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Add Consistency to Monoclonal Gammopathy Testing: N Latex FLC kappa and lambda Assays

White Paper

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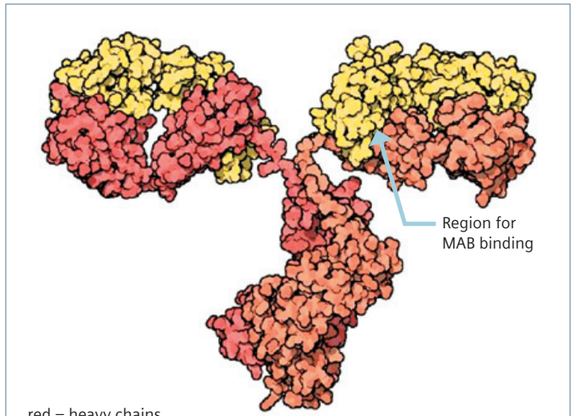
Monoclonal disorders—a wide spectrum of diseases

Monoclonal gammopathies range from asymptomatic, premalignant monoclonal gammopathy of undetermined significance (MGUS) to life-threatening manifestations such as light chain amyloidosis or fast-progressing multiple myeloma requiring highly aggressive therapy.

Until recently, the diagnosis of monoclonal gammopathies was based on the finding of an elevated level of a complete immunoglobulin. However, there are a substantial number of monoclonal gammopathies in which no increased expression of intact immunoglobulin molecules is seen, but rather increased levels of either the free light chain (FLC) kappa or lambda, resulting in an abnormally low or high FLC kappa/lambda ratio.

The majority of light chains are bound in complete immunoglobulins, but small amounts are circulating in a free form, as there is a slight excess of light chain synthesis versus heavy chain production. While there is approximately twice as many kappa produced as lambda, the smaller, monomeric FLC kappa (25 kD) is more efficiently cleared from circulation by glomerular filtration than the larger, dimeric FLC lambda molecule (50 kD).[1] In healthy individuals, the serum concentrations of both light chains are almost equal, resulting in a mean FLC kappa/lambda ratio of about 0.8 results.



IgG molecule

Clinical application of FLC testing

Nowadays, the determination of FLC kappa and lambda is included in several guidelines on multiple myeloma and related disorders. In 2009, the International Myeloma Working Group issued a guideline on the use of FLC testing, recommending the determination of FLC kappa and lambda for screening, determination of prognosis, and monitoring of monoclonal disorders.[2,3] In 2006, FLC testing was incorporated into uniform response criteria for assessment of therapy response.[3,4] The normalization of the FLC ratio defines a new category of stringent, complete response—the best possible outcome of therapy. Furthermore, in 2008 a prognostic staging recommendation was issued, based on serum FLC ratio, albumin, and $ß_2$-microglobulin.[3,5]

Application of FLC testing in clinical routine[2]	
• Screening for monoclonal gammopathies, in combination with serum protein electrophoresis and IFE • Determination of prognosis • Monitoring of hematological disease activity and therapy response	Monoclonal proliferative disorders: • Multiple myeloma • Smoldering myeloma • Plasmacytoma • AL amyloidosis • Monoclonal gammopathy of undetermined significance (MGUS)

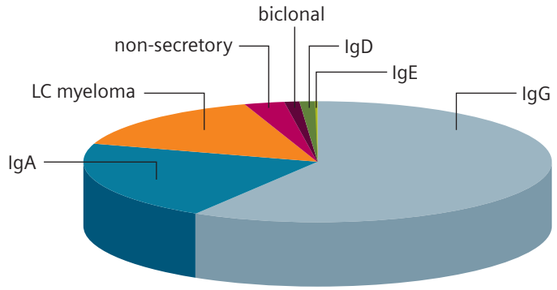
Diagnosis of monoclonal plasma cell dyscrasia

The diagnosis of monoclonal gammopathies requires a panel of tests, as no single test has optimal sensitivity. The tests focus on the cellular analysis of myeloid cells in the patient's bone marrow, and protein analysis in serum, and/or urine. For the serum analysis, the International Myeloma Working Group recommends a panel of serum protein electrophoresis, serum immunofixation electrophoresis (IFE), and serum FLC kappa and lambda assays for screening for pathological monoclonal proliferative disorders. For light chain amyloidosis (AL), an IFE is required in 24-hour urine in addition to all serum tests. If any serum assay indicates a monoclonal gammopathy, IFE and protein electrophoresis in 24-hour urine are required for additional confirmation; the determination of FLC in urine is not considered to be useful by the guideline.[2]

Recommended screening tests[2]
In serum: • FLC kappa and lambda • protein electrophoresis • IFE
In 24-hour urine: • IFE, only if screening for AL
Additional tests if any screen abnormal:
In 24-hour urine • protein electrophoresis and IFE

The most common forms of monoclonal gammopathies are the premalignant, asymptomatic MGUS, which may progress to a smoldering multiple myeloma or a symptomatic myeloma associated with increased serum calcium levels, renal disease, anemia, and/or bone lesions. In about 21% of multiple myeloma, only light chains are expressed, without an increase in any complete immunoglobulin type.

