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Evaluation of two serum free light chain quantitation methods, Freelite and Seralite, in the clinical laboratory with a view to switching immunoassay

Jennifer L J Heaney¹, Felix Ackermann², Michèle Paunet-Bobo³, Souad Mehlal⁴,
Emilie Jolly³, Johanna Hachem³, Aurélie Védrenne³, Marc Vasse⁵

1. Institute of Immunology and Immunotherapy, University of Birmingham, UK
2. Department of Internal Medicine, Hospital Foch, Suresnes, France.
3. Clinical Biology Department, Hospital Foch, Suresnes, France
4. Cerba Healthcare, Saint Ouen l'Aumône, France.
- 5 Clinical Biology Department and INSERM UMR_S 1176, Hospital Foch, Suresnes, France

Correspondence:

Professor Marc Vasse

Clinical Biology Department and INSERM UMR_S 1176, Hospital Foch, Suresnes,
France

T1. +33 (0)1 46 25 29 57

T2. +33 (0)6 27 83 67 59

m.vasse@hopital-foch.org

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1. Introduction

The efficacy of sFLC analysis and the $\kappa:\lambda$ ratio as a sensitive screening tool to detect multiple myeloma (MM) has been established in both retrospective and prospective studies that have included hundreds, or in some cases, thousands of patients [1-3]. Its utility has been further supported by studies demonstrating its prognostic value for myeloma patient outcomes [4-8]. sFLC quantitation now takes a key place in the panel of immunochemistry tests available for clinicians and is included in international and national clinical guidelines for not only myeloma but all plasma cell dyscrasias [9-14]. sFLC analysis supports diagnosis, prognostication and monitoring of amyloid light-chain (AL) amyloidosis [15-17]. FLC analysis also forms a key role in other clinical presentations, for example in severe renal impairment when the underlying pathology is unknown it is critical to assess if there is haematologic involvement [18].

Clinical guidelines are currently based upon the Freelite assay (The Binding Site) but several other commercial assays are now available for use in clinical chemistry departments. These include N Latex FLC (Siemens), KLoneus (Trimerio Diagnostics), Diazyme free light chain assay, Sebia FLC ELISAs and Seralite (Abingdon Health). Most these immunoassays use nephelometry, turbidimetry or microplate reader instrumentation. Nephelometry/turbidimetry are largely available in specialised clinical chemistry departments but smaller laboratories may have to send samples away to other sites, which can incur delays in results. As an alternative platform, Seralite is a dual κ and λ lateral flow device that is designed as portable, rapid test, free from large laboratory analysers, that could help speed up clinical results and intervention [19, 20]. This type of test may be preferable to

smaller laboratories, who receive lower numbers of samples, to enable in-house testing.

Method comparison studies between sFLC methods have revealed significant differences in quantitation. Regardless of employing monoclonal or polyclonal antibodies, each method appears to quantitate FLC differently and there is a consensus that tests are not interchangeable [5, 19, 21-25]. How tests differ has been predominately assessed in a retrospective manner in patients with a known diagnosis. Further clinical concordance has often been assessed using a single sample/time-point, which only provides a snapshot for clinical interpretation.

Prospective method comparison studies are limited and there is a lack of data concerning patient screening/monitoring in real time in the clinical laboratory. Yet these types of studies need to be performed by laboratories if they wish to switch assays. As there is no international standard available for FLCs, it is advised that to change methods the existing and new test should be run in parallel to establish any differences [22, 23]. However, there are limited published reports documenting the impact of this activity on results interpretation for individual patients. The present study performed Freelite and Seralite alongside each other in the clinical laboratory over 10 months and compared sFLC quantitation and patient results.

2. Materials and Methods

2.1. Patient samples and sFLC quantitation

Patient samples included in the study were those requested for sFLC quantitation by clinicians received by the clinical laboratory (Clinical Biology Department, Hospital Foch, Suresnes, France) during the period of June 2018–March 2019. Monoclonal gammopathy was diagnosed on the presence of a peak on the serum protein

electrophoresis performed on a Capillarys 2 Flex Piercing (Sebia France, Lisses), using the Capitest protein 6 kit (Sebia). Characterization of the monoclonal Ig was performed by immunofixation (IFE) (Hydragel IF, Sebia). Blood samples were centrifuged and the serum separated into aliquots. One aliquot was kept in the laboratory for Seralite analysis and another was sent to Cerba laboratory (Paris, France), which performs local routine testing for sFLC using Freelite. Testing was performed as samples were received, typically 2–3 times per week at both laboratories. Freelite (The Binding Site, Birmingham, UK) was performed using a Cobas® 6000 analyzer (Roche Diagnostics, Switzerland) according to the manufacturer's instructions. Freelite has undergone extensive validation and is incorporated into the international guidelines for plasma cell dyscrasias [13, 14]. Seralite (Abingdon Health Ltd, UK) has undergone clinical validation for diagnosis and monitoring of myeloma [5, 19, 20]. The present study was carried out in agreement with the French ethical laws and patients consented for residual blood samples to be used for research purposes.

