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Comparison of the Freelite serum free light chain (SFLC) assay with serum and urine electrophoresis/immunofixation and the N Latex FLC assay

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Summary

Few reports have compared available serum free light chain (SFLC) assays. Here, a retrospective audit of the Freelite SFLC assay compared results to electrophoresis (EP)/immunofixation (IFX) and the N Latex FLC assay. A total of 244 samples collected over 3.5 months were studied using the Freelite and N Latex FLC nephelometry assays. Results were compared with serum and/or urine EP/IFX. The precision and linearity of the N Latex FLC assay was examined. Detectable paraprotein by serum or urine EP/IFX was present in 94% of samples with kappa and 100% with lambda FLC restriction. The correlation between the assays was higher for kappa (rho = 0.97) than lambda (rho = 0.89) especially when lambda results were above the upper limit of normal (rho = 0.62). Agreement in the categorical diagnosis as measured by the Cohen's kappa statistic was good (0.70). The N Latex FLC assay displayed good precision and linearity. In discordant samples the Freelite and N Latex FLC assays had equivalent agreement with IFX. Traditional methods of EP/IFX detected paraproteins in the majority of cases. Correlation between the Freelite and N Latex FLC assay is better for kappa than lambda FLC. The two assays are not entirely equivalent. Care should be taken by interpreting physicians and laboratories considering switching assays.

Key words: Electrophoresis, immunofixation, myeloma, plasma cell dyscrasias, serum free light chains, SFLC.

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INTRODUCTION

Plasma cell dyscrasias (PCD) are frequently identified because of paraprotein detection. In the majority of patients this is an intact immunoglobulin; however, up to 20% of patients express immunoglobulin light chain alone.[1] Traditionally the detection of paraproteins has been by serum electrophoresis (EP) and immunofixation (IFX). Urine EP/IFX is particularly sensitive for detection of free light chains. In 2001 a new diagnostic test for the identification of paraproteins was released, based on the detection of serum free light chains (SFLC; Freelite; The Binding Site, UK). The test's reported sensitivity of 1.5 mg/L and 3 mg/L for kappa and lambda FLC, respectively, compares favourably to a serum EP/IFX level of detection of 50 mg/L[2] and urine EP/IFX level of detection of 20 mg/L.[3] The assay also offered the additional tool of the SFLC ratio (SFLCr, i.e., kappa FLC divided by lambda FLC) as a marker for monoclonality.

SFLC testing may obviate the need for 24 h urine collection which is associated with reduced patient compliance.[4–8] Further, SFLC analysis is less prone to storage and collection artifacts and is less subjective than interpretation of serum EP/IFX. Additionally, given the half-life of SFLC is 2–6 h it has been argued that SFLC analysis may provide better real-time information on disease progression and/or treatment response.[9] Major criticisms of the SFLC assay have been: high rates of false-positives,[8] the large number of samples displaying non-linearity or antigen excess, poor precision and high lot-to-lot variation.[10] Despite these limitations, SFLC testing has been widely adopted into clinical practice, and is recommended in guidelines for the diagnosis and management of PCD.[11] Measurement has also been recommended in AL amyloidosis where the majority of patients do not have detectable intact immunoglobulin by serum or urine EP/IFX. However, many of the studies that underpin these guidelines do not establish SFLC as superior to EP/IFX or control for the concentration of paraprotein.

In a large study of 2700 patients, McTaggart *et al.* analysed the performance of serum EP, urine EP/IFX and SFLC assays as isolated first line tests for PCDs, comparing individual tests results to a gold-standard of final diagnosis by a consultant haematologist using all available test results. Serum EP had the highest sensitivity (94.4%) and excellent specificity (97.9%). Urine EP/IFX had the highest specificity (99.2%) but lowest sensitivity (46.8%). The SFLC assay was highly specific (98.9%) but had low sensitivity (46.8%).[8]

In 2011 a second SFLC test became available (N Latex FLC kappa and lambda; Siemens, Germany).[12] This test utilised mouse monoclonal antibodies bound to polystyrene particles, as opposed to sheep (Fab)[2] polyclonal antibody fragments used in the Freelite assay. The newer assay is reported to have high precision, good lot-to-lot consistency and be less prone to the effects of antigen excess. There have been few studies comparing the diagnostic utility of SFLC testing and traditional EP/IFX methods and only three published reports directly comparing the two SFLC assays.[10,13,14] Two of these studies report lower correlation between lambda FLC compared with kappa, especially at higher levels of FLC.[13,14]

