DEVELOPMENT OF A NEW sFLC ELISA ASSAY FOR THE QUANTIFICATION OF SERUM FREE LIGHT CHAINS

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INTRODUCTION

In the last decade, the serum-free light-chain (sFLC) assay has been shown to be important in the diagnosis and management of plasma cell dyscrasias. Commercial nephelometric and turbidimetric assays are available to detect sFLC. Since its availability in 2001, there have been several publications discussing the clinical utility but also the numerous analytical limitations of these technologies for sFLC testing. We describe herein the validation of our new sFLC assays based on ELISA technology to overcome most of these very well known limitations.

METHODS

We industrialized a home-made ELISA assay (sebia FLC, Sebia, France) developed at the University of Nijmegen (The Netherlands) for both free kappa (K) and free lambda (L) quantification. This assay is a sandwich ELISA and uses polyclonal anti-FLC capture antibodies. This test was compared to the Freelite assay (The Binding Site, UK) on the BNII instrument (Siemens, Germany) on 156 samples. Sensitivity, linearity, measurement range, reproducibility, interference assessment and coherence to Serum Protein Electrophoresis (SPE) peak quantification were performed during validation. SPE was performed on CAPILLARYS 2 FLEX PIERCING (PROTEIN(E) 6 kit, Sebia, France). All experiments above were carried out in parallel on a manual ELISA and on a fully walkaway ELISA processor (AP22 Elite, das, Italy) using a specific validated program.

RESULTS

- All the study was carried out on both the manual technique and on AP22 Elite.
- The normal reference ranges of the sebia FLC test were established using healthy donors **(Figure 1)**.
- We show good correlation for sFLC K, L and K/L ratio between the 2 tested techniques for the tested patients (n=156) **(Figure 2)**.
- The sebia FLC assay has a sensitivity of 0.5 mg/L for both K and L. We show that the assay has a very good reproducibility with CV<15% (intra-assay, inter-assay and lot-to-lot).
- At the standard dilution, sebia FLC has a measurement range 5 times broader that the average measurement range of the nephelometric/turbidimetric techniques on the market and this allows obtaining 3 to 4 times less out-of-range samples in a batch of analysis leading to significantly decreased reagents' consumption **(Figure 3).**
- The sebia FLC assay shows a very good linearity for both Kappa and Lambda measurements **(Figure 4)**.
- We also show that sebia FLC is not prone to any of the tested interferences (Hemoglobin, Bilirubin, Triglycerides) (data not shown).
- This new test brings more coherent results with electrophoretic methods **(Figure 5)**.

Sebia FLC Kappa (mg/L)	Sebia FLC Lambda (mg/L)	Ratio
5.2 - 15.3	8.2 - 18.1	0.37 - 1.4