2.2. Data analyses

Concordance between methods was assessed using the κ : λ ratio. Agreement was defined on the basis of a low/normal/high ratio on both methods. Percentage concordance (proportion of agreement) is reported and Cohen's kappa as a measure of extent of agreement between methods, where .01–.20 indicates slight agreement; .21–.40 fair agreement, .41–.60 moderate agreement; .61–.80 substantial agreement and .81–.99 almost perfect agreement.

FLC quantitation was compared between tests within the MM and MGUS cohorts using Bland-Altman plots and Passing-Bablok regression. To compare differences between two consecutive patient samples, absolute and percentage change in FLC

difference (involved – uninvolved, dFLC), a key marker in monitoring patients, was assessed. Patients with consecutive samples were selected as case studies to compare patterns of disease activity over time. For patients with other conditions, FLC isotypes were classed as normal/elevated and κ : λ ratio as normal/abnormal, in accordance with reference ranges. The reference ranges employed were: FLC diagnostic ratio reference range 0.26–1.65 on Freelite [26] and 0.5–2.5 on Seralite; κ FLC: 3.3–19.4 mg/L and λ FLC: 5.7–26.3 mg/L (Freelite); κ FLC: 5.3–22.7 mg/L and λ FLC: 4.0–25.1 mg/L (Seralite). The Freelite renal reference ratio of 0.37–3.1 was also applied for renal impairment [27].

3. Results

3.1. Patients

189 patients were included in the study and categorised based on clinical condition according to the diagnosis from clinical notes. Patients analysed included: MM (n = 30); MGUS (n = 112), amyloidosis (n = 17), other plasma cell dyscrasias (n = 14) and renal impairment (n = 16).

3.2. Myeloma, MGUS and amyloidosis patients at diagnosis

36 samples were obtained from myeloma, MGUS or amyloidosis patients at disease presentation (Figure 1A). 53% (n = 19, Freelite) and 50% (n = 18, Seralite) of patients presented with an abnormal ratio. Concordance was seen between the methods for 28/36 patients (78% agreement; $\kappa = .647$, $p < .01$).

Of the patients analysed, 6 samples were obtained from MM patients and all patients had an abnormal ratio according to both methods (100% concordance).

23 patient samples were identified with MGUS: 43% (Freelite) and 39% (Seralite) of patients presented with an abnormal ratio at disease and agreement between

methods was found for 17/23 patients (74% concordance) Five discrepancies were due to marginal elevation of a FLC isotype on Freelite but not Seralite (3 cases) or elevation of a FLC according to both methods but only perturbing the ratio on Seralite (2 cases). The other discrepancy was due to a low ratio on Seralite but high ratio on Freelite. In this patient, both FLCs were elevated but methods disagreed regarding which FLC isotype was most perturbed (Freelite κ FLC = 59.7 mg/L and λ FLC = 29.7 mg/L; Seralite κ FLC = 58.0 mg/L and λ FLC = 179.0 mg/L). Electrophoresis identified an IgG κ peak of 4.6 g/L in this patient.

Seven samples were received patients with amyloidosis at diagnosis. Abnormal ratios were seen 3/7 according to both assays but differed in classification of individual patients. Concordance for ratio was seen for 5/7 patients (71% concordance). In the two patients where an abnormal ratio was observed on one assay but not the other, both FLCs were elevated on both methods and creatinine levels were > 110 mmol/L, indicative of renal impairment. If using the renal reference range for Freelite, assay agreement was seen for one of these patients.

3.3. Patient monitoring of myeloma, MGUS and amyloidosis

123 samples were obtained from myeloma, MGUS or amyloidosis patients as part of patient monitoring (Figure 1B). 67% (Freelite) and 57% (Seralite) of patients presented with an abnormal ratio. Concordance was seen for 93/123 patients (76% agreement; $\kappa = .591$, $p < .01$).

24/123 patients had MM and samples related to monitoring represented a variety of circumstances: remission, undergoing therapy, discontinued therapy and relapse. Most patients had measurable disease (FLCs > 100 mg/L) and both assays returned abnormal ratios for the majority of patients, with 79% concordance observed

between Freelite and Seralite for the FLC ratio. There were 5 discrepancies in 3/5 cases both κ and λ were elevated on both methods and creatinine was elevated (297–986 $\mu\text{mol/L}$) indicating renal impairment. These patients were classed as normal on Seralite and elevated on Freelite according to the diagnostic range. However, if the Freelite renal range (0.37–3.1) was applied, patients were classed as normal. In 1/5, both FLCs were normal (κ and λ FLCs between 6.81-17.3 mg/L on both assays) and ratios were similar on both assays (1.73 Freelite, 1.77 Seralite) but classed as abnormal Freelite and normal on Seralite. In 1/5, Seralite indicated elevated λ FLC (68 mg/L) and abnormal ratio but normal FLC levels and ratio was seen on Freelite.

