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# Monoclonal and Polyclonal Immunoglobulin Free Light Chains (FLC) detection in urine

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## *Intention of study*

The aim of this study was to determine whether immunoglobulin FLC nephelometric assay can be used to improve the detection of monoclonal FLC.

## *Samples and Methods*

Urine specimens from 300 patients were studied. Immunoglobulin free light chains (FLC) excretion by:

- patients with monoclonal gammopathies and
  - patients with proteinuria
- has been analysed in concentrated samples by good quality electrophoretic methods – agarose gel high resolution electrophoresis (HRE) and immunofixation electrophoresis (IFE).

In addition, we used a Nephelometric assay (IN-FLC) (Kit code: K.BNA.FRK.FRL – New Scientific Company, I - Cormano (MI); Nephelometry BNII – Dade Behring) in the unconcentrated urines for the quantitative estimation of FLC.

## *Results*

### Overall results by IFE

We were able to detect by IFE

- 147 monoclonal FLC (BJP) in 135 samples (12 samples contain both types Kappa and Lambda).
- Polyclonal FLC showing the ladder pattern were found in 166 urines.
- Both monoclonal and polyclonal immunoglobulin FLC were observed in 86/135 urines (64% of BJP cases).
- Intact immunoglobulins (Ig) were detected associated with the presence of BJP in 79/135 cases (58,5%) distributed as follows: polyclonal Igs in 33 cases, monoclonal Igs in 28 cases, and both mono- and polyclonal Igs in 18 cases.

### Analytical performance of Nephelometric method for FLC

Analytical performance of the nephelometric method for estimation of FLCs in urine was good:

- a. sample: unconcentrated urine, bar code identification, etc.
- b. limit detection close to 2.5 mg/L,
- c. no antigen excess (tested up to 50.000 mg/L)
- d. no cross reaction with intact Igs,
- e. analytical recovery near to 100% for both polyclonal and monoclonal FLCs,
- f. good precision
- g. preliminary data suggest parallelism in dose-response curves in the analytical range (from 5.0 to 80 mg/L) for both monoclonal and polyclonal FLCs.

## Comparison between IFE results and FLC ImmunoNephelometric results

Despite the good analytical performance characteristics of the nephelometric method for the estimation of immunoglobulin FLCs the method showed limitation:

### a. FLC-kappa (Table 1)

No detectable Kappa FLCs were found in 68/300 (22,6%) urines, being 10/68 (14,7%) samples positives for BJK and 13/68 (19,1 %) for ladder by IFE. The rate of false negatives for monoclonal and polyclonal FLCs was 23/68 (33,8%).

All samples with an IFE POSITIVE result for Monoclonal FLCs Kappa, were ***concentrated x 400*** fold, according to their low urine protein concentration.

We were able to detect FLCs in the concentrated samples by nephelometry.

### b. FLC-lambda (Table 2)

No detectable Lambda FLCs were found in 163/300 (53,6%) urines, being 26/161 (16,1%) samples positives for ladder and none sample positive for BJL by IFE. Thus the rate of false negatives was 16,1%.

The ***concentration factor*** varied ***from 50 to 400 x*** in function of the individual urinary protein content. Most positive cases have low urinary protein concentration.

## Monoclonal and Polyclonal FLC

The Nephelometric FLC method demonstrated some insensitivity to differentiate monoclonal from polyclonal FLCs in a wide range of values (2.5 mg/L to 100 mg/L) (see later)

### Polyclonal FLCs (Fig. 1, 3, 5, 6)

- a. are a common finding in proteinuria
- b. they are markers for tubular involvement
- c. show considerable variation in concentration (covering from < 2.5 mg/L up to 100 mg/L)
- d. kappa ladder pattern was present in 60% of the cases
- e. lambda ladder pattern was found in 24% of the cases
- f. the association between monoclonal and polyclonal FLCs is relatively frequent (60% of BJP cases)

### Monoclonal FLCs (Fig. 2, 4, 5, 6)

- a. similarly to polyclonal FLCs, show a wide concentration distribution
- b. in 80-90% of cases the concentration of monoclonal FLC was < 100 mg/L
- c. there is an overlap with polyclonal FLCs up to > 100 mg/L
- d. a concentration of free kappa greater than 250 mg/L strongly suggest monoclonality

FLC-κ/FLC-λ ratio

The use of the quotient FLC-κ/FLC-λ improves slightly the detection of monoclonality for FLCs:

- a. Values of FLC-κ/FLC-λ <1.0 indicates lambda FLCs monoclonality
- b. Values of FLC-κ/FLC-λ >4.0 suggest kappa FLCs monoclonality

However, the sensitivity for the detection of monoclonality was relatively low (Fig. 7):

- a. for a cut-off value of FLC-κ/FLC-λ >4.0, kappa monoclonality was detected only in 46,5% of the cases of BJK, and
- b. for a cut-off value of FLC-κ/FLC-λ <1.0, lambda monoclonality was detected in 60,0 % of the cases of BJL

The insert of the kit clearly states that the purpose of the method isn't the differentiation between monoclonal and polyclonal FLCs but the quantitative estimation of urinary FLCs concentration.

Other results

In 64 urines both FLCs Kappa & Lambda were < 2.5 mg/L. In 32/64 (50%) cases we can't demonstrate FLCs by IFE.

In 129 urines both Kappa & Lambda were > 2.5 mg/L. In 12/129 we can't demonstrate ladders and/or monoclonal FLCs by IFE.