Type distribution in multiple myeloma



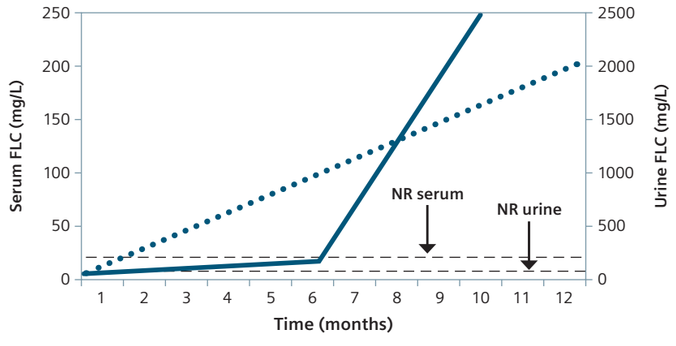
Waldenström's macroglobulinemia is associated with a monoclonal increase in IgM, which is characterized by different clinical symptoms and is less aggressive than multiple myeloma, and therefore considered as a distinct disease.

Monoclonal light chains may form precipitates in the form of amyloids, which cause deposits in several organs. AL amyloidosis is a protein confirmation disorder with deposition of light chain amyloids affecting primarily the heart, kidneys, liver, and the peripheral nervous system.

Excretion of light chains in urine

With advanced disease, FLC concentrations can increase in urine. Historically, urine was used for determination of light chains (Bence Jones protein), which provided better sensitivity with the use of antisera against total light chains (free + bound).

Serum FLC concentrations must increase manyfold before the tubular absorption mechanisms are overwhelmed and FLC concentration starts to increase in urine. With normal renal function, the kidneys are able to reabsorb up to 10–30 g of FLC per day by the proximal tubules.[6] Recent data demonstrated that increased levels of monoclonal FLC, and especially aggregated forms of FLC, may be highly toxic to the kidneys and induce renal impairment. Removal of circulation FLC by hemofiltration may reverse the FLC-induced renal impairment.[7] With progression of the monoclonal disease and increasing impairment of kidney function, the urinary excretion of FLC increases. Several studies have shown that serum FLC determination is more sensitive compared to urine FLC, particularly when monitoring residual disease, eliminating the need for urine analysis in the majority of patients.[1,2,6,8]



Changes in serum (dotted line) and urine (solid line) FLC with increasing tumor mass (NR = normal range)

(from www.specialtylabs.com, multiple myeloma 12/05)

Analytical requirements for FLC assays

As for every other tumor marker, FLC assays have stringent requirements for analytical performance. FLC assays should be:

Specific	**High selectivity of the antibodies used** As only small amounts of light chains circulate in the free form compared to the bound form in immunoglobulins, it is crucial that the antibodies used are highly selective for only the free form of light chains. **High clinical specificity** Specificity (true negative) is very important to prevent misdiagnosis of malignant disorders in non-affected individuals. The FLC kappa/lambda ratio should allow the differentiation of monoclonal from polyclonal increases, as are observed in renal disease or with immune stimulation.
Reliable	**High precision** High reproducibility over the entire measuring range is a prerequisite for reliable monitoring of patients; high precision in the very low concentration range is crucial when considering the FLC ratio for monitoring, because the non-involved light chain can be suppressed to subnormal levels. **Antigen-excess security** The range of serum FLC in disease spans over 4 logs, and very high levels may cause a high-dose hook effect in homogenous immunoassays, providing false-low results if not recognized appropriately.
Sensitive	**High analytical sensitivity** Within the screening panel for monoclonal gammopathies, FLC assays allow the quantification of even subnormally low FLC levels down to <1 mg/L. **High clinical sensitivity** Sensitivity (true positive) is very important to correctly diagnose malignant disorders in affected individuals. In patients with an elevated FLC, FLC testing provides a quantitative, precise tool for monitoring. However, in certain myeloma patients, e.g., in rare forms of non-secretory diseases, but also in some patients with complete immunoglobulin secretion, low or no detectable amount of light chains are excreted, resulting in a normal or only slightly abnormal FLC kappa/lambda ratio.
Consistent	**High lot-to-lot consistency** Patients with multiple myeloma and other monoclonal gammopathies require regular, long-term monitoring that may span over many years. When identifying disease regression or progression based on changes in FLC or FLC kappa/lambda ratio, and adapting or starting therapy accordingly, high lot-to-lot consistency is essential.

Previously available FLC assays are affected by limitations such as considerable lot-to-lot variability, antigen-excess risk, and gross overestimation in certain samples with FLC polymerization.[1,9–13] These limitations now can be overcome by high-performance assays based on unique monoclonal antibodies.