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We report a retrospective audit of SFLC testing using the Freelite assay over a 3.5 month period. We examined the agreement between SFLC results and serum and urine EP/IFX, and also directly compared the Freelite and N Latex FLC assays. The centre is a diagnostic pathology laboratory in a tertiary referral hospital servicing inpatients, outpatients and external providers including primary care physicians. The aims of the audit were to determine the usefulness of SFLC testing as an adjunct test to traditional methods, and to compare results of the Freelite and N Latex FLC assays in terms of absolute values and categorical diagnosis. This work was done as part of a broader consideration of adopting the N Latex FLC assay into diagnostic practice.

MATERIALS AND METHODS

Subjects

SFLC testing was completed on 244 samples from 192 patients over a 3.5 month period (December 2013–March 2014). Of these, 120 had sufficient stored serum so that the N Latex FLC test could be completed retrospectively. Seventeen of the 120 that were repeat samples were excluded from the comparison of the two assays, resulting in a final number of 103 single-tests-per-patient samples.

SFLC analysis

Collected serum was used to measure SFLC using the Freelite assay by nephelometry as per the manufacturer's instructions on a BNII analyser (Siemens) at Royal Prince Alfred Hospital, Sydney. Excess serum was stored at −20°C. The N Latex FLC assay (Siemens) was performed on thawed serum according to manufacturer's instructions at St Vincent's Hospital, Sydney, on a different analyser (Prospec; Siemens). The results of the two assays were therefore effectively blinded until data collection. Samples did not undergo multiple rounds of freeze-thawing prior to analysis. The in-built auto-dilution protocols for the two assays are identical apart from the fact that the Freelite lambda auto-dilution begins at 1:100 and the N Latex FLC auto-dilution begins at 1:20. Supplied commercial controls and an in-house pooled serum control were included on each run of the N Latex FLC assay. Quality assurance data for the Freelite assay during the study period was obtained, including results for supplied commercial controls and a separate immunoassay control (Level 3 MAS Immunology; ThermoScientific, Finland). Samples that contained very high amounts of kappa ($n = 5$) or lambda ($n = 5$) and a subset of samples that were discordant between the two assays ($n = 5$) on initial analysis underwent further investigation. These samples were manually diluted in serum diluent (Siemens) prior to subsequent analysis to assess linearity. The dilution factor was determined for each sample individually based on the SFLC concentration already obtained and the amount of available stored sample, in order that ≥ 3 serial dilutions could be obtained. The dilutions ranged from 1:2 to 1:5.

Serum and urine EP/IFX

Serum and urine EP was performed as standard of care using Sebia Hydragel β1-β2 kit (Sebia, France) and Hydragel 7 HR (Sebia), respectively, according to the manufacturer's instructions. Urine was considered as belonging to the same time-point as serum if collected within 24 h, and underwent routine static concentration (Minicon, Merk Millipore, Germany). Serum and urine IFX were performed using Hydragel 9 IF (Sebia) according to the manufacturer's instructions. Fourteen samples that were discordant between the two SFLC assays were studied using a Pentagel IFX (Sebia) as part of the research study only. All serum and urine EP and IFX were performed on a semi-automatic Hydrasys focusing machine (Sebia).

Statistics

The non-parametric Spearman's correlation and the Cohen's kappa statistic for agreement were calculated using SPSS version 22.0 (IBM, USA).

Ethics

This study was approved by the St Vincent's Hospital (Sydney) Human Research Ethics Committee.

RESULTS

SFLC testing patterns, patient characteristics and results

In the 3.5 months study period 244 SFLC tests were ordered. Most tests were ordered by haematologists (67%), nephrologists (9%), immunologists (6%), cardiologists (5%) and general practitioners (3%). A range of other specialties each accounting for ≤2% made up the remainder. The median age of patients tested was 69 years [interquartile range (IQR) 59–78]. The majority of patients tested were male ($n = 156$, 64%). Based on data provided on the request form, the most common indication for SFLC testing was multiple myeloma (MM; 37%) followed by detection of a paraprotein (8%), amyloidosis (6%) and monoclonal gammopathy of uncertain significance (MGUS; 5%). No clinical indication was listed on the request form for 25% of samples. A large range of other indications each accounting for ≤2% made up the remainder. Of the samples from patients with MM, 71% had a detectable paraprotein by either serum or urine EP/IFX at the corresponding time-point. The median serum paraprotein was 12 g/L and the median urine paraprotein was 52 mg/L. Six samples (7%) had light chain only disease.

