FLC assays were specific for FLC kappa and lambda. Bound light chains are present in high excess in serum when compared to the free light chains. With the addition of purified IgG, IgA and IgM, no more than 4.2 mg/L change was recorded in a serum of a healthy donor (Figure 3). At concentrations of 200 mg/L of FLC kappa or FLC lambda, there was < 2 mg/L change in the concentrations of the serum. We also tested interference from conjugated bilirubin.
Figure 1 Specificity of selected monoclonal antibodies for the free light chain kappa and lambda in a FLC-specific ELISA.
Panels A and B show two monoclonal antibodies against FLC kappa. Purified FLC kappa (●) and purified human IgG1-kappa (○). Panels C and D show two non-competing monoclonal antibodies against FLC lambda. Purified FLC lambda (●) and purified human IgG1-lambda (○).

Table 1 Reference ranges of 369 samples: 116 fresh serum samples and 253 fresh EDTA plasma samples from healthy lab donors and healthy blood bank donors. One sample only for each donor is included in this analysis.

<table>
<thead>
<tr>
<th></th>
<th>Kappa, mg/L</th>
<th>Lambda, mg/L</th>
<th>K/L ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>13.2 (4.0)</td>
<td>15.6 (5.0)</td>
<td>0.88 (0.23)</td>
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<tr>
<td>Median</td>
<td>12.9</td>
<td>14.7</td>
<td>0.86</td>
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<td>2.5th percentile</td>
<td>6.7</td>
<td>8.3</td>
<td>0.50</td>
</tr>
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<td>97.5th percentile</td>
<td>22.4</td>
<td>27.0</td>
<td>1.27</td>
</tr>
<tr>
<td>Min</td>
<td>4.5</td>
<td>6.4</td>
<td><strong>0.31</strong></td>
</tr>
<tr>
<td>Max</td>
<td>28.9</td>
<td>38.1</td>
<td><strong>1.56</strong></td>
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</table>

Bold values indicate the limits for results interpretation.

(342 and 1025 μM) and unconjugated bilirubin (342 and 618 μM), hemoglobin (2 and 5 g/L), cholesterol (3.9 and 13 mM), triglycerides (1.7 and 5.6 mmol/L), protein (40, 50, 60 g/L) and rheumatoid factor (2 samples with 2000 IU/mL), and found no interference on FLC kappa and lambda in serum (data not shown).

High-dose hook effect

The built-in pre-reaction of the BN™ systems secured the recognition of very high FLC concentrations above the initial measuring range. In Figure 4, the raw data of the pre-reaction of serial dilutions of very high samples (FLC kappa: 23,000 mg/L, FLC lambda 57,000 mg/L) are shown. For concentrations within the initial measuring range (FLC kappa 3.5–110 mg/L, FLC lambda 1.9–60 mg/L) the signal was well below the threshold. The threshold is defined as the highest bit value of the pre-reaction signal in the reference curve. When the concentrations of FLC kappa or lambda were above the initial measuring range (at the right side of the grey bar), the pre-reaction result was above the threshold and the system will automatically perform the measurement at a higher dilution. Our results indicated that even very high FLC concentrations were reliably recognized and correctly measured due to the implemented pre-reaction step.

Lot-to-lot consistency

N Latex FLC comes with separate, lot-independent kits for reagent, supplementary reagent, calibrator and controls. To determine lot-to-lot consistency, we tested three independent lots of each component. In Figure 5, we showed the lot-to-lot consistency of three independent reagent lots of N Latex FLC kappa (A) and lambda (B), in combination with three independent lots of calibrator and one lot of supplementary reagent. The observed normalized differences between all lots ranged between –4.9% and 3.8%, the slopes of the Pass-
Table 2 Method imprecision performed according to CLSI guideline EP5A2.