N Latex FLC kappa and lambda: Innovative FLC assays based on monoclonal antibodies

Specific → Reliable → Sensitive → Consistent

For any laboratory assays with high impact on patient management, and especially for tumor markers, reliability is the highest prerequisite. To fulfill reliability requirements, assays must be specific, robust, sensitive, and consistent in results.

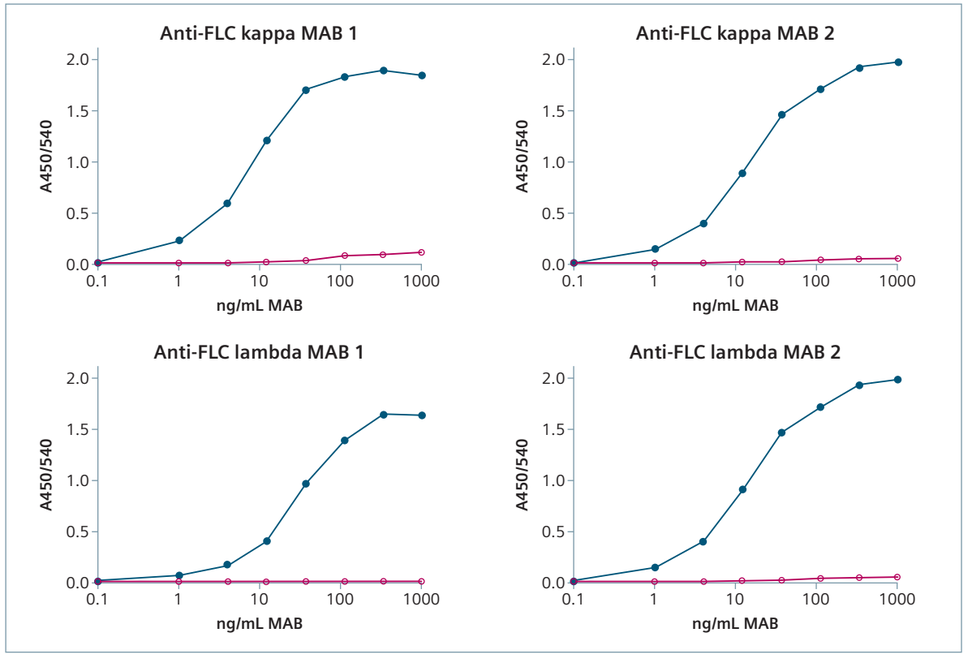
Specific → Reliable → Sensitive → Consistent

Specificity of the monoclonal antibodies used

From a panel of more than 150 monoclonal antibodies raised against FLC kappa and FLC lambda, two highly selective antibodies against the free form of light chains and not bound light chains were selected for each assay.

The graphs below show the reactivity of the kappa antibodies (top row) with FLC kappa (closed symbol) or IgG kappa (open symbol), and the reactivity of the lambda antibodies (bottom row) with FLC lambda (closed symbol) or IgG lambda (open symbol).

The very low reactivity with complete immunoglobulin demonstrates the high specificity of the monoclonal antibodies chosen.



(from te Velthuis H, Clin Chem Lab Med. 2011)[14]

Clinical specificity

Clinical specificity is defined as the proportion of true negatives for FLC kappa/lambda ratio in patients/individuals not affected by monoclonal disease. For determination of clinical specificity, a panel of 366 patients without signs of monoclonal disorders was tested. This panel also included patients with kidney disease and stimulation of the immune system, such as autoimmune disorders or chronic infections.

	Clinical Specificity	
	N Latex FLC ratio	FLC ratio competitor method
Patients submitted for screening without renal disease or polyclonal stimulation	99.4% (164/165)	97.6% (161/165)
Patients with renal disease	98.6% (143/145)	96.6% (140/145)
Patients with polyclonal stimulation	98.2% (55/56)	98.2% (55/56)
All patients screened (N=366)	98.9% (362/366)	97.3% (356/366)

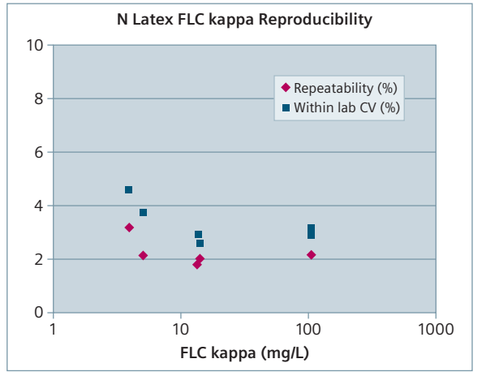
Furthermore, the high assay specificity of N Latex FLC kappa and lambda is further supported by the narrower reference range, especially for the FLC kappa/lambda ratio. This reference was determined by testing 369 healthy donors and is comparable to the reference range of the Freelite* assays:

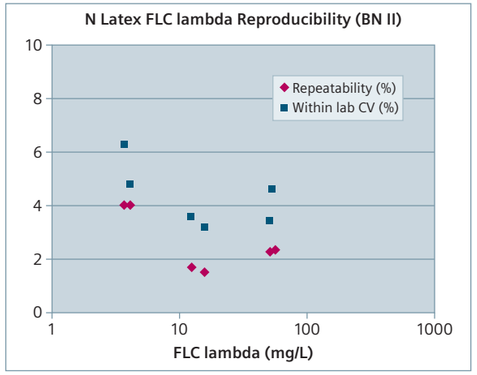
	Reference Range
N Latex FLC kappa (2.5th–97.5th range)	6.7–22.4 mg/L
N Latex FLC lambda (2.5th–97.5th range)	8.3–27.0 mg/L
N Latex FLC ratio (min–max range)	0.31–1.56

*Freelite is a trademark owned by The Binding Site Group Ltd.

High precision

For reliable monitoring of patients with monoclonal disease, precision of measurement is important not only for the elevated, affected light chain, but also for the non-involved light chain, which can be suppressed to subnormal levels. High impression in the very low range will directly affect the reproducibility of the FLC kappa/lambda ratio, which might induce clinical actions. By testing serum and EDTA plasma-pool samples twice per day in double determination over 20 days, total CVs in the range of 2–6% were measured.[14]



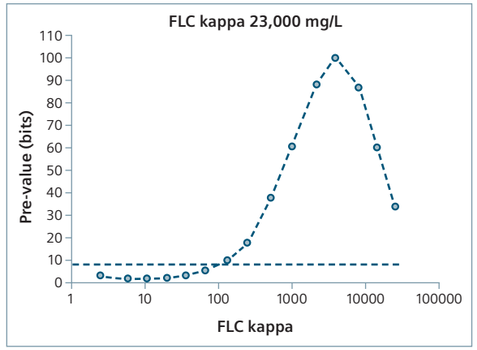


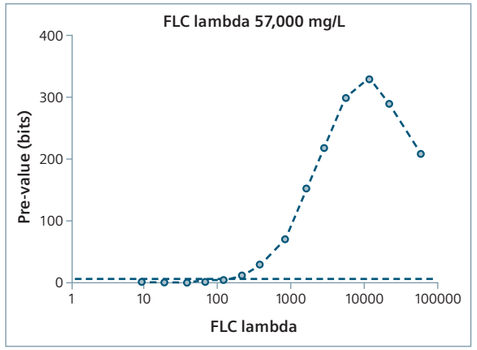
High antigen-excess security

Antigen-excess security is crucial because FLC concentrations can vary up to 10,000-fold. For detection of antigen excess, built-in pre-reaction protocols are used. In the pre-reaction phase, a small amount of the pre-diluted sample is incubated with the reagent for 2 min, followed by a check of the nephelometric signal. If the signal is below a predefined threshold established during calibration, the remaining amount of sample is added, and the measurement is completed. If the signal is higher than the threshold at the end of the pre-reaction phase, an antigen-excess situation is indicated.

In this case, the analyzer does not report a result and proceeds with the next-higher sample dilution automatically.

In the example below, two samples with very high monoclonal FLC (kappa 23,000 mg/L; lambda 57,000 mg/L) were serially diluted. These dilutions and the native sample were measured in the initial sample dilution (kappa 1:100; lambda 1:20). The predefined threshold for the pre-reaction is indicated by the horizontal dotted line. Even with very high FLC concentrations, the pre-reaction safely detected the antigen excess and triggered a further dilution of the sample by the analyzer.[14]





pre-reaction cutoff = antigen excess detection threshold

Analytical sensitivity

There is no reference method available for the measurement of FLC. Often the immunofixation is considered as the reference method, but the limit of quantitation of the nephelometric assays is much lower than the IFE. Samples positive by IF could also be identified by other methods.

In a comparison study, 60 IFE kappa-positive and 59 IFE lambda-positive samples were tested. All 60 IFE kappa-positive samples and 58 of 59 IFE lambda-positive samples showed abnormally high N Latex FLC kappa and lambda results, respectively, while the Freelite assays missed 1 sample in the kappa assay and 3 samples in the lambda assay.

Sensitivity versus IFE

IFE kappa positive	N Latex FLC kappa	Freelite kappa
N=60	60 (100%)	59 (98.3%)
IFE lambda positive	**N Latex FLC lambda**	**Freelite lambda**
N=59	58 (98.3%)	56 (94.4%)

The limit of quantitation (LoQ) for N Latex FLC was determined to be:

- 0.174 mg/L for N Latex FLC kappa
- 0.47 mg/L for N Latex FLC lambda

Clinical sensitivity

Clinical sensitivity is defined as the proportion of true positives for FLC kappa/lambda ratio in patients with monoclonal gammopathy. For determination of clinical sensitivity, a panel of patients with a pre-established diagnosis of monoclonal disease was tested, including patients with multiple myeloma, Waldenström's macroglobulinemia, AL amyloidosis, and MGUS.