Both serum and urine EP/IFX were completed from the same time-point for 103 (42%) SFLC tests. Serum EP/IFX alone was performed for 101 tests (41%); urine only EP/IFX was ordered for nine (4%) SFLC tests; and 32 SFLC tests were completed in the absence of any electrophoresis-based tests (13%).

Of the 244 tests, 68 tests (28%) had results consistent with restricted kappa light chains, e.g., elevated kappa FLC and an elevated SFLC ratio. Thirty-six samples (15%) had restricted lambda light chains, 34 (14%) had polyclonal increases in free light chains, i.e., elevated kappa and lambda FLC and normal SFLC ratio. Indicative of indeterminant results, 14 (6%) tests measured high kappa free light chains but a normal SFLC ratio and a further 29 (12%) returned similarly indeterminate results with at least one of the kappa, lambda, or SFLC ratio values outside the normal range. Four tests (1%) were consistent with impaired light chain production, i.e., reduced kappa and lambda FLC and normal SFLCr, and 59 (24%) of tests had all values within normal limits.

Concordance of SFLC assays with serum and urine EP/IFX

Concordance between the Freelite SFLC assay and serum and urine EP/IFX was examined in the 103 samples that had all tests performed. Thirty-four samples demonstrated restricted kappa FLC production. Of these 34 samples, 32 (94%) had a paraprotein detected on either serum or urine EP/IFX and only two samples (6%) had no abnormality detected by electrophoretic-based methods (Table 1). Ten samples demonstrated restricted lambda FLC production and all of these had a paraprotein detected by either serum or urine EP/IFX (Table 1).

Concordance between the N Latex FLC SFLC assay and serum and urine EP/IFX was also examined in the samples that had all tests performed. Fourteen samples demonstrated restricted kappa FLC production. Of these 14 samples, 13 (93%) had a paraprotein detected on either serum or urine EP/IFX, and only one sample (7%) had no abnormality detected by electrophoretic-based methods (Table 1). Four samples demonstrated restricted lambda FLC production and all of these had a paraprotein detected by either serum or urine EP/IFX (Table 1).

Table 1 Concordance between SFLC testing and serum and urine electrophoresis/immunofixation results

	n (%)	*n* (%)	*n* (%)	*n* (%)
Serum EP/IFX	–	–	+	+
Urine EP/IFX	–	+	–	+
Freelite assay				
Restricted kappa ($n=34$)	2 (6)	3 (9)	7 (20)	22 (65)
Restricted lambda ($n=10$)	0 (0)	0 (0)	2 (20)	8 (80)
N Latex FLC assay				
Restricted kappa ($n=14$)	1 (7)	3 (21)	1 (7)	9 (64)
Restricted lambda ($n=4$)	0 (0)	0 (0)	0 (0)	4 (100)

–, no paraprotein detected; +, paraprotein detected; EP, electrophoresis; IFX, immunofixation.

Precision of N Latex kappa and lambda FLC and Freelite assays

The precision of the N Latex FLC kappa and lambda assays was assessed by running the commercial low and high controls as well as an in-house control derived from pooled sera. The commercial low kappa control [$n=21$; mean = 13.18; standard deviation (SD) = 0.47; coefficient variant (CV) = 0.04] showed a 2_{2s} Westgard rule violation. The commercial low lambda control ($n=21$; mean = 12.95; SD = 0.47; CV = 0.04) showed a 1_{2s} rule violation. The commercial high kappa control ($n=21$; mean = 33.48; SD = 1.47; CV = 0.04) and lambda control ($n=21$; mean = 34.50; SD = 1.74; CV = 0.05) displayed high precision with no Westgard rule violations. An in-house pooled sera kappa control ($n=21$; mean = 10.11; SD = 0.22; CV = 0.02) and lambda control ($n=21$; mean = 10.90; SD = 0.50; CV = 0.05) displayed high precision with no Westgard rule violations.

