

Eliminating urine testing during initial investigations for monoclonal gammopathies

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Freelite is a highly sensitive nephelometric or turbidimetric assay for quantification of serum free light chains (FLCs). The assay represents a major breakthrough in the detection and monitoring of Multiple Myeloma (MM) and other B cell dyscrasia. Initial laboratory screening investigations for these conditions have traditionally required assessment of serum and urine [1].

Previous laboratory protocols have been based on the detection of whole immunoglobulin monoclonal protein (M-protein) in serum by serum protein electrophoresis (SPE). Subsequent immunofixation electrophoresis (IFE) confirms monoclonality and class of the M-protein. However, serum FLC assays are significantly more sensitive than electrophoretic methods and can quantify FLCs considerably below the detection limit of these other methods. As a consequence diagnosis of some patients will always be missed if electrophoretic techniques alone are used. Figure 1 shows the number of samples that would be reported as negative using SPE and IFE, but are detected with serum FLC assays [2].

	Kappa	Lambda
SPE	500-2000 mg/L	500-2000 mg/L
IFE	150-500 mg/L	150-500 mg/L
FLC	0.3 mg/L	0.5 mg/L

Table 1. Sensitivity comparison of free light chains with SPE and IFE.

Due to the low sensitivity of serum based electrophoresis methods for free light chains [Table 1], 24 hour urine samples are also recommended for analysis of Bence Jones Protein (BJP) by electrophoresis. Incorporation of serum FLCs into a laboratory screening protocol for monoclonal gammopathies and other B cell dyscrasia could eliminate the need for urine collections; only a single serum sample collection would be required. There are many positive reasons for using only serum as the medium analysed, including physiological, practical and analytical reasons.

Physiological reasons

Free light chains are small proteins that are filtered by the glomeruli of the kidney nephrons. FLCs are subsequently reabsorbed and broken down via the proximal tubules and as a result have a short half life of 2-6 hours (compared to a 5-21 day half-life of intact immunoglobulins). The kidney has a reabsorption capacity of 10-30g/day for FLCs. Healthy individuals typically produce 0.5g/day of FLCs and consequently very little passes into the urine. Urinary FLCs only become detectable after the serum level is sufficiently elevated to exceed the kidney's capability to break down and reabsorb them.

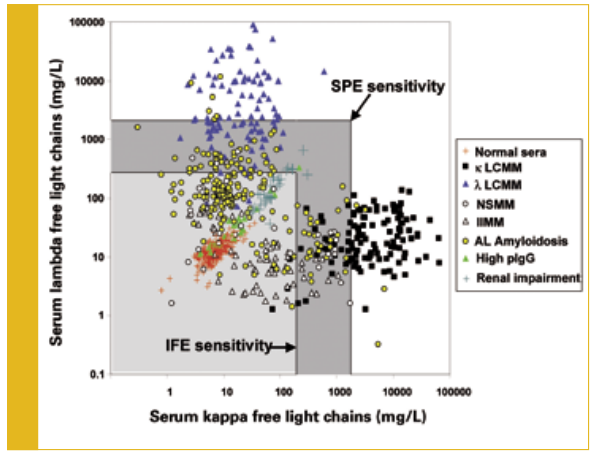


Figure 1. κ/λ graph of serum FLCs showing samples that would have been mis-identified as negative using SPE and IFE. (High pIgG: polyclonal hypergammaglobulinaemia).

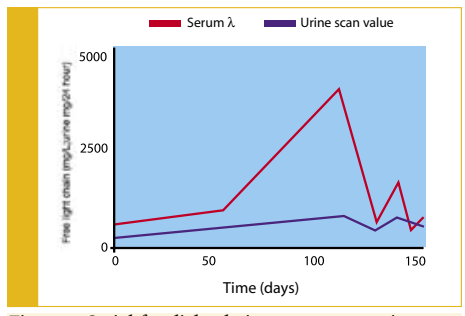


Figure 2. Serial free light chain measurements in serum samples from a Bence Jones lambda myeloma patient undergoing treatment, compared to urine densitometric scan levels (4).

In some patients with monoclonal gammopathies, the amount of FLCs produced remains below the reabsorption capacity of the kidneys, so it is possible for a myeloma patient to have an abnormal serum FLC ratio but no detectable urinary BJP. Urine tests are therefore potentially unreliable when the tumour mass is small, or free light chain synthesis is low [3, Figure 2].