Sensitivity in this group of 163 patients with a pre-established diagnosis of monoclonal gammopathy was 61.3% for N Latex FLC and 60.1% for the competitor method.[15]

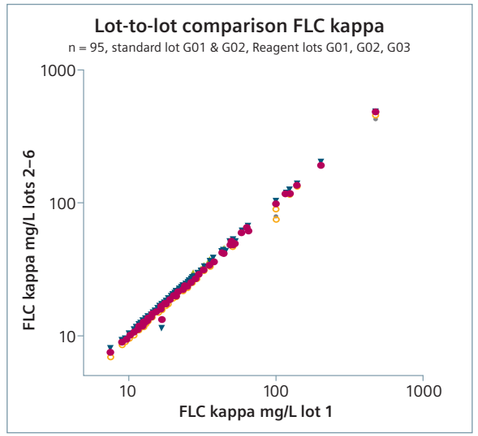
The relatively low sensitivity of both assays is explained by the inclusion of treated patients (therapy target is to bring FLC ratio back into the normal range) and MGUS patients (only 30–40% of MGUS patients show an abnormal FLC ratio), as well as the fact that not all patients excrete increased concentrations of FLC next to an intact immunoglobulin.

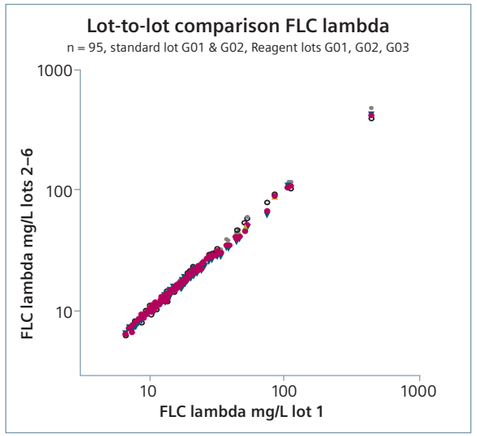
	Sensitivity	
	N Latex FLC ratio	FLC ratio competitor method
All patients with any form of monoclonal disorder (N=163)	61.3%	60.1%

Specific | Reliable | Sensitive | **Consistent**

High lot-to-lot consistency

Consistent results when different reagent batches are used during follow-up of patients over the long term are crucial for evaluation of disease progression and therapy response. The graphs below show the results obtained by combining three lots of reagent and two lots of calibrator in a total of six variations. Any differences were below 7.5%, with a correlation coefficient greater than 0.99, demonstrating excellent lot-to-lot consistency of results for N Latex FLC kappa and lambda.[14]





Method comparison of monoclonal versus polyclonal serum FLC determination: What can be expected; what should be considered?

Each monoclonal component is unique. This means that patients exhibit slightly different, specific immunoglobulins/light chains, and that the target antigen for FLC assays (i.e., the monoclonal light chain) differs from patient to patient. In addition to the variations in the light chain molecules, post-translational variations such as aggregation or complexation with other plasma proteins may occur. As a result of this diversity, the correlations between different assay systems are wider than for other, more consistent analytes.

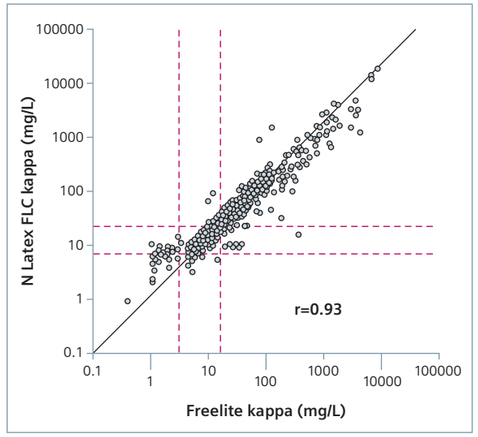
The difference in outcome between FLC assays is patient-specific and appeared to be constant during follow-up. Individual light chain clones can result in a 5- to 10-times higher reactivity in one assay when compared to another assay format. The difference in detection between methods may be explained by differences in epitope recognition of monoclonal antibodies compared to the polyclonal antibodies in the Freelite assays or the physical properties of the light chain molecules. Monoclonal light chains can aggregate or, conversely, be cleaved by activated enzymes, which may result in over- or underestimation of the analytes.

Correlation analysis

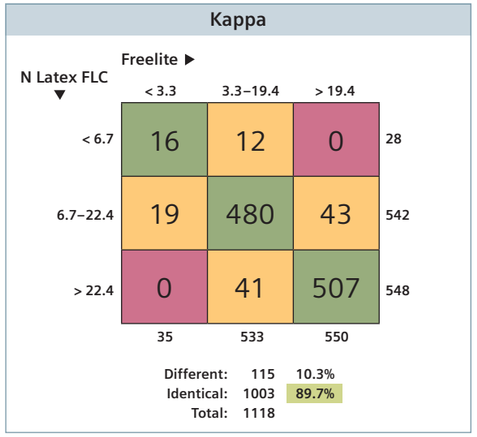
The concentration range of FLC kappa and lambda spans several potencies; levels can be below 1 mg/L or higher than 10 g/L. To illustrate the correlation of the entire range, a logarithmic scale is most appropriate.