Quality control data from the study period was obtained from the external laboratory in order to assess the precision of the Freelite assay. The precision of the Freelite kappa and lambda assays were assessed by running the kit low and high controls as well as a commercial immunoassay control. The summary data are as follows: commercial low kappa control ($n=83$; mean = 12.82; SD = 1.95; CV = 15.17); commercial high kappa control ($n=82$; mean = 25.36; SD = 2.20; CV = 8.68); commercial low lambda control lot #1 ($n=35$; mean = 29.37; SD = 1.53; CV = 5.20); commercial low lambda control lot #2 ($n=47$; mean = 24.60; SD = 1.14; CV = 4.62); commercial high lambda control lot #1 ($n=34$; mean = 53.36; SD = 1.95; CV = 3.66); commercial high lambda control lot #2 ($n=47$; mean = 50.45; SD = 1.80; CV = 3.56); commercial immunoassay control kappa ($n=78$; mean = 15.82; SD = 1.33; CV = 8.40); commercial immunoassay control lambda ($n=79$; mean = 19.94; SD = 1.56; CV = 7.82).

Correlation between Freelite and N Latex FLC assays

Of the total 244 samples, 103 had the N Latex FLC test performed. Values for kappa FLC were in general lower on

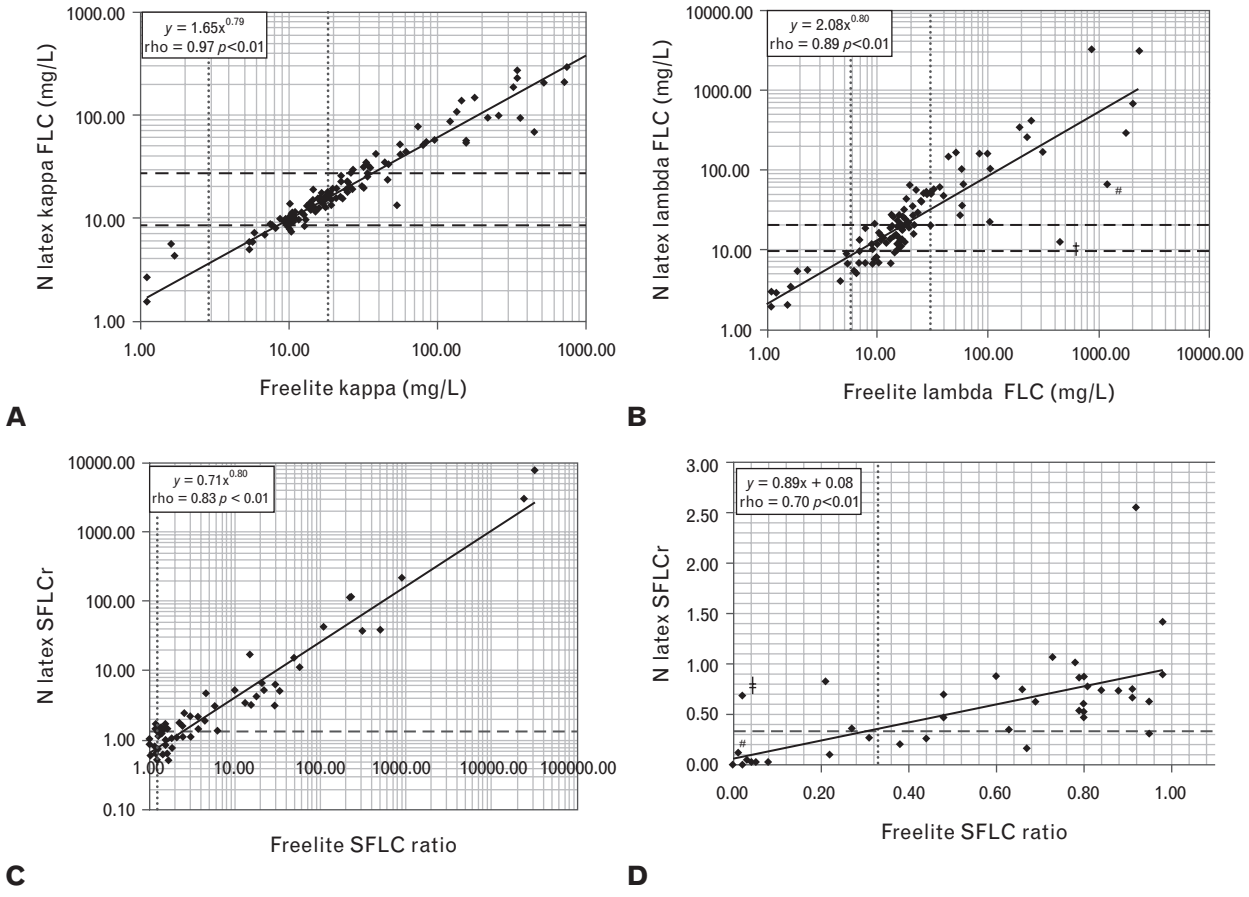

Fig. 1 Comparison of Freelite and N Latex FLC assays. Data from 103 serum samples run on two platforms are shown comparing (A) kappa FLC, (B) lambda FLC, (C) serum free light chain ratio (SFLCr) where the Freelite value was ≥1, and (D) SFLCr where the Freelite value was <1. Reference ranges for Freelite (dotted vertical line) and N Latex FLC (dashed horizontal line) are shown with upper and lower limits in (A) and (B), upper limit in (C) and lower limit in (D). In (B) and (D), # denotes samples from Patient 1 that underwent further analysis and ‡ denotes sample from Patient 2 that underwent further analysis.

the N Latex assay than the Freelite assay, however the correlation of the kappa FLC measurement was good between the two assays (Spearman's correlation rho = 0.97; $p < 0.01$; Fig. 1A) both within and above the reference range (3.3–19 mg/L). Values for lambda FLC were also lower on the N Latex FLC assay than the Freelite assay, and the correlation of the lambda FLC measurement between the two assays was lower than for kappa (rho = 0.89; $p < 0.01$; Fig. 1B). The correlation between lambda FLC values was higher for values that fell below the Freelite reference range upper limit of normal (26.3 mg/L; rho = 0.83; $p < 0.01$) than for those samples that were above this (rho = 0.62; $p < 0.01$).

