N Latex FLC Kappa and Lambda: Urine Application
Free light chains (FLCs) and the kidney

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Severe acute kidney injury with resulting renal failure is the major cause of morbidity for patients with multiple myeloma and AL amyloidosis. At diagnosis of multiple myeloma, 30–40% of patients show a reduced glomerular filtration rate.

With a typical molecular weight of about 22 kD for FLC kappa (monomeric) and about 44 kD for FLC lambda (dimeric), FLCs represent small proteins that are rapidly eliminated via free glomerular filtration, followed by tubular reabsorption and intracellular breakdown. The 500 mg of polyclonal FLCs normally produced per day are catabolized in the proximal tubulus.

However, in the case of high plasmatic FLC levels associated with monoclonal gammopathies, the tubulus cells cannot handle the high amount of molecules filtered due to limited reabsorption capacity, resulting in a so-called “overflow proteinuria,” or Bence Jones proteinuria, named after the author Henry Bence Jones, who first described this clinical picture in 1847.

Increased urinary FLC concentration is the cause of tubulo-intestinal renal injury. In the urine, FLCs build aggregates with Tamm-Horsfall protein, which are responsible for cast nephropathy with reduction of glomerular flow, tubular atrophy, and interstitial fibrosis.

Cast formation and precipitation is a complex process dependent on multiple variables such as tubule fluid, flow rate and concentrations of FLC and Tamm-Horsfall protein, and the polymeric form of the monoclonal FLC involved.

To reduce the nephrotoxic influence of high urinary FLC concentration, the following strategies are available:

• Maximization of urine output
• Avoidance of loop diuretics and nephrotoxic drugs such as radiologic contrast media
• Inhibition of light chain synthesis by chemotherapy (i.e., therapy of the underlying disease)
• Removal of FLC by hemodialysis or hemodiafiltration using highly permeable dialyzers

FLC in urine
In healthy individuals, urinary FLC concentrations are very low or even undetectable due to nearly complete tubular reabsorption capacity. With rising serum levels, urinary concentrations also increase, but a significant increase is only seen when the tubular reabsorption capacity is exhausted. As a result, the serum FLC offers higher and earlier sensitivity for the detection of monoclonal gammopathies than the urinary FLC measurement.

*Not available for sale in the U.S. Product availability varies by country.
Urine application for N Latex FLC: Performance

Reproducibility
Three urines of different concentrations were measured over 5 days in 8-fold determination on each day for estimation of repeatability and within-device CV.

<table>
<thead>
<tr>
<th>Pool</th>
<th>Mean (mg/L)</th>
<th>Repeatability (%)</th>
<th>Within-device CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N Latex FLC kappa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>5.4</td>
<td>6.7</td>
<td>8.7</td>
</tr>
<tr>
<td>Medium</td>
<td>19.7</td>
<td>6.5</td>
<td>8.1</td>
</tr>
<tr>
<td>High</td>
<td>60.3</td>
<td>8.5</td>
<td>9.7</td>
</tr>
<tr>
<td>N Latex FLC lambda</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>4.6</td>
<td>4.7</td>
<td>9.1</td>
</tr>
<tr>
<td>Medium</td>
<td>22.6</td>
<td>5.1</td>
<td>5.8</td>
</tr>
<tr>
<td>High</td>
<td>37.7</td>
<td>3.6</td>
<td>4.6</td>
</tr>
</tbody>
</table>

Urine sample stability
Urine samples can be stored for 2 days at 2–8°C. Freezing of urine samples is not recommended. Generally urine samples show reduced stability compared to serum samples due to the presence of proteolytic enzymes and potential bacterial contamination during collection. In addition, variation of pH may influence urinary analytes.

FLC levels were not influenced by pH in the range of 4–7. Outside this range, after freezing, or when stored for longer than 2 days, single samples can show larger deviations. Addition of a urine stabilizer to adjust for pH did not influence sample stability.

Urine sample stability

Urinary reference ranges
208 spot samples of urine were collected from ostensibly healthy individuals. To exclude the influence of an unknown tubular impairment, all urine samples were tested for α1-microglobulin. 26 samples with elevated α1-microglobulin levels were excluded, leaving 182 samples.