Samples were received from 89 confirmed MGUS patients as part of patient monitoring. An abnormal ratio was seen for 66% and 55% of patients on Freelite and Seralite with an overall concordance of 75%. For the majority of discrepancies, slight differences in quantitation resulted in a perturbed ratio on one assay but not the other. In 5 cases over 100 mg/L difference in involved FLC (iFLC) was seen between the two methods with one indicating high elevated where the other was normal/slightly elevated. The iFLC concentrations for these patients were: 1) λ FLC 253 mg/L Freelite vs 31 mg/L Seralite (IFE IgG λ); 2) λ FLC 182 mg/L Freelite vs 18.4 mg/L Seralite (IFE IgG λ); 3) λ FLC 112 mg/L Freelite vs 27 mg/L Seralite (IFE IgG λ); 4) λ FLC 35 mg/L Freelite vs 600 mg/L Seralite (IFE IgG λ); 5) κ FLC 10.5 mg/L Freelite vs 192 mg/L Seralite (IFE IgG λ). Patients with > 150 mg/L on Seralite without elevation on Freelite were explored for the possibility of antigen excess on Freelite. For example, the patient with 600 mg/L on Seralite underwent re-dilutions on Freelite and results did not indicate antigen excess.

Ten amyloidosis patients were assessed for patient monitoring. The majority had normal ratios on both assays: 4/10 had an abnormal ratio on Seralite; 3/10 had an abnormal ratio on Freelite. There was agreement regarding the isotype elevated, however, the patients identified as normal/abnormal differed between assays (70% concordance). Therefore, when analysing individual amyloid patients, as a result of quantitation differences, classification of the ratio as normal/abnormal of each test differed.

3.4. Methods comparison of FLC quantitation

Agreement between methods for FLC quantitation was examined using the first sample received for the MM and MGUS cohorts (n = 142). Bland-Altman plots are shown in Figure 2 and show that variation between methods depends on the magnitude of measurements. Mean bias (95% limits of agreement) for κ FLC was 1.7 mg/L (-180.7 and 177.3 mg/L). Good agreement was seen up to 100 g/L and variation increased thereafter as concentrations increased, with several data points falling outside the 95% limits of agreement. Up to 120 mg/L Seralite was often higher but above this concentration Freelite predominantly measured higher, substantially so in highly elevated samples. λ FLC mean bias was 1.4 mg/L (-221.0 to 217.8 mg/L) with very good agreement up to 50 mg/L and maintaining within limits up to 200 mg/L; above this level, 4 points fell outside limits of agreement, falling below and above in equal proportion. Mean bias for FLC ratio was 3.82 (-68.0 and 60.0). Good agreement was observed up to a ratio of 50 but became poor once ratios were highly elevated, with Freelite returning higher values. A mean bias of -13.5 mg/L (-292.1 and 265.1 mg/L) was returned for iFLC; there was good agreement at the lower end with disparity increasing as the iFLC concentration increased. At the lower end (< 70

mg/L) Seralite mostly returned higher values, followed by an even distribution of higher/lower values on both methods between 70-200 mg/L, then switching to predominately higher values on Freelite above 200 mg/L.

Passing and Bablok regression analysis (Freelite x-axis, Seralite y-axis) returned the following intercepts (systematic differences) and slopes (proportional differences) with 95% CI, respectively: κ FLC -7.0 (-13.3 to -3.1) and 1.0 (0.8 to 1.2); λ FLC 4.6 (3.3 to 6.2) and 0.6 (0.4 to 0.6); $\kappa:\lambda$ FLC ratio -0.1 (-0.5 to 0.07) and 0.9 (0.7 to 1.2); iFLC -11.7 (-25.6 to -2.0) and 1.2 (0.9 to 1.5). The intercepts indicated that the methods differed by at least a constant amount for all parameters, except the $\kappa:\lambda$ FLC ratio. Slope CI's returned no proportional differences between methods, except for λ FLC where a difference was observed.