To investigate the correlation between the SFLC ratio between the two assays, separate analysis of samples that had Freelite SFLC ratio ≥1 and samples that had a Freelite SFLC ratio <1 was undertaken. The correlation between SFLC ratio between the two assays was high when the ratio derived from the Freelite assay was ≥1 (rho = 0.83; $p < 0.01$; Fig. 1C), while the correlation between the two assays was low when the ratio derived from the Freelite assay was <1 (rho = 0.70; $p < 0.01$; Fig. 1D).

Linearity of N Latex SFLC assay

The linearity of the N Latex FLC assay was assessed using samples that had very high kappa FLC ($n = 5$) or lambda FLC ($n = 5$) measurements. These samples underwent manual serial dilutions prior to analysis. Good linearity was demonstrated for both N Latex kappa (Fig. 2A) and lambda (Fig. 2B) FLC assays. Additionally five samples that displayed discordant results between the two SFLC assays for lambda FLC were manually diluted prior to re-analysis on the N Latex FLC system to determine if antigen excess and/or non-linearity was a contributing factor. All these discordant samples displayed good linearity using the N Latex FLC assay (Fig. 2C) including two patients who were the most obvious outliers (see Patient 1 and Patient 2 in Fig. 1B).

Agreement in categorical diagnosis

Agreement in the categorical diagnosis of 'restricted light chain detected' or 'no restricted light chain detected' was measured. Fifty-three samples were negative on both tests, 35 samples were positive on both tests, 11 samples were positive on the Freelite assay but negative on N Latex FLC assay, and four samples were positive on N Latex FLC and negative by the Freelite assay. The Cohen's kappa statistic found good agreement (0.70; 95% CI 0.56–0.84).

Investigation of samples with discordant categorical diagnosis between the Freelite and N Latex FLC assays

Fourteen samples (14%) had restricted kappa or lambda FLC by one assay but not the other and were further investigated for presence of a paraprotein using serum IFX as part of the research study. There was agreement between the Freelite FLC assay and serum IFX in seven samples (50%) and agreement between the N Latex SFLC assay and serum IFX in seven samples (50%) as shown in Table 2.

DISCUSSION

SFLC tests have become widely adopted into the clinical practice for diagnosis and monitoring of PCD and AL amyloidosis. Despite this, there are few reports directly comparing the two available assays. Here we conduct a retrospective audit of SFLC testing, in part to assess how adoption of a new assay and platform would compare with existing results.