<table>
<thead>
<tr>
<th>Pool</th>
<th>N Latex FLC</th>
<th>FReeLITE®*</th>
</tr>
</thead>
<tbody>
<tr>
<td>FLC kappa</td>
<td>≤25.8</td>
<td>1.35–24.19</td>
</tr>
<tr>
<td>(95 percentile)</td>
<td></td>
<td>(2.5–97.5 percentile)</td>
</tr>
<tr>
<td>FLC lambda</td>
<td>≤11.3</td>
<td>0.24–6.66</td>
</tr>
<tr>
<td>(95 percentile)</td>
<td></td>
<td>(2.5–97.5 percentile)</td>
</tr>
<tr>
<td>FLC ratio</td>
<td>1.43–6.24</td>
<td>2.04–10.37</td>
</tr>
<tr>
<td>(2.5–97.5 percentile)</td>
<td></td>
<td>(min–max range)</td>
</tr>
</tbody>
</table>

*FReeLITE urine reference ranges according to IFU, N=29

Method comparison/correlation analysis
A total of 701 urine samples submitted for routine FLC testing have been investigated by N Latex FLC and FReeLITE.

The following correlations were obtained:

Figure 3a: FLC kappa

Figure 3b: FLC lambda

Figure 3c: FLC ratio

Very high correlations were obtained for both FLC kappa and FLC lambda assays. FLC kappa concentrations agree well between both methods. For FLC lambda, about 2.5-fold higher concentrations are measured with N Latex FLC lambda consistently over the entire range, which is consistent with the higher reference-range levels determined for this assay.

Based on the higher FLC lambda levels obtained with N Latex FLC, the FLC ratio calculated for the N Latex FLC assay system is consistently lower than the FReeLITE ratio.
Comparison of FLC kappa to lambda in serum and urine

When FLC lambda is plotted over FLC kappa, the graphs below are obtained for FReeLITE and N Latex FLC assays.

Figures 4a and 4b: FLC kappa versus lambda for FReeLITE and N Latex FLC assays.

In the FReeLITE graph, many results below 1 mg/L, as well as undetectable levels (0.1 mg/L), can be seen. In contrast, for N Latex FLC, only a few results below 1 mg/L are seen, and no samples have a result below the measuring range for the 1:20 sample dilution.

The wide scattering of the FReeLITE results in the low range can be explained by FReeLITE’s high CV in this low range (>20%), as well as the limited ability of the FReeLITE method to provide reliable sample results at the low end of the measuring range, followed by a switch to the next-lower dilution (see wikilite.com, chapter 4.2, G. Precision). TBS claims “an uncharacterised substance(s) that interferes with the latex assays” to be responsible, an interference that obviously is not present in the N Latex FLC assays.

Further, with N Latex FLC, the FLC kappa-to-lambda ratio for the normal and polyclonal increase samples is much more narrowly distributed in the diagonal, and more similar in serum and urine.

Why are FLC lambda levels higher with N Latex FLC lambda?

While urinary FLC kappa levels measured by N Latex FLC and FReeLITE agree well in healthy donors, the urinary FLC lambda levels determined by N Latex FLC are 2- to 3-fold higher compared to FReeLITE.

This difference in the FLC lambda levels detected may reflect the difference in reactivity with smaller and larger FLC lambda aggregates of the two assay systems.

Typically, FLC lambda forms dimers in plasma. However, in gammopathies, FLC lambda may form larger aggregates (large multimers). In urine, only small FLC lambda molecules can be expected (primarily monomers and dimers), because larger aggregates are too big for filtration. When reduced glomerular filtration causes a polyclonal increase of FLC, an almost-parallel increase of FLC lambda molecules can be expected (primarily monomers and dimers), because larger aggregates are too big for filtration. In the case of FLC lambda, however, reacts less strongly with these multimers.

The “over-reactivity” of FReeLITE assays with larger FLC aggregates is acknowledged by The Binding Site on its website, where TBS states, “FLC polymerisation may also lead to over-estimation of antigen concentrations by [FReelite] immunoprecipitation assays”. Two studies are cited, which report an overestimation by 1.5- to 3.5-fold for purified dimers and higher polymers, and a greater than 7-fold overestimation in certain samples when compared with CZE in association with polymers of up to 200 kDa. 

Neither “uncharacterized substances that interfere with immunoassays” nor overestimation caused by FLC polymerization are observed with N Latex FLC kappa and lambda assays—the precise, reliable, and consistent solution for FLC testing in serum, plasma, and urine.

In summary, FLC kappa and lambda assays are performed by N Latex Kappa and Lambda, which provides a precise, reliable, and consistent solution for FLC testing in serum, plasma, and urine.