6 - Freelite reference intervals

Summary:
- sFLC concentrations and κ/λ ratios are maintained within narrow limits in normal individuals.
- Ethnicity appears to have minimal influence on sFLC normal ranges.
- sFLC concentrations increase substantially and κ/λ sFLC ratios increase slightly with decreasing renal function. Use of a modified renal reference interval for the ratio increases the diagnostic specificity for detecting monoclonal FLC production in patients with renal impairment.
- FLC concentrations in serum are less variable than in urine.

6.1. Freelite® serum reference intervals

The most substantial study of sFLC concentrations in normal individuals using Freelite was published by Katzmann et al. [1]. Serum samples were obtained from 127 healthy blood donors (21 - 62 years) and 155 older, normal individuals (51 - 90 years). The κ and λ sFLC concentrations for the 282 serum samples are plotted in Figure 6.1 and the normal range data is summarised in Table 6.1. The 95% reference ranges for κ and λ sFLC concentrations and κ/λ sFLC ratio were 3.3 - 19.4 mg/L, 5.7 - 26.3 mg/L and 0.3 - 1.2, respectively. It was proposed that the 100% reference range for the ratio (0.26 - 1.65) should be used diagnostically, in order to minimise the number of false-positive results when screening for monoclonal FLC production. This 100% diagnostic range has now been generally adopted and is incorporated into guidelines (Chapter 25). The utility of Freelite sFLC measurements for identifying FLC monoclonal gammapathies is discussed in Chapter 23.

<table>
<thead>
<tr>
<th>Normal adult serum</th>
<th>Mean</th>
<th>Median</th>
<th>95% reference range</th>
<th>100% diagnostic range</th>
</tr>
</thead>
<tbody>
<tr>
<td>κ sFLC</td>
<td>8.4 mg/L</td>
<td>7.3 mg/L</td>
<td>3.3 - 19.4</td>
<td></td>
</tr>
<tr>
<td>λ sFLC</td>
<td>13.4 mg/L</td>
<td>12.7 mg/L</td>
<td>5.7 - 26.3</td>
<td></td>
</tr>
<tr>
<td>κ/λ sFLC ratio</td>
<td>0.63</td>
<td>0.59</td>
<td>0.3 - 1.2</td>
<td>0.26 - 1.65</td>
</tr>
</tbody>
</table>

Table 6.1. Mean/median values and ranges for FLC concentrations and κ/λ sFLC ratios in the sera of 282 normal individuals [1].
Katzmann et al. observed that κ sFLC concentrations tended to be lower than λ, giving a median κ/λ sFLC ratio of 0.59. This is because serum FLC levels are dependent upon the balance between production and clearance. There are approximately twice as many plasma cells producing κ FLC as there are producing λ FLC. However, as κ molecules are normally monomeric (25 kDa), their renal clearance is faster than λ molecules, which tend to be dimeric (50 kDa). Consequently the serum half-life of κ FLCs is shorter than λ, and κ FLCs accumulate less in serum leading to lower concentrations of κ sFLC in normal individuals (Chapter 3).

In elderly people there was a trend towards higher sFLC concentrations (Figure 6.2.a,b and Table 6.2) [1]. The same trend was observed for the renal function marker, cystatin C, which was measured in the same samples (Figure 6.2,d). When the results were expressed as sFLC κ/λ ratio or a ratio of sFLC concentration/cystatin C concentration, the effect of age was eliminated or reduced (Figure 6.2 c,e,f). This indicates that the higher sFLC values seen in older people can be largely explained by small reductions in glomerular filtration rate (GFR) [1]. Whilst several other groups have similarly observed no significant age-related differences in the sFLC ratio [2][3], others have reported an increase in the κ/λ sFLC ratio reference range in elderly populations, prompting the suggestion that age-dependent reference ranges should be considered [4][5].

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>κ sFLC (mg/L)</th>
<th>λ sFLC (mg/L)</th>
<th>κ/λ sFLC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 - 29</td>
<td>6.3</td>
<td>12.4</td>
<td>0.49</td>
</tr>
<tr>
<td>30 - 39</td>
<td>7.2</td>
<td>13.6</td>
<td>0.55</td>
</tr>
<tr>
<td>40 - 49</td>
<td>7.5</td>
<td>12.8</td>
<td>0.58</td>
</tr>
<tr>
<td>50 - 59</td>
<td>6.4</td>
<td>11.3</td>
<td>0.59</td>
</tr>
<tr>
<td>60 - 69</td>
<td>6.9</td>
<td>11.8</td>
<td>0.70</td>
</tr>
<tr>
<td>70 - 79</td>
<td>8.0</td>
<td>11.9</td>
<td>0.65</td>
</tr>
<tr>
<td>80 - 90</td>
<td>9.1</td>
<td>15.1</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Table 6.2. Median values for sFLCs and κ/λ ratios in different age groups [1].