This study demonstrated that serum and urine EP/IFX will detect paraproteins in most cases. Of 37 samples that demonstrated restricted kappa FLC, the large majority (93–94%) had a detectable paraprotein by either serum or urine EP/IFX (Table 1). Two samples (6%) had results consistent with restricted FLC by the Freelite assay but no abnormality detected by serum or urine EP/IFX (Table 1). Both of these samples were additionally tested on the N latex assay. One of the samples returned all values within normal limits and the

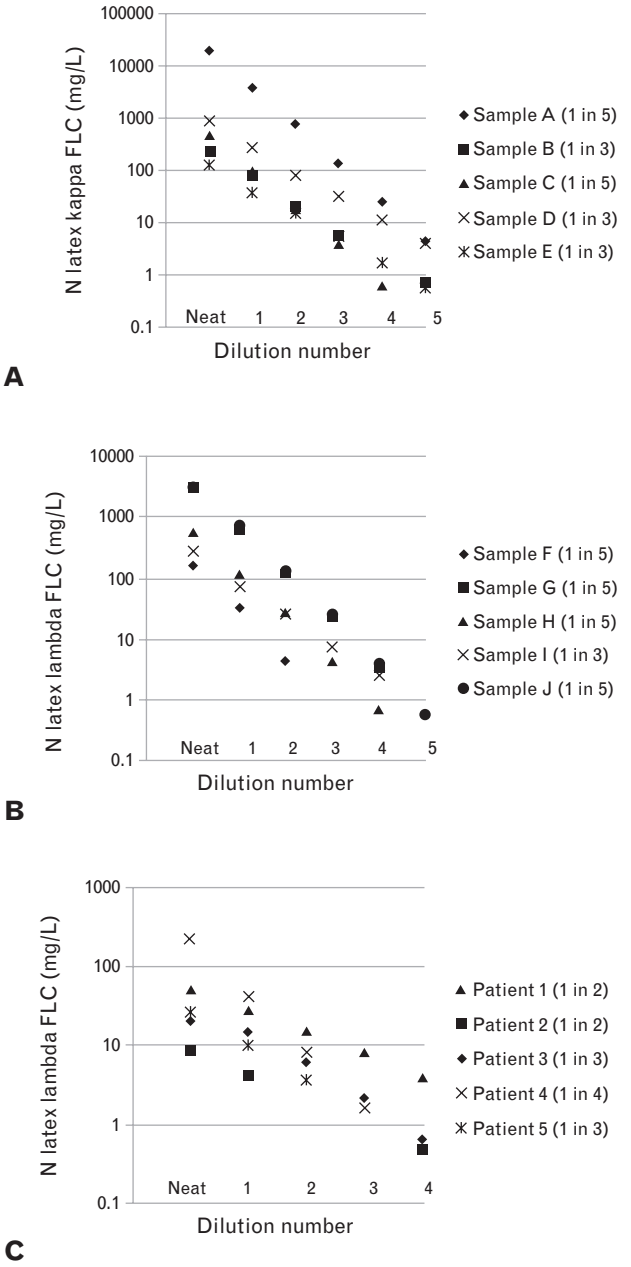

Fig. 2 Linearity of the N Latex FLC assay. Assay linearity was assessed by running samples neat and following serial dilutions as shown in the figure key. The dilution factor used was determined by the estimated FLC concentration and the volume of remaining serum available. (A) Samples with high kappa FLC, (B) samples with high lambda FLC, (C) samples that displayed marked discordance between the Freelite and N Latex FLC assays for lambda. Patients 1 and 2 are those referred to in Fig. 1B and D with # and ‡, respectively.