Practical reasons

The most comprehensive screen for B cell disorders includes SPE, IFE and serum FLC assays plus urine IFE and PE [5]. There are several practical

issues with urine collections, such as poor patient compliance, storage of large volumes of urine and difficulty in obtaining matched serum and urine samples. In one hospital, despite publicity from the laboratory, concurrent urine and serum samples were received from fewer than 40% of patients [6]. Hill *et al.* investigated the use of SPE and serum FLCs only, as part of the initial screening protocol for possible B cell disorders. It was shown that no substantial pathologies would have been missed by replacing assays for urine BJP with those for serum FLCs. As a result of this study, a urine sample is no longer required as part of the initial screen at the centre involved. Katzmann *et al.* concluded

that a diagnostic algorithm using only serum samples showed normal serum FLC values in just two (0.5%) of 428 monoclonal gammopathies cases with urinary monoclonal proteins [5]. These two cases required no medical intervention. This paper also recommended the discontinuation of urine testing from the screening algorithm.

Table 2 shows the detection rate for all Multiple Myelomas, for AL amyloidosis, Light Chain Multiple Myelomas and Nonsecretory Multiple Myelomas using different combinations of screening tests [3,7,8,9]. It can be seen that the optimal detection rate can be achieved by using simply SPE and Freelite. The two studies discussed were conducted in the UK and USA, and involved 923 and 428 patients respectively. The authors mentioned several benefits of using serum Freelite assays over urine tests. Katzmann *et al.* discussed the reduction of laboratory costs, the fact that patients were spared the inconvenience of collecting urine samples, and the provision of prognostic information in patients with Monoclonal Gammopathy of Undetermined Significance (MGUS) [5]. Hill *et al.* mentioned both an improved quality of diagnostic service and the fact that substantial clinical information was gained by addition of serum FLC assays to SPE. A reduction in turnaround

Protocols	% of Paraproteins detected			
	*Myeloma	AL	LCMM	NSMM
SPE/CZE alone	90	50	45	0
SPE/CZE, serum IFE	95	70	75	0
SPE/CZE and UPE	95	75	90	0
SPE/CZE, UPE, serum and urine IFE	97	90	95	0
FLC alone	96	98	100	82
SPE/CZE and FLC	99	98	100	82
SPE/CZE, FLC and serum IFE	99	98	100	82

Table 2. Accuracy of different diagnostic protocols. *Myeloma is inclusive of samples from patients identified with Intact Immunoglobulin Multiple Myeloma, Light Chain Multiple Myeloma (LCMM) and Nonsecretory Multiple Myeloma (NSMM). UPE: urine protein electrophoresis, AL: AL amyloidosis.

