8.6. Clinical performance

8.6.1. Comparison of absolute values

A number of studies and EQA schemes have compared the absolute values returned by the Freelite and N Latex FLC assays (Section 39.3). All have reached similar conclusions: the two assays do not compare well and are not interchangeable. Therefore, clinical cut-offs based on Freelite cannot be applied to N Latex FLC assays (Section 8.7).

A well-designed study by Lock et al. compared quantitative results of Freelite and N Latex FLC assay for 327 serum samples submitted for analysis to four routine diagnostic laboratories in the UK; a total of 79% were from patients with known monoclonal gammopathies. Comparing the results produced by the two assays, standard linear regression gave $r^2$ values of 0.86 and 0.71 for $\kappa$ and $\lambda$ sFLCs, respectively. This level of agreement is well below the requirements of the Clinical Laboratory Standards Institute (which requires an $r^2 \geq 0.95$ to establish that two assays are equivalent) [16]. Bland-Altman plots identified 17/327 (5.2%) samples that had the most discrepant results (Figure 8.8). This included 14 patients whose sera contained clonal FLC that were "poorly detected" by the N Latex FLC but not the Freelite assays [1]. Of most concern was the fact that 2 of the 14 patients had LCMM (prior to treatment) but their N Latex FLC assay results indicated normal sFLC ratios while their Freelite assay results were clearly abnormal (Table 8.8). These 2 patients and other examples of missed diagnoses are discussed in Section 8.6.2.

Campbell et al. compared sFLC values reported by Seralite-FLC and Freelite assays for 120 patients with polyclonal FLCs and 209 newly diagnosed MM patients. In general, absolute $\kappa$ and $\lambda$ values reported by the lateral flow assay were lower than those reported by Freelite. Consistent with these findings, a follow-on study by the same group defined measurable disease by the Seralite-FLC assay as a dFLC $>20$ mg/L, which is substantially lower than the equivalent Freelite definition in international guidelines (iFLC $\geq 100$ mg/L with an abnormal sFLC ratio, Sections 8.7 and 25.3).

Jacobs et al. conducted a method comparison of Sebia FLC and Freelite assays using 501 patient sera and 208 healthy blood donors. The values reported by the assays correlated reasonably well, but absolute $\kappa$ and $\lambda$ values reported by the Sebia FLC assays were lower than Freelite, and these differences were particularly evident at high concentrations of sFLCs. Similar discrepancies have been reported by Caillon et al. in a comparison of 368 samples from 177 IFM DFCI 2009 trial patients. Jacobs et al. hypothesised that samples containing the highest Freelite concentrations may contain FLC polymers that may not be optimally recognised by the ELISA assays. The authors also state that Sebia FLC values were more coherent with those obtained by SPE, but SPE is not considered the gold standard for light chain quantitation as the technique is subjective.
insensitive and inaccurate (Section 4.2.3).

In summary, all comparison studies published to date have reported a poor concordance of results generated using Freelite and other commercially available FLC assays. This lack of agreement can be clinically important and is discussed further below.

8.6.2. Diagnostic performance in MM

One major drawback of using monoclonal antibody-based FLC assays is that these assays have the potential to miss a particular monoclonal FLC clone. This is most serious when there is the greatest reliance on sFLC assays to identify a monoclonal protein that would otherwise be hidden or absent by standard electrophoretic techniques. This is often the case in LCMM and NSMM. An increasing number of publications suggest that both N Latex FLC and Seralite-FLC assays can fail to detect certain FLC tumour clones.

In a total of six studies published to date, which incorporated approximately 80 LCMM patients, the Siemens N Latex FLC assays failed to detect abnormalities in six λ LCMM and one κ LCMM patients (Table 8.7) [1][5][7][23][24][25]. By comparison, the same six studies reported that all of these LCMM patients were correctly identified by the Freelite assays. The best described cohorts of confirmed cases of LCMM were included in the studies by Schneider and Hoedemakers [7][25] in which 3/26 of confirmed LCMM cases were not detected by the N Latex FLC assays. In contrast, an abnormal Freelite κ/λ sFLC ratio has been detected in all of 692 LCMM patients at diagnosis (Chapter 15).