In addition to correlation analysis, the qualitative comparison of results is of interest to determine the agreement rate at which both assay systems provide the same classification (abnormal low, normal, abnormal high). To quantify the rate of agreement, the Cohen's kappa coefficient is calculated, which ranges from -1 to +1. A kappa coefficient of greater than 0.6 indicates "substantial agreement" and greater than 0.8 "almost perfect agreement."

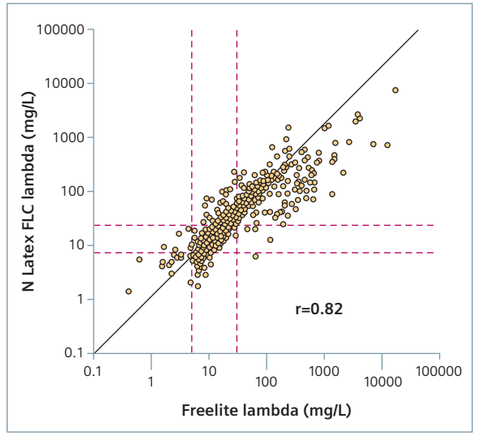
The pooled analysis of method-comparison data obtained at three different trial sites is shown below:



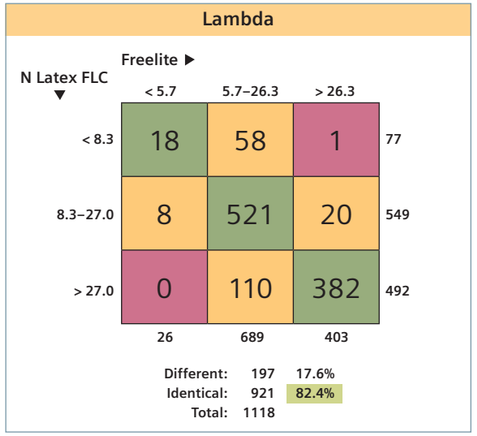
FLC kappa method comparison



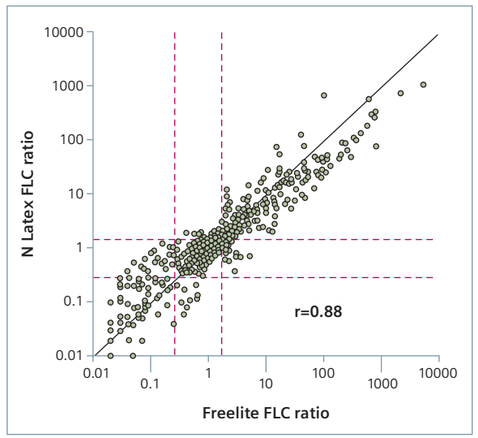
Cohen's kappa coefficient: 0.80



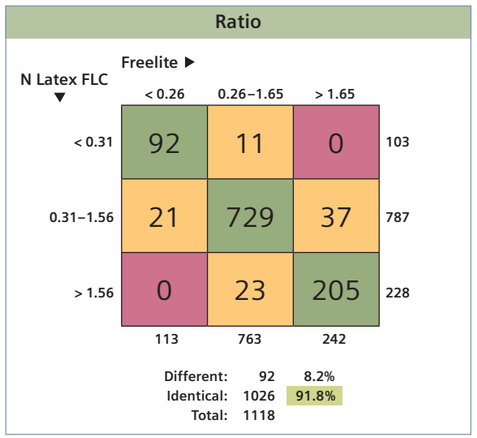
FLC lambda method comparison



Cohen's kappa coefficient: 0.67



FLC ratio method comparison



Cohen's kappa coefficient: 0.82

In general, a higher correlation between the two assay systems is observed for FLC kappa compared to FLC lambda, resulting in a closer correlation of higher FLC kappa/lambda ratios (i.e., kappa monoclonals) and a wider scattering for lower FLC kappa/lambda ratios (lambda monoclonals).

In contrast to FLC kappa (which typically circulates as a monomer), FLC lambda typically form dimers in the circulation, but can also form higher-molecular aggregates (this appears to be patient-specific). This heterogeneity of FLC lambda is associated with a more-variable reactivity in different test systems.

Furthermore, the correlation of FLC kappa/lambda ratio can be severely affected by the Freelite values measured for the non-involved FLC, which can be suppressed to subnormal levels. In fact, in the lower portion of the Freelite reference curves, no samples are measured! This phenomenon is well-documented by TBS on www.wikilite.com (see Figure 4.10).