*Tables and Figures*

Table 1		Samples IFE positive Kappa			
Urine Total Protein mg/L	Nephelometry free Kappa < 2,5 mg/L Unconcentrated urines	IFE Results			
		Samples Concentration	Monoclonal Kappa	Monoclonal K + Polyclonal K	Polyclonal K
100	32	x 400	4	5	7
150	5		1	0	3
200	2		0	0	0
250	1		0	0	0
500	8		0	0	1
1000	13		0	0	4
2000	3		0	0	0
5000	5		0	0	1
> 5000	0		0	0	0
	69			5	5

Table 2		Samples IFE positive Lambda			
Urine Total Protein mg/L	Nephelometry free Lambda < 2,5 mg/L Unconcentrated urines	Urine Concentration	IFE Results		
			Monoclonal Lambda	Monoclonal L + Polyclonal K	Polyclonal L
100	59	from x 400 to x 50	2	4	6
150	20		1	2	4
200	11		1	1	0
250	5		0	1	1
500	20		1	1	1
1000	26		0	1	2
2000	11		0	0	0
5000	8		0	0	0
> 5000	0		0	0	0
	160			5	10

Figure 1

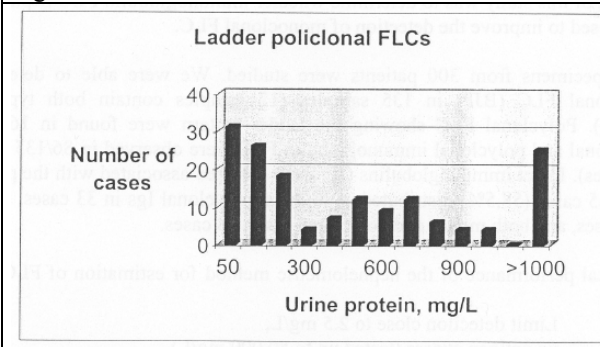


Figure 2

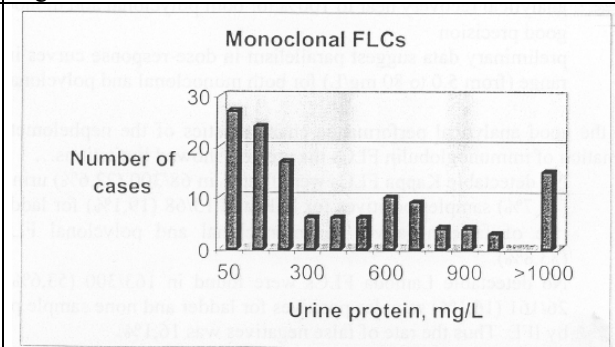


Figure 3

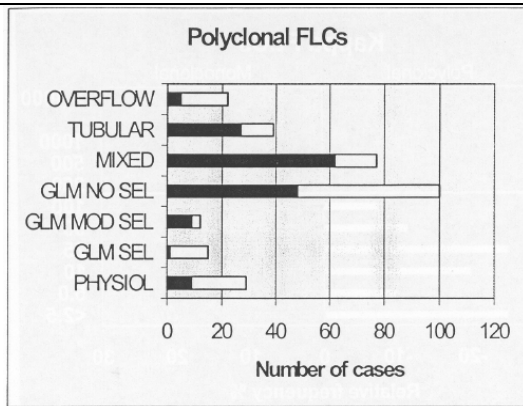


Figure 4

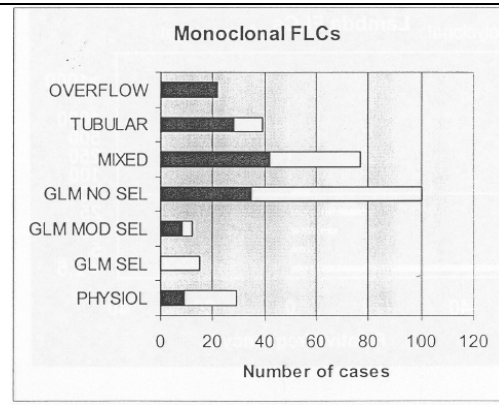


Figure 5

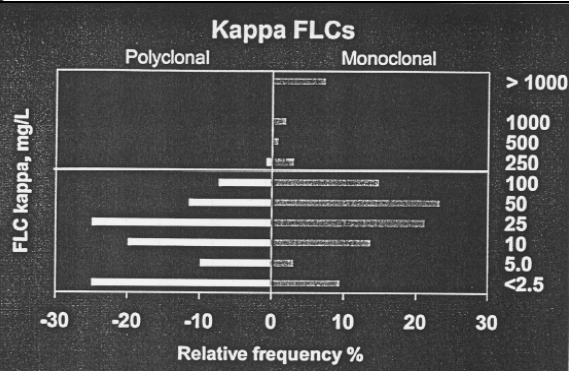


Figure 6

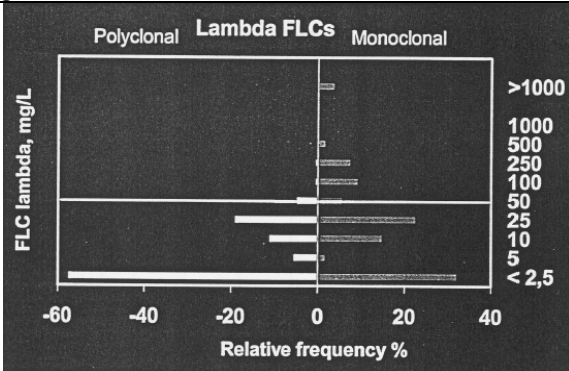


Figure 7