The normal ranges published by Katzmann et al. were very similar to those previously reported by Bradwell et al., who observed a mean κ/λ sFLC ratio of 0.6 and a 95% reference range of 0.35 - 1.0 [6]. Slightly lower κ and λ sFLC concentrations (95% reference ranges of 4.2 - 13.1 mg/L and 9.2 - 22.7 mg/L, respectively) observed in the Bradwell study may be attributed to a younger study population (17 - 71 years).

6.1.1. Ethnic influences

The Freelite normal ranges published by Katzmann et al. were established in an American, predominantly white, population [1]. Known ethnic differences in the normal ranges of total immunoglobulins (Chapter 10) have prompted a number of laboratories to determine normal sFLC ranges for their local populations.

European research groups have reported Freelite reference ranges for normal individuals that are comparable to Katzmann et al. [7][8]. Similarly, sFLC concentrations in a small South African cohort including Black (57/113), mixed-race (44/113) and Caucasian (12/113) subjects were not significantly different, both for the local population as a whole, and for the Black and mixed-race populations independently [9]. In a Han Chinese population of 326 subjects, although a narrower κ/λ sFLC ratio normal range was observed (0.32 - 1.52), this local range and the Katzmann range (0.26 - 1.65) provided the same diagnostic sensitivity and
specifity for multiple myeloma (MM) (area under ROC curve 0.99 in both cases) [9].

A multi-centre Indian normal range study [10] reported slightly higher concentrations of κ and λ sFLCs compared with those reported by Katzmann et al. [11] (both p<0.01). The concentration of κ sFLCs was slightly higher than that of λ sFLCs, and consequently the κ/λ sFLC ratio (0.36 – 2.33, 95% range) was also higher than that reported by the American study [9]. The authors suggest that their findings are consistent with the lower glomerular filtration rates of normal Indian subjects than those of Western populations (Section 6.3).

6.2 Borderline Freelite results

All laboratory tests can produce borderline results, which should be considered in their clinical context and alongside other laboratory test results. Borderline κ/λ sFLC ratios may be attributed to a variety of causes. Increases in FLC concentrations and borderline elevated ratios due to renal impairment in the absence of monoclonal gammopathy are well documented [12][13]. For such patients, the use of a renal reference interval for the κ/λ sFLC ratio may reduce the number of false-positive results (Section 6.3).

Borderline high κ/λ sFLC ratios have also been reported in conditions associated with polyclonal inflammatory responses, such as infections, inflammation and autoimmune diseases [13][14][15]. In an audit reported by Marshall et al., 4.9% (47/955) of individuals tested had a borderline abnormal κ/λ sFLC ratio (between 1.67 and 3.2) with no known plasma cell disorder [13]. In the majority of cases this could be attributed to renal impairment or an inflammatory process. The authors commented that borderline low sFLC ratios were infrequently associated with renal impairment or inflammatory states, and that such borderline low ratios should prompt further investigation.

In addition to renal impairment and inflammatory conditions, borderline abnormal Freelite results may occur in a variety of monoclonal diseases encompassing intact immunoglobulin MM, many lymphomas and leukaemias (Chapters 31 and 33), AL amyloidosis (Chapter 28) and monoclonal gammopathy of undetermined significance (MGUS) (Chapter 13). It is now recognised that the probability of a malignant plasma cell disorder increases in relation to the degree of abnormality of the κ/λ sFLC ratio (Chapter 7).

6.3 Freelite renal reference intervals

Severe renal impairment changes the dynamics of FLC clearance. As GFR reduces, the clearance of FLCs decreases and becomes more dependent upon the reticulo-endothelial system, which shows no size preference and clears both κ and λ FLCs at the same rate. Therefore, as renal impairment increases, the serum half-life of κ FLCs approaches that of λ FLCs and their serum levels become more influenced by their underlying production rates. Consequently, the increase in the concentration of κ sFLCs is greater than λ sFLCs, and in a minority of patients the κ/λ ratio can increase above the normal reference interval in the absence of monoclonal gammopathy. This was demonstrated in a study using Freelite on serum samples from 688 patients with chronic kidney disease (CKD) and no evidence of monoclonal gammopathy [12]. As both κ and λ sFLC concentrations increased in patients with deteriorating renal function, their relative amounts changed slightly (Figure 6.3A). As a consequence, with increasing CKD stage, the median sFLC ratios were found to increase progressively from 0.6 to 1.1, with a 100% range of 0.37 - 3.10 (Figure 6.3B). Therefore, a κ/λ sFLC reference interval of 0.37 - 3.10, termed the "renal reference interval", was proposed for patients with renal impairment [16]. A similar trend was observed by Galvani et al. [17].
The diagnostic accuracy of the Freelite renal reference interval was assessed in an unselected group of 142 patients who presented with dialysis-dependent acute kidney injury (AKI) of unknown cause. All 41 patients with MM had abnormal \( \kappa/\lambda \) sFLC ratios by both the published reference range and renal reference range (Figure 6.4). Receiver operating characteristic (ROC) analysis showed that application of the renal reference range for the ratio increased the specificity from 93% to 98% with no loss of sensitivity. The diagnostic utility of the \( \kappa/\lambda \) sFLC renal reference range was further demonstrated by Park et al. A combination of sFLC analysis (using the renal reference interval) in combination with SPE, was the optimal screening algorithm for detecting MM in patients with renal impairment (Section 23.3.1, Table 23.4).