In this graph of 4,000 abnormal samples, a discontinuous distribution is displayed, indicating that, in the lower end of the reference curve, no sample results are obtained. The phenomenon is batch-dependent and may be observed for both kappa and lambda. In general, samples with FLC concentrations in the "non-detectable" or "gap" region will give results at lower dilutions. The poor performance of the Freelite assays when samples are measured at different dilutions is well-documented.[13] The Freelite assays may result in concentrations 5- to 10-fold lower than the N Latex FLC assays at the low end, which has dramatic effects on the FLC kappa/lambda ratio.

Measuring gaps of Freelite assays

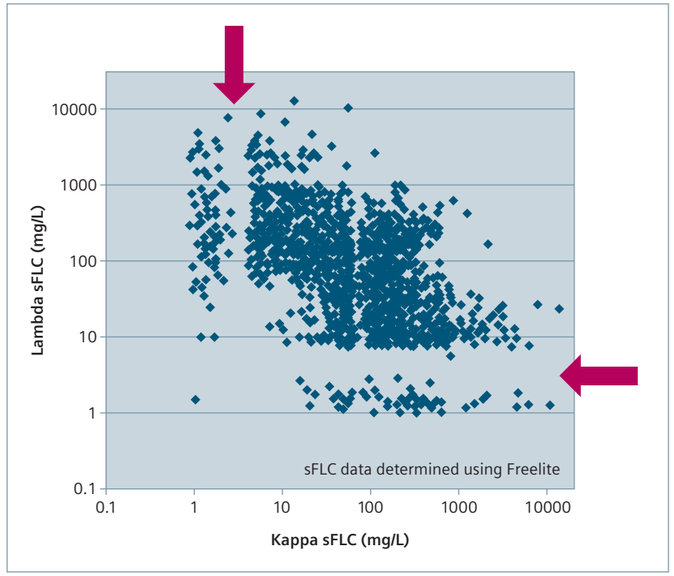
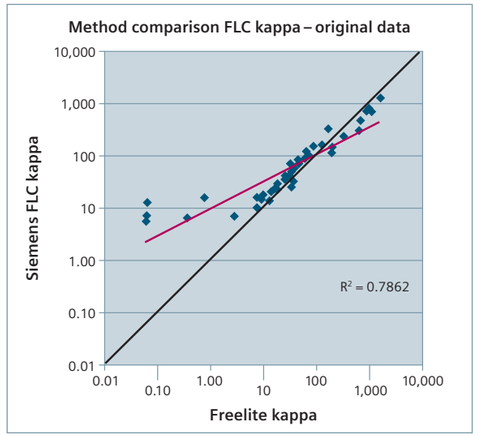
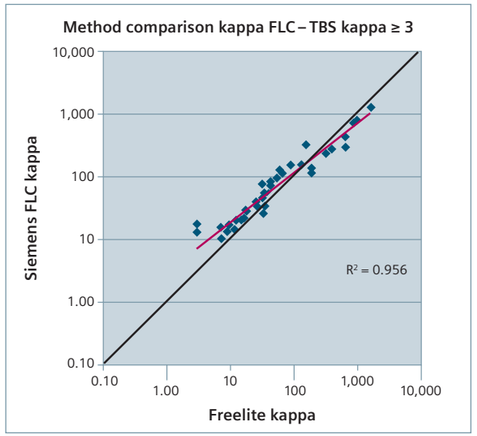


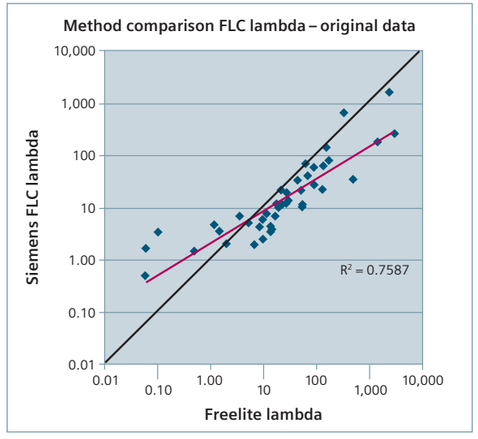
Figure 4.10. Kappa/lambda log plot of approximately 4,000 abnormal sFLC samples showing "gaps" at the lower and upper ends of the instrument's assay range

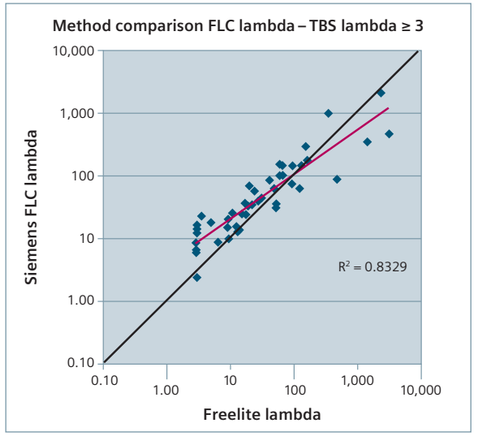
Influence of 1:5 measurements on method comparison with the Freelite assays