Table 2 Immunofixation of samples with discordant SFLC results

Sample	Freelite κ mg/L (3.30–19.40)*	Freelite λ mg/L (5.71–26.30)*	Freelite SFLCr (0.26–1.65)*	Freelite SFLC assay interpretation	N Latex FLC κ mg/L (6.70–22.41)*	N Latex FLC λ mg/L (8.30–27.00)*	N Latex SFLCr (0.31–1.56)*	N Latex FLC assay interpretation	Pentagel IFX result
1	**25.10 (H)**	13.6	**1.85 (H)**	Excess kappa FLC	20.30	25.90	0.78	NAD	PP
2	**24.60 (H)**	9.98	**2.46 (H)**	Excess kappa FLC	18.00	15.60	1.15	NAD	PP
3	**22.90 (H)**	10.9	**2.10 (H)**	Excess kappa FLC	15.70	14.40	1.09	NAD	PP
4	**31.70 (H)**	17.50	**1.81 (H)**	Excess kappa FLC	19.80	19.80	1.00	NAD	PP
5	**155.00 (H)**	24.80	**6.25 (H)**	Excess kappa FLC	**56.20 (H)**	**40.90 (H)**	1.37	Polyclonal	PP
6	**20.80 (H)**	8.74	**2.38 (H)**	Excess kappa FLC	19.40	11.80	**1.64 (H)**	Indeterminant	PP
7	10.50	**5.22 (L)**	**2.01 (H)**	Excess kappa FLC	9.55	8.95	1.07	NAD	NoPP
8	**34.90 (H)**	9.52	**3.67 (H)**	Excess kappa FLC	**30.70 (H)**	21.10	1.45	Indeterminant	NoPP
9	**33.60 (H)**	18.50	**1.82 (H)**	Excess kappa FLC	**25.30 (H)**	22.90	1.10	Indeterminant	NoPP
10	9.80	**450.00 (H)**	**0.02 (L)**	Excess lambda FLC	8.61	12.40	0.69	NAD	PP
11	**95.40 (H)**	**104.00 (H)**	0.92	Polyclonal	**57.10 (H)**	22.30	**2.56 (H)**	Excess kappa FLC	PP
12	**34.40 (H)**	**51.50 (H)**	0.67	Polyclonal	**25.80 (H)**	**165.00 (H)**	**0.16 (L)**	Excess lambda FLC	PP
13	18.50	**59.60 (H)**	0.31	Indeterminant	17.70	**65.40 (H)**	**0.27 (L)**	Excess lambda FLC	PP
14	13.40	**30.50 (H)**	0.44	Indeterminant	13.40	**51.10 (H)**	**0.26 (L)**	Excess lambda FLC	PP

Serum samples that displayed discordant results on the two SFLC assays ($n = 14$) in regard to detection of a free light chain excess were further studied using immunofixation for detection of a paraprotein for the purposes of this research study.

* Reference ranges for assays are shown in parentheses.

κ, kappa; λ, lambda; FLC, free light chain; H, high; L, low; NAD, no abnormality detected; NoPP, no paraprotein detected; PP, paraprotein detected.

other again had restricted kappa FLC. Further information regarding these samples was obtained. One patient had been treated for MM and had achieved remission. This patient developed a urine Bence-Jones protein by EP/IFX one month after the study period, with no detectable paraprotein by serum EP/IFX. This patient had restricted FLC by both the Freelite and N Latex FLC assays. The other patient had hyper immunoglobulin-G (IgG) and overproduction of IgG4 without the clinical features of IgG4 disease and no diagnosis of a PCD. This patient had restricted FLC by the Freelite assay and normal results by the N Latex FLC assay (see Table 2, Sample 9). Therefore SFLC testing found one case of relapsed PCD that would be missed by traditional methods.

In the 13 cases of excess lambda light chain production detected by the Freelite assay, where SFLC testing, serum and urine EP/IFX were completed, electrophoresis-based methods identified all 13 cases. Of the four samples additionally tested on the N Latex FLC assay, all four demonstrated lambda restriction (Table 1). This work supports the results of the largest study in this area which found the addition of SFLC testing to serum EP increased the sensitivity of diagnosis of PCDs from 94.4% to 100%[8] using a gold-standard of final diagnosis by a haematologist. These data suggest that SFLC analysis is more sensitive to detecting restricted kappa light chain production, although further work is needed to confirm this.

Analysis of the N Latex FLC assay demonstrated high precision when assessed by both commercial and in-house controls. In comparing the two assays there was higher correlation for kappa FLC (Fig. 1A; rho = 0.97), than lambda FLC (rho = 0.89). The correlation of lambda FLC results was satisfactory up to the upper limit of the reference range (rho = 0.83), but samples higher than this displayed markedly reduced correlation (Fig. 1B; rho = 0.62). Correspondingly the correlation of the SFLCr was good when the Freelite value was ≥ 1 (indicating normal range to excess of kappa light chains; rho = 0.83), and only moderate for Freelite assay values <1 (indicating normal range to excess lambda light chains; rho = 0.70).