<table>
<thead>
<tr>
<th>Device/assay</th>
<th>Pool description</th>
<th>mg/L</th>
<th>Repeatability, % (SD)</th>
<th>Between-run, % (SD)</th>
<th>Within-day, % (SD)</th>
<th>Between-day, % (SD)</th>
<th>Within Device/lab, % (SD)</th>
</tr>
</thead>
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<td>BN™II FLC kappa</td>
<td>Serum pool low</td>
<td>5.1</td>
<td>2.1 (0.1)</td>
<td>0.9 (0.05)</td>
<td>2.3 (0.1)</td>
<td>2.9 (0.1)</td>
<td>3.7 (0.2)</td>
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<td></td>
<td>Plasma pool low</td>
<td>3.9</td>
<td>3.2 (0.1)</td>
<td>0 (0)</td>
<td>3.2 (0.1)</td>
<td>3.3 (0.1)</td>
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<tr>
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<td>2.6 (0.4)</td>
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<td></td>
<td>High serum pool</td>
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<td>2.0 (2.1)</td>
<td>2.9 (3.0)</td>
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<td>1.2 (1.2)</td>
<td>2.4 (2.5)</td>
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<td>3.9 (0.2)</td>
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<td>3.9</td>
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<td>2.4 (0.1)</td>
<td>3.9 (0.2)</td>
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<tr>
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<tr>
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<td>1.4 (0.2)</td>
<td>1.6 (0.2)</td>
<td>2.2 (0.3)</td>
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<td>High serum pool</td>
<td>106.6</td>
<td>1.8 (1.9)</td>
<td>0 (0)</td>
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<td>2.0 (2.1)</td>
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<tr>
<td></td>
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<td>2.4 (1.4)</td>
<td>2.7 (1.6)</td>
<td>3.7 (2.1)</td>
</tr>
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</table>

The results of serum and EDTA plasma pools of BN™II and BNProSpec® are given.

Figure 2 Examples for linearity for FLC kappa (A) and FLC lambda (B) on BN ProSpec®. Serial dilutions of serum samples were made with concentrations of FLC kappa and lambda just above the initial measuring range (gray line on the left side). Black continuous line indicates the linear fit, dotted line indicates the non-linear (cubic) fit.

BN™II and BN ProSpec® comparison

For comparison of BN™II and BN ProSpec®, 120 serum and 40 EDTA plasma samples were measured on both analyzers in parallel. Outlier analysis excluded two samples for FLC kappa (n = 158) and one sample for FLC lambda (n = 159) and for ratio (n = 158). Two outlier samples for FLC kappa...
Figure 3 Cross-over reactivity of the FLC kappa assay (A) and the FLC lambda assay (B) to intact immunoglobulins. A serum sample from a healthy donor was spiked with purified FLC kappa, FLC lambda, IgG-K, IgA-K, IgM-K, IgG-L, IgA-L and IgM-L. Mean of triplicate analysis with ranges.

Method comparison

For the N Latex FLC kappa assay, one outlier was removed and none were removed for the FLC lambda assay. The FLC kappa method comparison was performed with 119 samples, the FLC lambda assay with all 120 samples, and the ratio with 119 samples. The normalized difference for the outlier FLC kappa sample was 0.08 (limit for outliers was ≤0.07). When comparing N Latex FLC kappa and lambda directly with the Freelite™ assays, the correlation was only moderate (Figure 6), with a slope of 1.11 (95% CI: 1.0–1.2) (Passing and Bablok), intercept 1.4 (95% CI: 0.3–3.1), r = 0.94, and 19.7% normalized difference for FLC kappa, and a slope of 1.05 (95% CI: 0.95–1.15), intercept –1.0 (95% CI: –3.0 to 0.51), r = 0.77 and 32.7% normalized difference for FLC lambda. Discrepancies were observed, especially in the high concentration range for FLC kappa; Freelite™ results were much lower in the range to 100 mg/L. However, for FLC lambda, the opposite was observed, some samples showing considerably lower results than with N Latex FLC lambda.

A moderate correlation of r = 0.73 was obtained for the method comparison of FLC ratios. However, the general mean agreement is good between both methods (Passing-Bablok slope: 1.08). Alternatively, results were categorized as abnormal low, normal, or abnormal high. Comparison of both methods on this basis (Table 3) reveals an ‘almost perfect’ agreement for FLC kappa, FLC lambda and FLC ratio, as indicated by Cohen’s kappa coefficients, a measure of agreement between methods versus the expected agreement by chance, all above 0.80 (11).

Discussion

The detection of monoclonal light chains in serum from patients with plasma cell disorders has become an important asset for screening patients suspected of monoclonal gammapathy, with amyloidosis, for therapy monitoring as well as for prediction of disease progression (9, 12–14). In this study, we introduce new assays for the quantification of FLC kappa and FLC lambda in serum and plasma designed for the Siemens BN™ systems. We covalently coupled a selection of highly specific monoclonal antibodies to FLC kappa and lambda. The detection of monoclonal light chains in serum from patients with plasma cell disorders has become an important asset for screening patients suspected of monoclonal gammapathy, with amyloidosis, for therapy monitoring as well as for prediction of disease progression (9, 12–14). In this study, we introduce new assays for the quantification of FLC kappa and FLC lambda in serum and plasma designed for the Siemens BN™ systems. We covalently coupled a selection of highly specific monoclonal antibodies to FLC kappa and lambda.
and FLC lambda to latex particles to obtain a nephelometric reagent. With the use of the automated BN™ systems of Siemens, the assays were selective, sensitive for quantitation of FLC, reproducible, and secured for antigen excess.