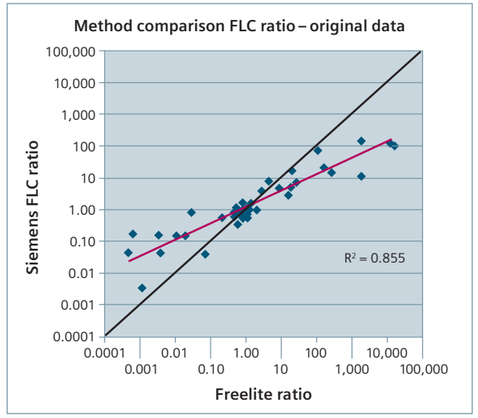


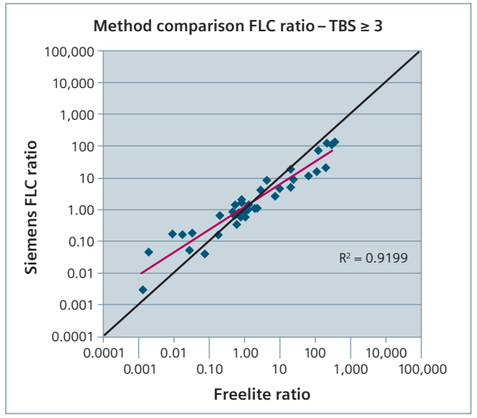
Method comparison: FLC kappa





Method comparison: FLC lambda





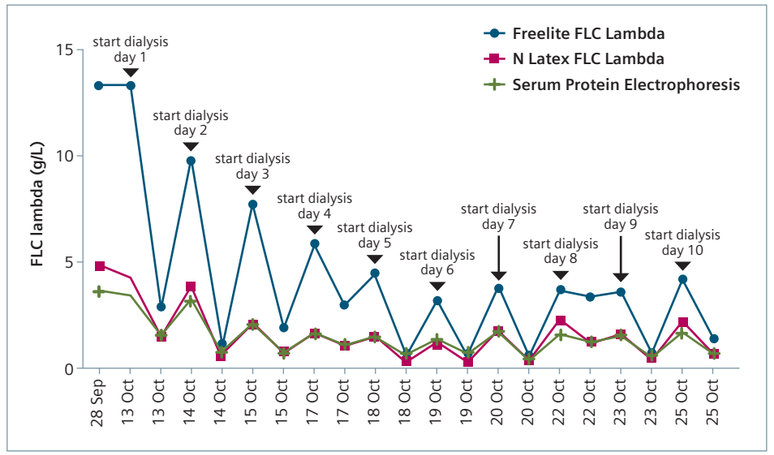
Method comparison: FLC kappa/lambda ratio

Left-hand graphs: Original data, including results from 1:5 and undiluted samples with the Freelite assays
Right-hand graphs: All results <3 mg/L reset to 3 mg/L

The lack of a pre-reaction protocol for the Freelite assays regularly causes false-normal results in pathological samples with high FLC concentrations. On the other hand, over-estimation in Freelite assays is a well-described issue,[9-13,16] especially in samples with higher aggregates of FLC, or when complexed with other proteins.

In method-comparison studies of N Latex FLC and Freelite, discrepancies between the methods can be found for certain samples with very high serum concentrations of FLCs. The largest differences were observed for FLC lambda, with results for TBS of 10 g/L and higher. In most cases, results for N Latex FLC lambda were much lower, and repeated samples from the same patient display the same effect.

The case report illustrated below (from Gentzer W, poster at AACC 2011) shows a patient with light chain lambda multiple myeloma undergoing hemodialysis with a Campro™ filter for removal of FLC lambda from serum. Serum measurements before and after each dialysis session were performed by N Latex FLC lambda, Freelite lambda, and quantitative serum protein electrophoresis. The three methods concordantly indicate the efficient removal of FLCs by the applied dialysis, followed by a redistribution of FLC lambda from the extra vascular fluids as observed at the start of the next dialysis session. The Freelite levels measured were much higher than the results obtained by the Siemens method; the N Latex FLC lambda results were in good agreement with the quantification from serum electrophoresis.



Patient with light chain lambda myeloma on dialysis with Campro filter

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Ordering Information

Catalog No.	Description	Contents
OPJA03	N Latex FLC kappa	3 x 37 tests
OPJB03	N Latex FLC lambda	3 x 37 tests
OPJC03	N FLC Supplement Reagent	3 x 0.5 mL Supp A, 3 x 2 mL Supp B
OPJD03	N FLC Standard SL	3 x 1 mL
OPJE03	N FLC Control SL1	3 x 1 mL
OPJF03	N FLC Control SL2	3 x 1 mL