3.5. Monitoring individual patients

Patients with two consecutive samples (11 MGUS and 19 MM) were evaluated to compare percentage change in dFLC between the subsequent and preceding sample. The dFLC concentrations were typically different between assays; in some cases, over ten-fold difference was seen in quantitation. Similarly, the percentage change differed between the assays. However, the same pattern of disease activity (higher/lower dFLC compared to the previous time point) was observed for the majority of patients (22/30). In the 8 patients where the direction of percentage change differed between Freelite and Seralite differences did not impact upon clinical interpretation. Four patients had very low FLC levels (< 20 mg/L) on both assays at both timepoints; 3 patients were also in remission and fluctuations in FLCs were small (< 30 mg/L). One patient had a large difference in dFLC and percentage change between the two assays due to a large difference in iFLC for the first sample

(35 mg/L Freelite vs 600 mg/L Seralite), highlighted previously. Two patients indicated disease progression according to Freelite (> 25% increase in dFLC and absolute increase > 100 mg/L); these same thresholds were also seen on Seralite for these patients.

Patients with ≥ 3 samples were examined. Figure 3A1/2 compares results at diagnosis and follow-up of a light chain only myeloma patient. At diagnosis, the patient had highly elevated κ FLC and abnormal ratio according to both assays, although Freelite returned double the concentration of Seralite. Following initiation of therapy, at 3 weeks post-diagnosis an 81% reduction and 69% reduction was seen in dFLC for Freelite and Seralite, respectively. At 6 weeks-post diagnosis, Freelite displayed a 91% and Seralite a 88% reduction from the baseline sample. Therefore, despite Freelite returning consistently higher values for iFLC and dFLC at all time points, the assays provided similar information regarding percentage reduction in FLC and good response to therapy.

Figure 3B–E show case studies of longitudinal patient monitoring from any time during follow-up. These patients represent both monoclonal FLC isotypes and include stable, increasing and decreasing disease activity. For Patient B, both FLC tests indicated stable disease, followed by a decrease, and subsequent increase. For patient C, both assays showed a reduction in iFLC followed by an increase back to original (Freelite) or above original values (Seralite). For patient D, both methods showed a decrease in iFLC then fluctuate in a similar manner. In the final sample, FLC are normal (Seralite)/just above normal (Freelite). Patient E showed stable disease followed by a decrease to normal levels according to both assays.

3.7. Other plasma cell dyscrasias

Overall, good agreement between methods was seen for 12/14 (86% concordance) for patients with other plasma cell dyscrasias. In patients with Waldenstrom's Macroglobulinemia, for 5/6 κ FLC or both FLC isotypes were elevated according to both methods. This resulted in an elevated ratio for 5 and 3 patients on Freelite and Seralite, respectively. For the 5 solitary plasmacytoma patients analysed, 4/5 had a normal ratio and 100% concordance was seen between methods. The other patients (Burkitt lymphoma, acute plasma cell leukemia or monoclonal B lymphoproliferation) had normal ratios according to both methods. The patient with acute plasma cell leukemia had highly elevated creatinine (613 nmol/L) and both assays returned elevated κ and λ FLC.

3.8. Renal impairment

Samples from 16 patients with renal impairment or chronic renal failure were analysed without evidence of haematological malignancy. Of these, 13/16 had elevated/highly elevated creatinine (120–699 $\mu\text{mol/L}$). On Seralite no patients had a perturbed ratio according to the diagnostic range. On Freelite, half of patients had an abnormal ratio according to the diagnostic range (0.26-1.65) but all were normal when applying the renal reference range (0.37–3.10).

4. Discussion

This long-term study performed in real-time in clinical practice provides an insight into how Freelite and Seralite compare in the clinical interpretation of individual patients with disorders associated with FLC abnormalities. For patients overall, good

clinical concordance was seen between these two methods, with substantial and moderate agreement observed at diagnosis and for monitoring, respectively.

Approximately 95% of all MM patients have an abnormal $\kappa:\lambda$ ratio at diagnosis [28, 29]. A small number of samples received were from MM at disease presentation; all had an abnormal ratio on both sFLC tests. In the clinical laboratory, sFLC assays should have nearly 100% agreement on the ratio at diagnosis but due to the different antibodies used, occasionally individual patients may be missed on one test but picked up by another - this should be rare based on retrospective studies [5]. With any sFLC test, detection of abnormal ratios is critical for diagnostic sensitivity and should be expected to be seen in nearly all patients with measurable disease with confirmed MM based on full clinical and laboratory work-up.

More patient samples were revealed to have MGUS rather than MM, as expected with a much more common disease. The number of MGUS patients seen with an abnormal ratio in the present study is comparable to larger studies of 728 and 1148 MGUS patients at diagnosis that reported abnormal $\kappa:\lambda$ ratios in 47% and 33% of patients, respectively [30, 31]. Unlike MM, with MGUS there is less than a 50% chance of patients having an abnormal ratio at diagnosis. Not having close to full agreement between tests could be seen as less clinically important, particularly as MGUS is typically monitored rather than treated with therapy. However, an abnormal ratio at baseline is a risk factor for progression to MM [30-32] and therefore the prognostication of individual patients for high risk of MM may differ slightly between Freelite and Seralite.

