

Free Light Chains

Additional Information

BNA/BNII Nephelometric Kit - Urine

K.BNA.FRK.FRL

The protocol and the Reagents and Calibrators used are standard. The information on the lots used, the location of the analyser, the calibration curves on which the evaluations are based and all the analytical documentation are kept in the appropriate marked file.

Sensitivity Limit

The PBS was used as sample and was measured 30 times. The sensitivity limit was calculated by applying the Average+3DS formula.

The values obtained were: FRK = 0.080 mg/dl – FRL = 0.051 mg/dl

Linearity Limit

We used the positive Bence Jones samples, which the Forlì and Liguria Commissions selected as “provisional controls” and are named BJ-kappa Lavagna, BJ-lambda Bellaria.

Given that the calibration curves stretch from 8 mg/dl to 0.5 mg/dl, the samples were diluted in PBS as follows: BJ-kappa Lavagna 1:50, BJ-lambda Bellaria 1:10. Of the samples diluted thus, ten dilutions were obtained, from 100% to 10% in 10% steps.

All the determinations were carried out twice over, and the average of the two determinations was taken. The CV% was always below 3%.

The 100% dilution was regarded as assigned value concentration and therefore the measurement range controlled was FRK: 5.49 – 0.55 mg/dl, FRL: 5.95 – 0.59 mg/dl.

The values obtained were: FRK Correlation = 0.9991 – FRL Correlation = 0.9986

Imprecision - Repeatability

We used the positive Bence Jones samples, which the Forlì and Liguria Commissions selected as “provisional controls” and named: BJ-kappa Lavagna, BJ-lambda Bellaria.

Nine repeats were carried out on six dilutions of the abovementioned samples. The results are shown in the following table:

FRK	BJ-kappa Lavagna					
Dilution %	100	80	60	50	30	10
Average mg/dl	5.56	4.46	3.44	2.83	1.78	0.50
CV %	3.04	1.92	1.35	1.87	0.93	4.29
FRL	BJ-lambda Bellaria – high			BJ-lambda Bellaria – low		
Dilution %	100	80	60	100	60	20
Average mg/dl	5.95	5.03	4.01	3.23	2.20	0.76
CV %	3.19	1.68	2.11	1.44	2.01	3.49

Matrix Effect

As well as the Calibrators, we used the ATAB “CA4” Reference Serum diluted in PBS from 1:5 to 1:40 and ten samples of urine, five with BJ-kappa and five with BJ-lambda. The test was performed substituting the Antiserum Reagent with the PBS.

The results obtained were all well below the sensitivity limit.

Correlation BNII - Immage

For each type of Bence Jones, 24 samples were examined – 8 low, 8 medium and 8 very high – both on the BNII (Dade Behring) nephelometer and also on the Immage (Beckman Coulter) nephelometer with the appropriate kit.

The results were:
(under way)

Cross-Reaction with Bound Light Chains

We used the ATAB “CA4” Reference Serum as sample, diluted in PBS from 1:5 to 1:40.

The concentration of Ig-kappa and Ig-lambda was measured with the Beckman anti Total Light Chains reagent, kappa and lambda respectively, and the result was divided by 3.33 to obtain the Free Light Chain expression.

The Free Light Chains were repeated twice.

The results achieved are shown in the table.

FRK	“CA4” Reference Serum			
Dilutions	1:5	1:10	1:20	1:40
Ig-kappa mg/dl	55.25	28.00	13.80	7.20
Free kappa – average – mg/dl	0.049	0.017	0.025	0.052

FRL	“CA4” Reference Serum			
Dilutions	1:5	1:10	1:20	1:40
Ig-lambda mg/dl	24.90	13.45	6.85	3.66
Free lambda – average – mg/dl	0.0152	0.165	0.060	0.020

Given the trend of the results, the measurement of the Blank Sample (matrix effect) was considered superfluous.

It should be noted that **the existence of small quantities of FLC in the reference serum cannot be excluded.**

In conclusion, **it can be excluded that in real samples of urine the reaction may be affected by cross-reaction with Bound Light Chains and consequently with intact Immunoglobulin.**

Cross-Reaction with Free Light Chains of the opposite type

We measured the kappa Calibrator with the lambda Reagent and viceversa, and the result was below the Sensitivity Limit of the method.

The lack of this type of cross-reaction is confirmed by the results obtained on the samples with the exclusive presence of BJ-kappa with the lambda Reagent and viceversa.