<table>
<thead>
<tr>
<th>Study</th>
<th>Total no. of LCMM patients</th>
<th>Missed LCMM</th>
<th>N Latex FLC</th>
<th>Freelite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>κ FLC mg/L</td>
<td>λ FLC mg/L</td>
</tr>
<tr>
<td>Lock 2013</td>
<td>n.s (189 MM)</td>
<td>λ LCMM</td>
<td>19.5</td>
<td>59.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>λ LCMM</td>
<td>12.8</td>
<td>40.2</td>
</tr>
<tr>
<td>Hoedemakers 2011</td>
<td>3 κ LCMM, 6 λ LCMM</td>
<td>λ LCMM</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>Schneider 2013</td>
<td>17 LCMM</td>
<td>λ LCMM</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td></td>
<td></td>
<td>κ LCMM</td>
<td>n.s</td>
<td>n.s</td>
</tr>
<tr>
<td>Sabatino 2017</td>
<td>1 λ LCMM</td>
<td>λ LCMM</td>
<td>16.5</td>
<td>23.0</td>
</tr>
<tr>
<td>Pretorius 2012</td>
<td>n.s</td>
<td>λ LCMM</td>
<td>29</td>
<td>84</td>
</tr>
</tbody>
</table>

Table 8.7. Summary of missed cases of LCMM by N Latex FLC assays. Patients were categorised as misclassified if the N Latex FLC assays reported a normal κ/λ ratio (n.s. = not stated).

Heaney et al. [20] compared Freelite and Seralite-FLC results in 325 light chain MM (LCMM) and 37 nonsecretory MM (NSMM) patients. All 325 LCMM patients had abnormal Freelite κ/λ ratio at diagnosis, but 3/325 patients had a normal Seralite-FLC ratio. In all three cases, the iFLC Freelite concentration was >100 mg/L, suggesting that the Seralite-FLC assay either missed epitopes or had insufficient analytical sensitivity. Similar results were found in NSMM at diagnosis: 49% (18/37) patients had an abnormal Freelite sFLC ratio but only 32% had an abnormal Seralite-FLC sFLC ratio.

The recommendation that sFLC analysis plus serum electrophoresis constitutes a suitable primary screening protocol for monoclonal gammopathies [26][27] should, arguably, only be applied to FLC analysis with Freelite assays and not N Latex FLC or...
Seralite-FLC assays, which have the potential to miss patients and are not validated in this context.

8.6.3. Rationale for the diagnoses of LCMM missed by monoclonal antibody-based FLC assays

The clinical studies discussed above have provided a number of examples where patients known to have LCMM have had normal N Latex FLC results but abnormal results with Freelite assays. There are two reasons why FLC assays may fail to identify the presence of monoclonal sFLCs: 1) antigen excess (Section 8.4.4), or 2) complete non-reaction, in which the monoclonal antibodies fail to recognise a particular FLC clone.

Pretorius et al. [5] performed a comparison of Freelite and N Latex FLC results for 116 samples sent to the laboratory for routine FLC analysis (after exclusion of samples with Freelite <50 mg/L), and investigated any samples that were highly discordant. For 6/116 (5.2%) samples, N Latex FLC assays gave markedly higher ς results (Figure 8.9). When these six samples were further diluted and retested using Freelite assays, the results significantly increased, consistent with antigen excess in the Freelite assays. In contrast, 4/116 samples (3.4%) had markedly higher λ Freelite results (Figure 8.9B). Further dilution did not increase the N Latex FLC λ results for these samples. These four patients included one with confirmed λ LCMM for whom the κ/λ sFLC ratio was normal by N Latex FLC assays, but abnormal by Freelite (Table 8.8).

It is noteworthy that the majority of diagnoses that were missed by the N Latex FLC assays were of λ type. Whilst the κ constant domain is typically encoded by a single C gene segment, the λ constant domain is encoded by one of a number of C gene segments (Section 3.3). It is probable that the monoclonal antibody-based assays fail to detect all polymorphic forms of FLCs, particularly λ FLCs that are more genetically diverse. Further evidence of the difficulties associated with raising monoclonal antibodies to recognise all λ light chain types was reported by Mollee et al. [28], and is discussed in Section 8.6.6.

A patient with λ LCMM who was not diagnosed by the N Latex FLC assays is presented as a clinical case study below. In this example, serum immunofixation confirmed the presence of monoclonal FLCs that were detected by the Freelite sFLC assays, but not the monoclonal antibody-based N Latex FLC assays.

Clinical case history

A patient with λ LCMM identified by Freelite but not N Latex FLC assays.

A 47-year-old woman was admitted to the Istituto Nazionale Tumori, Naples, with bone pain. An X-ray of her pelvis revealed osteolytic lesions, and a serum protein electrophoresis (SPE) was ordered but this revealed no obvious monoclonal protein. Subsequently, sFLC analysis was performed using both Freelite and N Latex FLC assays. Whilst the Freelite assay identified monoclonal λ sFLCs, the N Latex FLC assay results were normal (Table 8.9). High-resolution agarose electrophoresis of serum and urine samples identified a monoclonal protein band (Figure 8.10A), which was typed by immunofixation electrophoresis (IFE) as monoclonal λ FLCs (in the absence of intact immunoglobulins including IgD/IgE; Figures 8.10B and C). A bone-marrow biopsy confirmed the diagnosis of λ LCMM. The patient was admitted to the Hematology-Oncology Unit, and one month after the start of therapy, serum and urine IFE became negative and the
8.6.4. Monitoring MM

A number of studies have compared N Latex FLC and Freelite for monitoring MM. Many report that whilst the two assays show a similar trend in results, differences in absolute values may lead to significant discrepancies in clinical interpretation. For example Lutteri et al. report a case of λ LCMM in which progressive disease was confirmed by Freelite before N Latex FLC (Figure 8.11).