In 2001, with availability of the Freelite™ assays new possibilities became available for the diagnosis and monitoring of patients with plasma cell dyscrasia (2, 15). The determination of FLC has been implemented into the guidelines for multiple myeloma and related disorders (9). Criteria for a stringent complete response nowadays include a return of FLC ratio into the normal range. Since an international reference preparation for FLC does not exist, and as the wide clinical use of the Freelite™ FLC assays has established commonly used reference ranges in clinical routine, we decided to harmonize the calibration of our new FLC assays with the Freelite™ FLC assays. However, this approach for standardization confronted us with the poor lot-to-lot consistency of the Freelite™ assays (9, 16–18). For this reason, we calibrated our highest standards against one single lot of the Freelite™ assays, and fixed these concentrations for all derived standards thereafter. The aim of our standardization was to have comparable ranges to those published by Katzmann et al. with the Freelite™ assays (11). The minimum to maximum range for the N Latex FLC ratio was 0.31–1.56, which is very close to the range of the Katzmann study of 0.26–1.65. For FLC kappa and FLC lambda, we also found comparable ranges, but slightly more narrow at the low end.

As the basis for our FLC assays, we selected highly specific monoclonal antibodies against FLC kappa and FLC lambda, which were raised against purified FLC kappa and lambda, including only those with the highest selectivity and reactivity in our assay format. The specificity and linearity of the assays was further improved with the addition of the assay-specific supplementary reagent containing mouse immunoglobulins.

Patients with multiple myeloma require long-term monitoring to evaluate the efficacy of therapy and to recognize relapses or the increase of FLC to a range that can be nephrotoxic (19). We demonstrated lot-to-lot consistency with variation of three independent lots of calibrator and reagent, which is a crucial requirement in long-term patient monitoring. We showed excellent repeatability, between-run and between-device precision for both assays on both BN™ systems. To check for antigen excess on the BN™ systems, we used a short ‘pre-reaction’ with a small volume of serum and all the reagent for one determination. Within 120 s, the system detects analyte concentrations above the calibrated range, and automatically starts with a higher dilution when the values are out of range. We demonstrated the detection of high protein FLC concentrations in serum with our built-in pre-reaction. There was very low cross-over reactivity of purified intact immunoglobulins on the final FLC assays. We demonstrated this high selectivity after the addition of purified immunoglobulins. According to Nakano et al. this high selectivity can only be achieved with monoclonal antibodies against FLC instead of polyclonal antibodies (20, 21).

The method comparison between the Freelite™ assays and the new N Latex FLC assays confirmed that we are comparative in calibration for both FLC kappa and FLC lambda. However, direct comparison between both methods showed moderate correlations for especially FLC lambda. For the Freelite™ assays there have been reports of sometimes extremely high values for both FLC kappa and FLC lambda, which do not correspond to the estimated protein concentrations on SPE (6, 17, 22, 23). In our study, certain samples showed by a factor 3–10, lower results with the new FLC assays. Polyclonal FLC lambda at concentrations just above the reference range was higher with our new FLC lambda. The K/L ratio also showed a moderate correlation, but the concordance between both methods was 95%. We observed
Figure 6  Method comparison of the new FLC assays and the Freelite™ assays.
(A1) FLC kappa comparison, (A2) FLC kappa comparison with focus on samples with <100 mg/L of FLC kappa in serum, (B1) FLC lambda comparison, (B2) FLC lambda comparison with focus on samples with <100 mg/L of FLC lambda comparison, (C) Kappa/lambda ratio comparison. Circles indicate concordance in ratio between the methods and crosses indicate ratios that are different. Dotted lines indicate the limits of the reference ranges for both methods and the straight line indicates the y = x line.
the same or higher concentrations of accordance for FLC kappa and FLC lambda which gave the classification of ‘almost perfect agreement’ for Cohen’s kappa coefficient.

We developed a new automated assays for FLC kappa and lambda for the Siemens BN™ systems based on monoclonal antibodies. The assay showed good performance characteristics for selectivity, sensitivity, precision, linearity, antigen excess security, and lot-to-lot consistency. The reference ranges were within the ranges of the Freeelite™ assays. The method comparison showed moderate correlations, but have almost perfect agreements on concordance. Further studies are needed to demonstrate the clinical value of the new FN assays.

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Conflict of interest statement

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