Samples from MM patients as part of monitoring were associated with different stages of disease reflecting the expected heterogeneity when analysing samples as

they are received by laboratory. Most patients in the present study returned abnormal ratios and elevated FLC levels when monitored. Even when patients are in remission, low levels of FLC at the bottom end of calibration curves and close to the limits of the assays sensitivity can produce abnormal ratios. Further, oligoclonal plasma cell reconstitution after high-dose melphalan can lead to abnormal ratios. Therefore, when monitoring patients the ratio needs to be considered alongside iFLC and dFLC concentrations.

This study demonstrates the importance of analysing consecutive samples to compare clinical interpretation of sFLC techniques more robustly. When assessing individual patients, there were clear differences in the dFLC and iFLC between the assays and similarly in the percentage change between time points. Case studies also highlighted obvious differences in FLC quantitation, which was supported by Passing Bablok analysis of the overall cohort. Bland-Altman plots showed typically small mean bias but increasing variation between methods proportional to the magnitude of FLC concentrations. Findings are consistent with a growing body of evidence that demonstrates sFLC assays quantitate FLC differently in patients with monoclonal gammopathies [5, 19, 21-25]. Despite differences in quantitation, when investigating individual patients over time the assays generally confirmed the same pattern of change relative to preceding samples. When discrepancies in direction of change were highlighted, these tended to be when patients had very low levels of FLCs or changes were not of a magnitude that would infringe upon clinical decision points.

Previous investigations that have flagged cases where antigen excess was evident on Freelite but not on Seralite [19, 21]. Consequently, in patients with high concentrations on Seralite but normal on Freelite, antigen excess was explored as a possibility. Discrepancies tended to occur more commonly in patients in IgG λ patients. In all cases re-dilutions showed that antigen excess was not the cause of the discrepancy in FLC quantitation. In terms of the other way round (low on Seralite high on Freelite), Seralite® utilizes a competitive inhibition format, which means that as FLC levels increase, available binding sites on the gold-labelled mAb become saturated and the mAb is inhibited from binding to the immobilized FLC antigen on the membrane. This competitive format means antigen excess is not possible [33] and thus did not account for the discrepancies of higher concentrations of Freelite compared to Seralite, which likely reflect quantitative differences.

The current investigation compared the assays in AL amyloidosis, where sFLC assays have been used in clinical practice to support diagnosis and prognosis. At diagnosis, 84-97% of amyloidosis patients have been reported to have an abnormal ratio [15-17, 34]. Here, we found just under (Seralite) or just over (Freelite) half of patients had an abnormal ratio, although as a rare condition, only a small number of samples were received and statistics could not be performed on this cohort.

However, the proportion of patients with agreement between methods was poorer than MM/MGUS cohorts, with patients identified with abnormal ratios differing between the assays. Although the proportion of patients with abnormal ratios is likely to be similar for Freelite and Seralite, the individual patients identified with an abnormal ratio will vary. In practice, this means that amyloid cases missed by one assay may be picked up by the other and vice versa.

Samples were also received from other rare plasma cell disorders and Freelite and Seralite agreed on sFLC classification for the majority of patients. When investigating samples from individuals aged ≥ 60 approximately a quarter of patients can be expected to have mild–moderate renal impairment, and thus elevated sFLCs, as a result of age-related comorbidities [35-37]. Good concordance between the assays was seen for patients with renal impairment without plasma cell dyscrasias. Importantly, perfect concordance was only seen when applying the Freelite renal reference range rather than the standard diagnostic range. This stresses the use of the broader range in cases of known renal impairment to prevent unnecessary investigation for monoclonal gammopathy. All patients had a normal ratio according to the Seralite diagnostic range. An alternative reference range has been suggested for Seralite for patients presenting with acute kidney injury to help identify myeloma as the cause [20]; however, an alternative range has not been proposed in patients with less severe renal impairment or chronic disease and the findings of this study imply one is not required.

If users wish to change analytical platform then it is necessary to analyse samples using both the current and new method to enable new baselines to be set for individual patients. The current study used the manufacturer reference ranges. Clinical interpretation of results could differ between manufacturer and locally established ranges [38]; in reality it is not feasible for individual laboratories to establish their own reference ranges and most will apply limits set by the manufacturer. The sFLC criteria used in international guidelines are based upon the Freelite assay and any non-Freelite method requires aligned criteria for results interpretation [39]. Preliminary analysis has proposed Freelite equivalent cut-points for the Seralite assay [5]. In practice, sFLC criteria need to be used in conjunction

with other clinical information to confirm responses to therapy and disease progression.