Palladini et al. studied the performance of the renal reference interval in 982 newly diagnosed AL amyloidosis patients of whom 16% (160/982) had severe renal dysfunction (eGFR<30 mL/min/1.73m²). Use of the renal reference interval in patients with renal failure improved the overall diagnostic sensitivity from 70% to 74%. This was due to an increase in the diagnostic sensitivity in patients with \( \lambda \) clones, but a decrease in the performance in \( \kappa \) clones. The authors concluded that "patients with AL amyloidosis and severe renal failure whose \( \kappa/\lambda \) sFLC ratio is <0.37 likely harbour a \( \lambda \) clone".

In conclusion, use of the Freelite renal reference interval in routine clinical practice may lead to increased diagnostic accuracy for the diagnosis of monoclonal gammopathy. For patients with CKD, a \( \kappa/\lambda \) sFLC ratio of 1.66 - 3.1 is likely to be caused by the change in FLC clearance and further investigation is only warranted if there is a significant clinical suspicion of monoclonal gammopathy.

### 6.4 Freelite urine reference intervals

Bradwell et al. measured \( \kappa \) and \( \lambda \) concentrations in early morning urine samples from 66 normal individuals using Freelite immunoassays (Figure 6.5 and Table 6.3). When compared to sFLC data from the same study, the range of urine FLC concentrations was much wider than for serum, and \( \kappa/\lambda \) urine FLC ratios were more variable. Presumably, this reflects differences in renal handling, urine dilution and mucosal secretion of FLCs between individuals.

<table>
<thead>
<tr>
<th>Normal adult urine</th>
<th>Mean</th>
<th>95% reference range</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \kappa ) FLC</td>
<td>5.4 mg/L (± 4.95)</td>
<td>0.39 - 15.1 mg/L</td>
</tr>
<tr>
<td>( \lambda ) FLC</td>
<td>3.17 mg/L (± 3.3)</td>
<td>0.81 - 10.1 mg/L</td>
</tr>
<tr>
<td>( \kappa/\lambda ) ratio</td>
<td>1.85</td>
<td>0.46 - 4.0</td>
</tr>
</tbody>
</table>

Table 6.3. Mean values (± standard deviation) and ranges for FLC concentrations and \( \kappa/\lambda \) ratios in early morning urine samples from 66 normal individuals [6].

A wide range of normal urine FLC concentrations was similarly observed by Snyder et al., who established a 95% reference range for the urine Freelite ratio of 1 - 19 using 91 healthy adult donors. The authors attributed the relatively poor diagnostic sensitivity of the urine FLC assay (80%) to the wide reference ranges and a high background of polyclonal FLC in the urine. It is important to note that international guidelines do not recommend the use of urinary FLC immunoassays. Arguments in favour of serum over urine FLC assays are further discussed in Chapter 24.
Figures

**Figure 6.1.** κ and λ FLC concentrations in 282 normal sera plotted on a logarithmic scale.

In general λ sFLC concentrations are higher than κ in normal individuals. (A) Axis at the normal κ/λ sFLC ratio of 0.6, and (B) axis at a κ/λ ratio of 1.00. (Reproduced with permission from the American Association for Clinical Chemistry).

View source:

- 6.1. Freelite® serum reference intervals

**Figure 6.2.** Effect of age on sFLCs.
(a) κ sFLC concentration, (b) λ sFLC concentration and (c) κ/λ sFLC ratios versus age (years) in 282 normal serum samples together with (d) cystatin C results in the same patients. sFLC/cystatin C ratios (e) and (f) show no change with age, confirming renal deterioration as the cause of increased sFLC levels in elderly individuals. (red = fresh sera, black = frozen sera). (Reproduced with permission from the American Association for Clinical Chemistry).

View source:
- 6.1. Freelite® serum reference intervals
- 35.2. Chronic kidney disease

Figure 6.3. (A) κ (white) and λ (grey) sFLC concentrations and (B) κ/λ sFLC ratio in CKD stages 1 – 5 plus patients on peritoneal dialysis (PD), haemodialysis (HD) and controls (Con).

Data presented as box plots (1st – 3rd inter-quartile ranges, central line is median value) with whiskers (5th – 95th percentile values). (Courtesy of Colin Hutchison).

View source:
- 6.3 Freelite renal reference intervals
- 35.2. Chronic kidney disease

Figure 6.4. sFLCs in 142 patients presenting with dialysis-dependent, acute renal failure.
Data presented as box plots (1st – 3rd inter-quartile ranges, central line is median value) with whiskers (5th – 95th percentile values). (Courtesy of Colin Hutchison).

View source:
- 6.3 Freelite renal reference intervals

Figure 6.5. Comparison of FLC measurements in serum and early morning urine samples from healthy individuals.

(Courtesy of J.A. Katzmann)

View source:
- 6.4 Freelite urine reference intervals

References
3. Abadie JM, Bankson DD. Assessment of serum free light chain assays for plasma cell disorder screening in a Veterans