Our data are consistent with two previous reports showing higher correlation for kappa FLC than lambda FLC.[13,14] The one previous report showing higher correlation for lambda FLC excluded 162 of 278 samples with a Freelite value of <50 mg/L,[10] where no justification for this exclusion criterion was given. In the present report we did not exclude any sample based on clinical or SFLC data and samples were only excluded if insufficient serum was available. This is in contrast to other papers that excluded patients either on the basis of not having a well-defined diagnosis[13] or low SFLC values.[10] Including all samples may provide less biased data in comparing the two assays.

We investigated for non-linearity, a phenomenon previously reported in SFLC assays.[10] The N Latex FLC assay displayed good linearity for kappa FLC and lambda FLC across and above the reference interval. Additionally non-linearity was not found in samples that were discordant between the two assays on the N Latex FLC assay (Fig. 2). We were unable to assess non-linearity in these samples on the Freelite assay, and this would have been an interesting comparison. The N Latex FLC assay displayed good consistency over three lots. Overall there was good agreement between the two assays in the categorical diagnosis of restricted FLC production with a kappa statistic similar to that reported by Lock *et al.*,[14] but significantly lower

than another study which excluded a large number of samples at the lower end of the Freelite assay.[10]

Restriction in either kappa or lambda FLC by one SFLC assay but not the other was found in 14 of 103 (14%) samples. These discordant samples were further investigated for presence of a paraprotein by IFX for the purposes of this research study only. The Freelite assay found restricted FLC in three samples when there was no paraprotein by IFX, and did not find restricted FLC in four cases where there was detectable paraprotein by IFX. In comparison, the N Latex FLC assay did not report restricted FLC in any samples where there was no paraprotein by IFX, but did report no excess of FLC in seven samples with detectable paraprotein by IFX (Table 2). In this small data set, using the IFX result as the reference standard, the Freelite assay had both false-positives and false-negatives, while all of the N Latex assay's errors were false-negatives. These data are consistent with another study that found the Freelite assay had higher sensitivity (i.e., agreement with IFX) while, conversely, the N Latex FLC assay had higher clinical specificity.[13] It is possible that this error pattern is due to the limited epitope specificity of the monoclonal antibodies in the N Latex assay.

This study compared the performance of two SFLC assays in the setting of a diagnostic laboratory. The fact that our samples originate from diverse clinical settings including in-patient, outpatient and primary care likely makes our data fairly representative of the general population. Certainly previous work in this area has shown higher sensitivity and specificity of SFLC assays in studies based solely in haematology departments, compared with those based in more generalised settings.[15]

Several shortcomings and potential sources of bias in the present study should be noted. Firstly the samples were run on different nephelometers; however, the two machines are produced by the same manufacturer and are considered highly comparable. Secondly the Freelite assay was conducted on fresh serum, while the N Latex FLC assay was performed on serum that was frozen pending analysis. While this could influence the results, and possibly account for the lower numerical results on the N Latex FLC assay, preliminary experiments showed that up to four freeze-thaw cycles did not significantly affect the results using the N Latex FLC assay (data not shown). Because the Freelite assay was run external to the research site we were unable to interrogate this assay in the same way the N Latex FLC was examined, e.g., in terms of linearity.

SFLC testing likely plays an important role as an adjunctive test in PCD and other light chain diseases. This study demonstrates that the large majority of PCD can be diagnosed by serum and urine EP/IFX and the gains from addition of SFLC testing are small. The N Latex FLC assay displayed good precision and linearity. The N Latex FLC assay has good correlation with the Freelite assay for kappa FLC, with lower correlation for lambda FLC. The poorer correlation for lambda FLC, now demonstrated in three studies, may be due to lower sensitivities of detection antibodies, or may reflect greater variation or abnormalities in lambda FLC production compared with kappa FLC in PCD. In samples that were discordant between the two SFLC assays, the Freelite assay displayed similar rates of false negatives and false positives. In contrast the N Latex FLC assay had a higher rate of false negatives. We hypothesise that the discordance between the N Latex FLC assay and the Freelite SFLC assay (or indeed IFX) is likely due to differences in the detection antibody system in regard to the ability to recognise light chain epitopes of PCD, as it does not seem to be a function of imprecision or non-linearity. This work supports the argument that patients cannot be monitored effectively by differing methods, and care should be taken by laboratories and ordering physicians considering switching assays.

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