Cases of myeloma increased globally by 126% between 1990 and 2016; incidence is still rising with another 20% projected increase by 2025 [40, 41]. The need for sFLC analysis within the clinical laboratory will increase accordingly. Freelite and Seralite offer two very different platforms. In the present study, Seralite was considered as a rapid method to permit in-house testing, avoiding associated time-delays and costs of sending samples away, and was compatible with the numbers of sFLC requests received by the laboratory. There are now several commercially available assays for sFLC analysis and the choice of assay, and consideration of switching assays, is likely to incorporate user's testing needs (such as volume of samples and turnaround times), resources (both equipment and staff) and cost-effectiveness. This study highlights important issues that should be taken into account if laboratories are contemplating switching sFLC techniques. In-depth analysis beyond basic quantitative differences needs to be explored over time in order to assess agreement between methods and the impact on clinical interpretation for individual patients to ensure accurate reporting of results to clinicians.

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Figure captions

Figure 1. Concordance between Freelite and Seralite for the κ : λ FLC ratio

Data is shown for patients with myeloma, MGUS or amyloidosis at diagnosis ($n = 35$) (A) and for patient monitoring ($n = 123$) (B). Patients are categorised according to a low, normal or high ratio using Freelite and Seralite. Concordance between methods was 78% at diagnosis ($\kappa = .591$, $p < .01$) and 76% for patient monitoring ($\kappa = .647$, $p < .01$). Grey shaded boxes indicate the number of patients with agreement between methods regarding a normal/abnormal ratio.

Figure 2. Bland-Altman representation of FLC quantitation according to Freelite and Seralite

Bland-Altman graphs are presented by plotting the difference between method vs. the mean of the methods for κ FLC (A), λ FLC (B) FLC ratio (C) and involved FLC (D) in 142 patients with MM or MGUS. Agreement between methods for FLC quantitation was examined using the first sample received for the MM and MGUS cohorts ($n = 142$). Mean bias (95% limits of agreement) were: κ FLC 1.7 mg/L (-180.7 and 177.3 mg/L), λ FLC 1.4 mg/L (-221.0 to 217.8 mg/L), FLC ratio 3.82 mg/L (-68.0 and 60.0 mg/L) and iFLC 13.5 mg/L (-292.1 and 265.1 mg/L). Overall good agreement was seen up to 100-200 mg/L with variation increasing proportional to FLCs concentrations.

Figure 3. Patient monitoring of patients with multiple myeloma with consecutive samples analysed using Freelite and Seralite

Panels A1 and A2 show a patient with light chain only myeloma at diagnosis and follow-up after initiation of therapy. Data is shown for the involved κ FLC (A1) and for FLC difference (A2; dFLC- involved minus uninvolved FLC) with percentage change compared to diagnosis. At diagnosis, the patient had highly elevated κ FLC and abnormal ratio according to both assays, although Freelite returned double the concentration of Seralite. Following initiation of therapy, at 3 weeks post-diagnosis an 81% reduction and 69% reduction was seen in dFLC

for Freelite and Seralite, respectively. At 6 weeks-post diagnosis, Freelite displayed a 88% and Seralite a 91% reduction from the baseline sample. Therefore, despite Freelite returning consistently higher values for iFLC and dFLC at all time points, the assays both indicated a good response to therapy. Panels B–E show longitudinal monitoring of different patients with monoclonal λ FLC (B and D) or κ FLC (C and E). Differences in iFLC quantitation can be seen between Freelite and Seralite (with Freelite returning higher FLC concentrations) but the assays confirm the same pattern of change relative to preceding samples.

Seralite	Freelite		
	Ratio low (n = 5)	Ratio normal (n = 17)	Ratio high (n = 14)
Ratio low (n = 6)	4	1	1
Ratio normal (n = 18)	1	14	3
Ratio high (n = 12)	0	2	10

A: Overall Concordance = 78%

Seralite	Freelite		
	Ratio low (n = 14)	Ratio normal (n = 41)	Ratio high (n = 68)
Ratio low (n = 13)	10	3	0
Ratio normal (n = 53)	4	32	17
Ratio high (n = 57)	0	6	51

B: Overall Concordance = 76%

Figure 1. Concordance between Freelite and Seralite for the $\kappa:\lambda$ FLC ratio