Popat et al. compared Freelite and N Latex FLC assays to monitor 59 MM patients treated with Bortezomib-based regimens. The proportion of patients who had measurable disease was much higher by Freelite than by N Latex FLC assays (64% vs. 37%, n=59), and for the subgroup of patients with measurable disease by both assays (n=20), the agreement in responses assigned using the two tests was only moderate (Weighted Kappa = 0.54). Similar results have been reported by others. Popat et al. reported that during follow-up, Freelite identified clonal disease in 4/61 MM patients when the N Latex FLC ratio had normalised. In each case, clonal disease was confirmed by the presence of a monoclonal protein on serum IFE suggesting that the N Latex FLC assays may lack sensitivity to detect residual disease. Similar results were reported by others.

Heaney et al. compared the ability of Freelite and Seralite-FLC to monitor 132 LCMM patients. At presentation, absolute dFLC levels were higher on Freelite than Seralite-FLC (median values: 3207.8 mg/L vs. 657.5mg/L), but at maximum response a similar percentage reduction in dFLC was observed. However, the authors noted a significant difference in assigned response categories: fewer patients achieved a complete response (defined as normalisation of the sFLC ratio) by Freelite (41.7%) than by Seralite-FLC (52.3%). This may reflect insensitivity of the lateral flow-based assay. At disease relapse, the increase in dFLC by Freelite was consistently higher than the increase by Seralite-FLC (median values: 558 mg/L vs. 101.2 mg/L), and the authors proposed a Seralite-FLC cut-off value to define progressive disease (>30 mg/L). This is much lower than the definition in IMWG guidelines, which are based on Freelite values (25% increase in dFLC, provided the absolute increase iFLC >100 mg/L, see Sections 8.7 and 25.3).

Jacobs et al. compared Freelite and Sebia FLC assays to monitor four patients with LCMM. Both assays showed a parallel trend in results at all sampling points, but there were substantial differences in the absolute values reported by both FLC assays. A larger study by Caillon et al. compared Freelite and Sebia FLC ELISA results for 47 IIMM and 112 LCMM patients. The two assays did not give analogous response classification, with a clinical concordance of 75% for IIMM and 71% for LCMM samples. Progressive disease classification was concordant in 77% of patients, however, 16 patients (21%) had a dFLC increase.
≥100 mg/L by Freelite, but a <100 mg/L increase when measured with Sebia FLC. All but one of the discordant patients met progression criteria by Freelite but not by Sebia FLC ELISA. The authors also noted a “time lag” between detection of relapse by Freelite and detection by Sebia FLC ELISA \[22\].

In summary, quantitative differences between polyclonal and monoclonal antibody-based FLC assays may lead to significant differences in clinical interpretations when assessing response to treatment and disease relapse \[33\]. For this reason, the different FLC assays should not be used interchangeably, nor should it be assumed that international guidelines developed with Freelite assays can be applied to N Latex FLC, Seralite-FLC or Sebia FLC ELISA.

### 8.6.5. Diagnostic performance in cast nephropathy

The International Kidney and Monoclonal Gammapathy Research Group (IKMGRG) recommend the use of SPE and sFLC analysis to screen for monoclonal disease in patients presenting with acute kidney injury (Chapter 27) \[14\]. The IKMGRG suggest that if the concentration of monoclonal FLCs is ≥500 mg/L in patients with acute kidney injury (AKI), a diagnosis of tubular interstitial pathology is likely, and the most common renal lesion in such cases is cast nephropathy \[16\]. For such patients, further haematological work-up and prompt treatment, to reduce FLC production, is essential.

In the only study performed to date using the Siemens N Latex FLC assays in the context of AKI, the authors concluded that the IKMGRG recommendations could not be carried out satisfactorily \[35\]. In this study, five of the 28 patients (18%) with AKI secondary to MM were misclassified by the N Latex FLC assays. For one patient, the N Latex FLC assay reported a λ FLC concentration of 1 mg/L, whereas a value of 1810 mg/L was reported by the Freelite assay. Once again, this suggests that the pathogenic monoclonal λ FLC clone was not recognised by the monoclonal antibody-based assay.