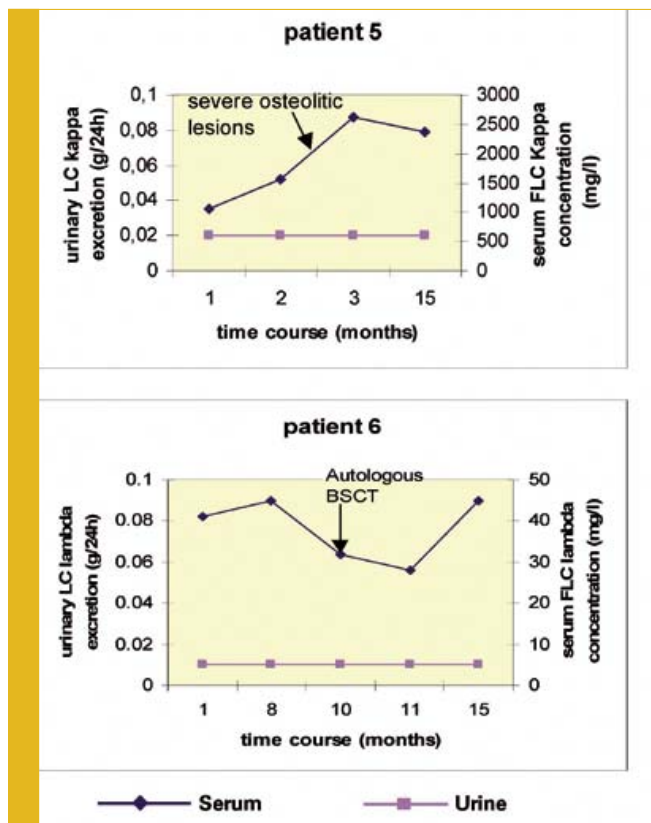


Figure 3. Alyanakian *et al.* concluded “Immunonephelometric measurement of serum free light chains are a reliable method for follow up of patients with light chain secreting monoclonal gammopathies.” Also that for cases featuring hardly measurable amounts of light chain in the urine “...the serum free light chain assay proved sensitive enough for correlation with clinical events.”

times was also noted as FLC results were mostly available the same day compared to approximately one week for urine BJP plus urine IFE [6].

Analytical reasons

There are several analytical issues surrounding urine analysis. Urine samples for IFE can have very high protein concentrations, particularly in the case of Multiple Myeloma patients. This can lead to a number of analytical problems [10] such as:

- Increased background noise
- Appearance of light chain ladders
- Loss of FLCs during urine concentration
- Prozone effects due to high concentrations of FLCs

Such effects can make interpretation of urine IFE difficult, and subsequent dilution steps may be required [11]. Furthermore urine IFE analysis is only qualitative, unlike measurement of serum FLCs which is quantitative. Interpretation of results from IFE can therefore be subjective.

Nowrousian *et al.* showed that out of 378 paired urine and serum samples from 82 patients, 54% of abnormal serum samples from all patients and 48% from patients with Bence Jones proteinuria at presentation were associated with no detectable BJP in the urine. This was apparent at diagnosis as well as during the course of the disease [11]. Figure 3 illustrates two Light Chain Multiple Myeloma patients with urine FLC concentrations that were too low for detection. However serum FLCs were quantifiable throughout [12].

Laboratory investigation with Freelite

In all recent studies the addition of serum FLC assays to SPE as a first line test increased the

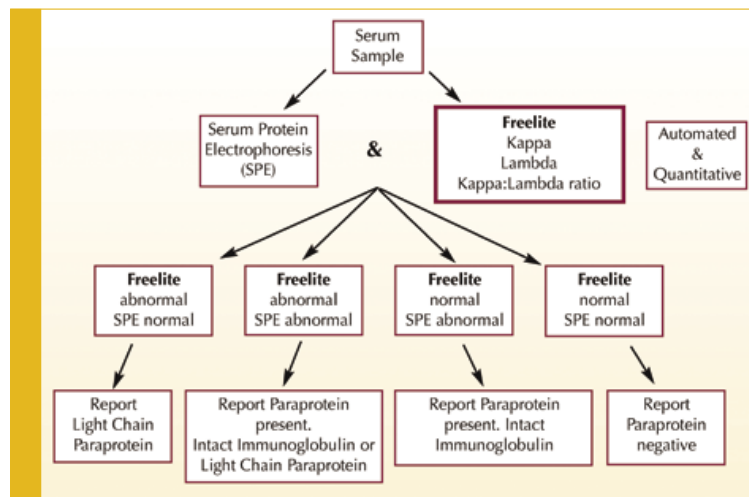


Figure 4. A suggested screening protocol for B cell dyscrasia with the incorporation of serum FLC assays and the exclusion of urine sample tests.

detection of B cell dyscrasia. Bakshi *et al.* also demonstrated the benefit of adding serum FLC assays to Capillary Zone Electrophoresis (CZE) [13]. A prospective analysis of 312 serum samples in a veteran population showed that the adoption of the FLCs plus SPE protocol resulted in the additional detection of 15 cases of Multiple Myeloma, one case of lymphoma and

one patient with bladder transitional cell carcinoma [14]. Figure 4 illustrates a suggested screening protocol for B cell dyscrasia with the incorporation of serum FLC assays and the exclusion of urine sample testing.

Conclusion

There is strong evidence to suggest that urine testing is no longer required during screening for monoclonal gammopathies and other B cell dyscrasia, when replaced by serum free light chain assays. Elimination of urine testing in screening and inclusion of the free light chain assay provides quantitative, precise results from a single serum sample and removes the analytical, practical and physiological problems surrounding urine testing.

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