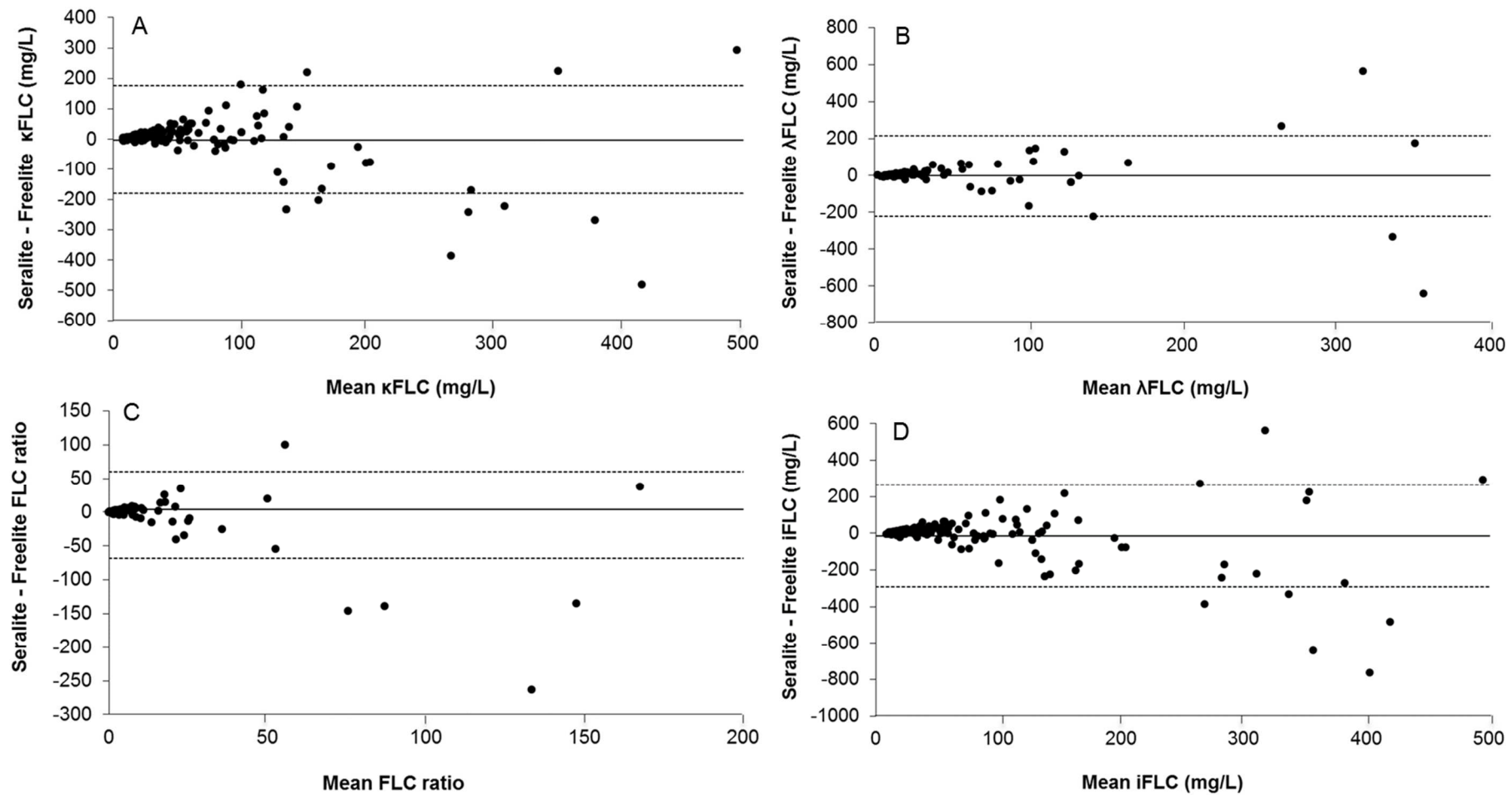


Figure 2. Bland-Altman representation of FLC quantitation according to Freelite and Seralite