### 8.6.6. Diagnostic performance in AL amyloidosis

The largest study that has compared the performance of N Latex FLC and Freelite assays in AL amyloidosis was published by Palladini et al. \[36\]. This included 426 patients with newly-diagnosed AL amyloidosis from two specialist centres (Pavia, n=353; and Limoges, n=73). When the median sFLC concentrations in λ patients were compared, similar values were obtained with Freelite and N Latex FLC assays (197 mg/L vs. 187 mg/L). However, for κ patients, κ sFLC values were significantly higher using Freelite (378 vs. 244 mg/L, p=0.038); this difference was most pronounced at high κ sFLC concentrations. Overall, the diagnostic sensitivity of Freelite (82%) and N Latex FLC (84%) assays was similar, and both improved to 98% in combination with serum and urine immunofixation. Similar results were reported by Mollee et al. \[9\].

Mahmood et al. \[37\] confirmed that absolute values reported by Freelite and N Latex FLC assays do not always compare well in AL amyloidosis. In this study, the agreement between κ sFLC results at presentation (n=90) was better than that of λ (κ sFLC R2 = 0.91; λ sFLC R2 = 0.52), as discussed above (Section 8.5.3.). κ/λ sFLC ratios were also discordant between the two assays: 10/90 patients were abnormal by Freelite but normal by N Latex, and 11/90 abnormal by N Latex but normal by Freelite. Similar results were reported by Mollee et al. \[9\], but the reason for these differences is not understood. A subsequent publication by the same author discussed the difficulties associated with raising monoclonal antibodies to recognise all λ light chain types in AL amyloidosis \[28\]. This study compared the performance of the Seralite ELISA with N Latex FLC and Freelite assays. All AL amyloidosis patients with monoclonal κ FLCs had an abnormal ratio by all three assays, but for λ patients, the diagnostic sensitivity was much lower for the Seralite ELISA than N Latex or FLC assays (58% vs. 70% and 79%). The authors conclude that the monoclonal antibody-based ELISA assay appears to react poorly with λ sFLCs and is not suitable for diagnosis or monitoring of AL amyloidosis. They also describe how attempts to modify the Seralite ELISA assay to improve λ reactivity failed to improve results. Unsurprisingly, the Seralite ELISA is no longer commercially available.

### 8.6.7. Monitoring AL amyloidosis

Freelite sFLC assays are well established for monitoring haematological response in AL amyloidosis, and have been incorporated into a number of national and international guidelines (Section 28.6). By contrast, FLC assays from other manufacturers, including N Latex FLC assays, have not been formally validated. A study by Mahmood et al. \[37\] was the first to compare haematological responses assigned (using consensus criteria) with Freelite or N Latex FLC assays in 90 newly diagnosed AL amyloidosis patients. Although there was broad agreement in responses at 2, 4, and 6 months, the N Latex assay response was often earlier: 18 patients reached a PR or VGPR earlier by N Latex FLC than by Freelite. The authors highlight that patients classed as early responders by the N Latex FLC assay may get under-treated, and hence carry a risk of shorter time to disease progression.

Palladini et al. \[36\] reported that during follow-up, the reduction in dFLC concentration measured using either method was of
prognostic significance in AL amyloidosis, although the optimal cut-off values, determined by ROC analysis were different (>50% dFLC decrease by Freelite; >33% dFLC decrease by N Latex FLC). In conclusion, Freelite and N Latex assays cannot be used interchangeably when monitoring haematological response in AL amyloidosis, and new response criteria would need to be derived and validated for the N Latex FLC assays if they are to be used in routine clinical practice.

Figures

**Figure 8.10. Serum and urine electrophoresis.**

(A) High-resolution agarose electrophoresis of serum and urine samples (B) Serum IFE (C) Urine IFE. (Reproduced with permission from Bioclinical Clinica)

**View source:**

- 8.6.3. Rationale for the diagnoses of LCMM missed by monoclonal antibody-based FLC assays

**Figure 8.11. Freelite indicates LCMM progressive disease before N Latex FLC.**

Progressive disease was defined as a 25% increase in dFLC, with an absolute increase iFLC >100 mg/L (courtesy of L. Lutteri)
Figure 8.8. Bland-Altman plots comparing results for Freelite and N Latex sFLC assays. (A) $\kappa$ sFLC, (B) $\lambda$ sFLC.

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Figure 8.9. Scatter plot comparing Freelite and N Latex sFLC results.

(A) $\kappa$ sFLC. Six samples with markedly elevated N Latex FLC results are indicated (1 – 6). (B) $\lambda$ sFLC. Four samples with markedly elevated Freelite results are indicated (7 – 10). Further dilution did not increase the N Latex FLC $\lambda$ results. Dotted lines: normal reference intervals. (Reproduced by permission of SAGE Publications Ltd., London, Los Angeles, New Delhi, Singapore and Washington DC, from [©Pretorius, 2012]).
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