Figure 1. Normal reference range for the Sebia FLC

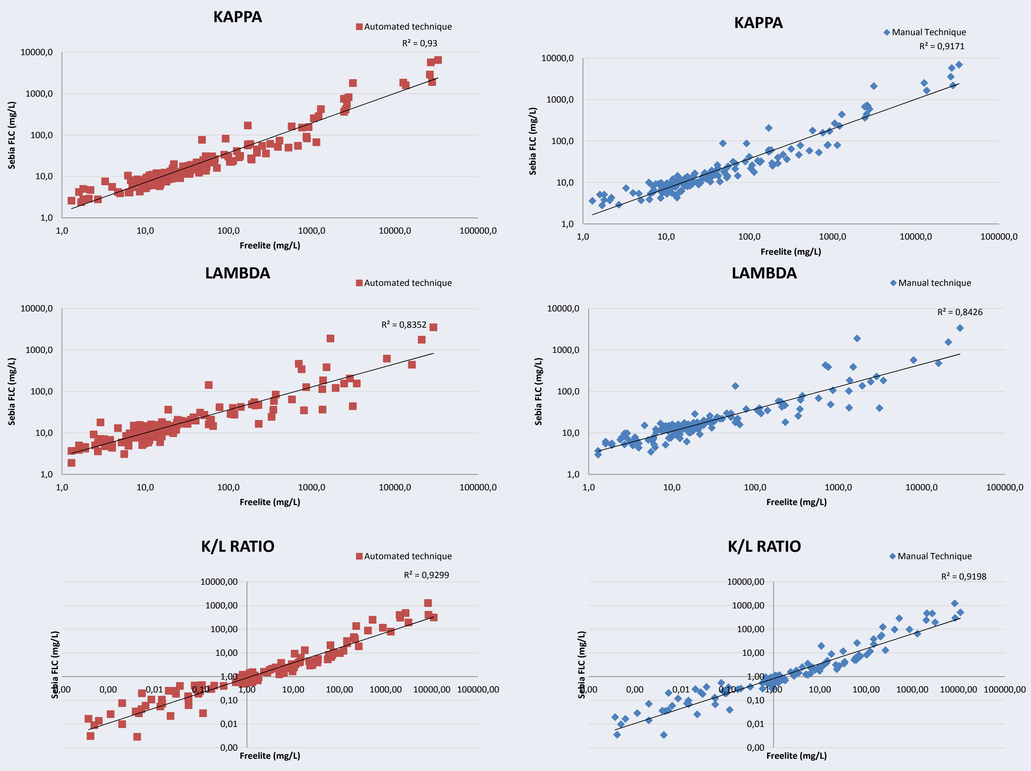


Figure 2. Sebia FLC correlates very well with Freelite on both manual and automated techniques (n=156)

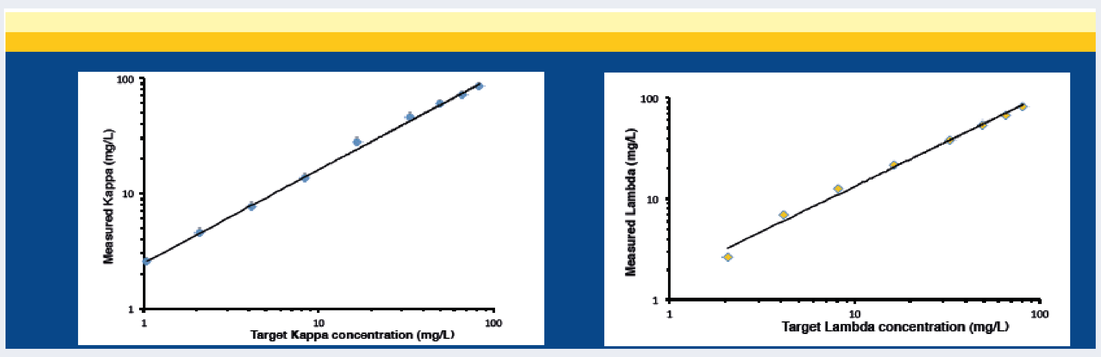


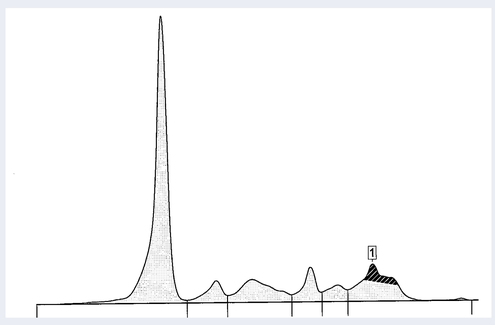
Figure 4. Linearity of the Sebia FLC assay. The linearity was tested on 2 serum samples serially diluted in the dilution buffer.

% of retest Kappa		% of retest Lambda	
Sebia	Freelite	Sebia	Freelite
9%	33%	8%	36%
Results consolidation done on 5 Laboratories ⇒ N = 1244 samples			

Figure 3. Retest rate of the Sebia FLC and Freelite

Dilution	Kappa free mg/L
1:10	85,67 mg/L
1:100	1300 mg/L
1:1000	>16570 mg/L
1:10000	**36400 mg/L**

Dilution	Kappa free mg/L
1:1000	>98 mg/L
1:10000	> 980 mg/L
1:100000	**4817 mg/L**



SPE peak	Sebia FLC	Freelite
3200 mg/L	4817 mg/L	36400 mg/L

Figure 5. The Sebia FLC results are coherent with those obtained with the FLC peak quantification on SPE.

CONCLUSION

We describe here a new generation of fully automated FLC assay based on ELISA. This assay has been validated in comparison to historical methods. Moreover, it brings more coherence with electrophoretic techniques and generates less retest for out-of-range samples.

sebia BEYOND SEPARATION FLC Line