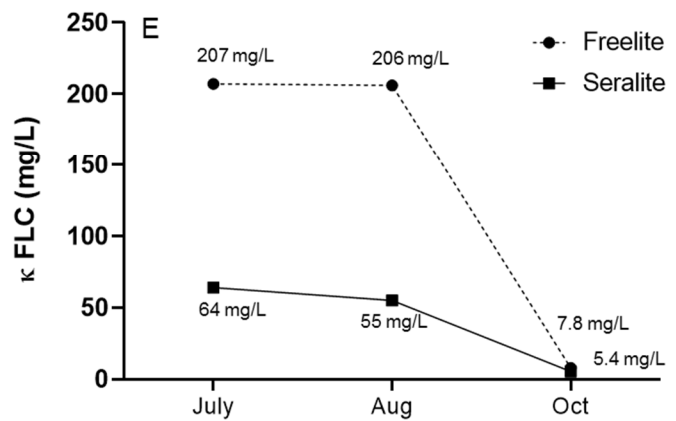
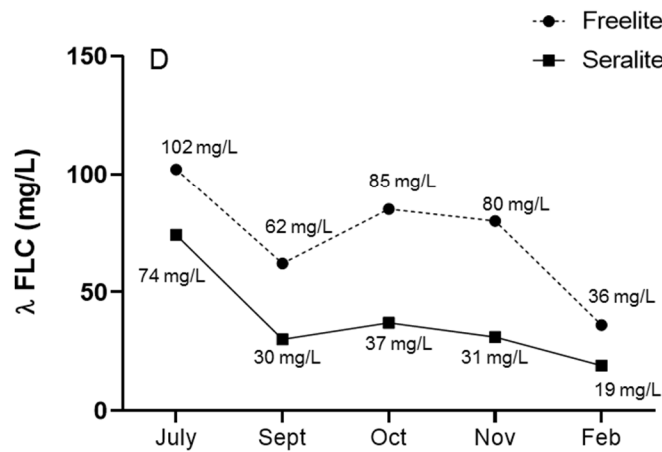
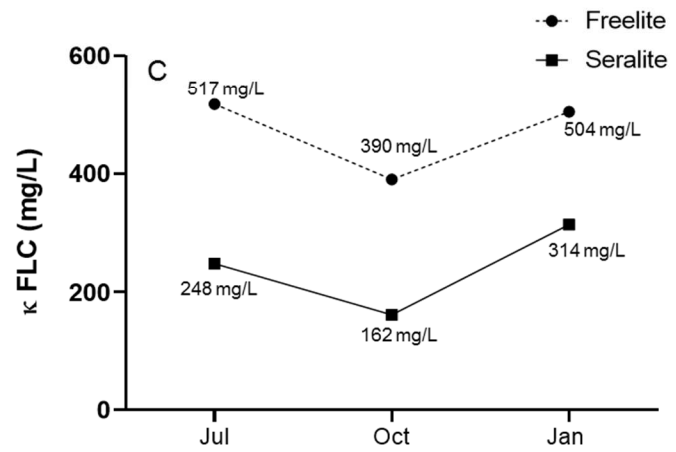
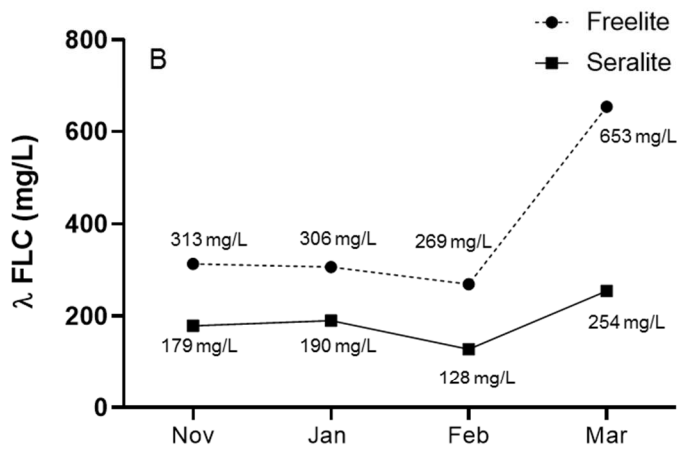
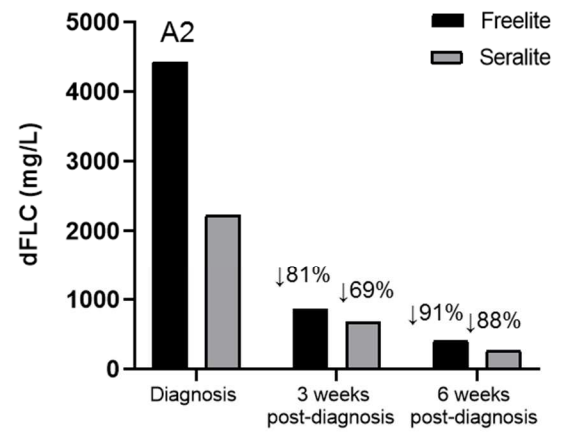
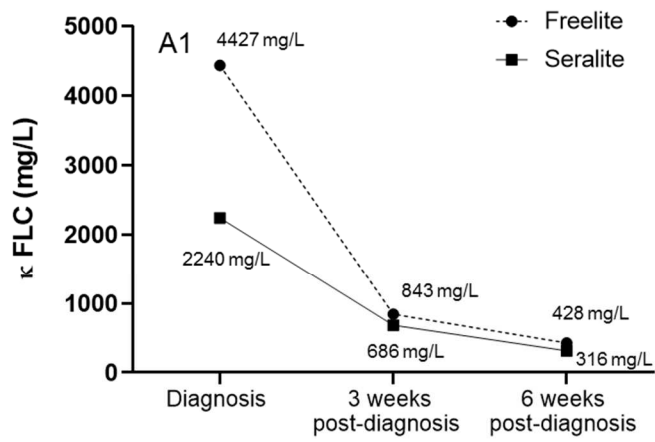


Figure 3. Patient monitoring of patients with multiple myeloma with consecutive samples analysed using Freelite and Seralite