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Multi-Centre Evaluation of Routine Commercial Methods for the Detection of Bence Jones Proteins in Urine – 2001 Results

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Non standard abbreviations	
BJP	(Bence Jones Proteins) Proteine di Bence Jones
BJ	Bence Jones
FLC	(Free Light Chains) Catene Leggere Libere
FRK	(Free Light Chains kappa) Catene Leggere Libere kappa
FRL	(Free Light Chains lambda)Catene Leggere Libere lambda
FLC-pc	(policlonal-Free Light Chains) Catene Leggere Libere policlonali
LC(b&f)	(Light Chains bound & free) Catene Leggere Totali libere & legate
IFE	Immunofissazione

Key Words
Bence Jones Protein, Kappa Free Light Chains, Lambda Free Light Chains, Free Light Chains

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Summary

The objective of the study groups is to verify the possibility of making uniform the evaluation, interpretation and reporting of Bence Jones Proteins (BJP) and, more generally, of Free Light Chains (FLC) in urine.
It has emerged from the "Forlì" Group's previous work (1) and the subsequent multi-centre evaluation carried out over the last few years both by the "Forlì" and the "Liguria" Groups that for the detection of BJP the following are unreliable:

- the stick for the dosage of Total Proteins in urine
- the dosage of Total Proteins in urine.

The comprehensive results obtained by the multi-methodological multi-centre evaluation of three scaled dilutions of two urine samples are presented, one with BJP lambda and one with BJP kappa, which were carried out by the "Forlì" and "Liguria" Study Groups in 2000 (25 Laboratories). For both samples, the concentration of BJP in the dilutions distributed is between approx. 2 mg/dl and 0.3 mg/dl. These results have been integrated with those obtained during 2001 by the "Lazio" and "Toscana-Umbria" Groups (27 Laboratories) on two of the aforementioned dilutions, that is those of higher concentration.
The results show us that the best performances were obtained on Nephelometry/Turbimetry with reagent for the Free Light Chains, immediately followed by that with reagent for Total Light Chains (Bound & Free); ImmunoFixation and Electrophoresis, which produced some negative results even on "A1" samples with BJP concentration of about 2 mg/dl, are clearly well behind.
From the evaluation, two dilutions of the BJP-kappa Lavagna sample and two of the BJP-lambda Bellaria sample were defined as "reference samples", at least in "positive/negative" terms. For both samples, the BJP concentration of the two proposed pre-packed dilutions was estimated at approx. 2 mg/dl and 0.5 mg/dl.

Introduction

At present, research into Free Light Chains (FLC) is for the detection of Bence Jones Proteins (BJP), that is Monoclonal Free Light Chains in urine, whilst Polyclonal Free Light Chains (PFLC) are considered to be of secondary importance, unless one specifically intends carrying out their determination as tubular proteinuria index. In this case, the choice method would seem to be the direct quantitative determination with ImmunoPrecipitation in Liquid Phase (IPL), turbimetry or nephelometry, using the specific FLC reagents.

An adequate standardisation of methods, protocols, references, checks and reporting – all of which are quite rightly felt by the operators as absolutely essential, together with the opportunity to compare concrete experiences on the subject – does not appear to match the importance of BJP (and FLC) research.

This need, along with the opportunity to compare concrete experiences, is strongly felt by operators in the sector.
New Scientific Company (NSC) has collated these needs, taking care of the organisational part of the study groups as they are set up.
The "Forlì" Group has held ten meeting since 1993 with an average of 30 Laboratories from Emilia-Romagna and other regions; the "Liguria" Group, with an average of 10 Laboratories has had four meetings since 1997. The "Lazio" and "Toscana-Umbria" Groups have recently joined them.

Method of Work

The study groups periodically hold round-table and open debate "co-ordination meetings".
At these meetings the results of previous work are examined and discussed, and the work for the subsequent meeting is set out.

General Objectives and Strategies

The objective of the study groups is to assess the possibility of making uniform:

- evaluation,
- interpretation and
- reporting

of Bence Jones Proteins (BJP) and, more generally, of Free Light Chains (FLC) in urine.
It would in fact be hoped that a sample resulted at least "BJP-positive" or "BJP-negative" in all the Laboratories regardless of method or protocol; that is, from the screening and second stage methods utilised.
The strategy adopted is that of comparing the different and manifold dimensional, structural and organisational operating experiences in the daily routines, starting with the verification of the *"real characteristics"* of the methods practicable.
With this aim, it was decided to evaluate the "sensitivity" and the "precision" of the individual methods and to then include them in the strategy for the detection of BJP.
Evaluation of the "precision" is not only preliminary to the possibility of an eventual quantitative determination but is also essential for the correct evaluation of the "sensitivity".
For an initial verification, we used the elementary but linear procedure, made up of dilutions of samples with a significant quantity of BJP and only traces of other proteins.
This enables the evaluation and comparison of "sensitivity and precision" of the methods in detecting ***"abnormality of the sample"*** without any consideration of the absolute concentration of the BJP.
This approach was thought to be an indispensable starting point despite not being complete due to the heterogeneity of the BJP and FLC:

- The BJP amongst themselves are antigenically diverse even for the same type, and this can determine variations in results in the techniques of ImmunoPrecipitation in Liquid Phase (IPL) (Nephelometry, Turbidimetry) and ImmunoFixation (IFE), whilst it does not influence Electrophoresis (EF).
- Polyclonal Free Light Chains (PLFC) will be highlighted with less sensitivity on EF and IFE compared to an equal quantity of BJP since for the distribution on a wider surface compared to the narrow band typical of BJP, the PFLC will have a lower concentration per surface unit.

Desired Characteristics for Methods and Protocols

The **"perfectly effective"** method and protocol for BJP and FLC should be characterised by:

a) **no "false negative" – Sensitivity and Precision**
This is the principal and the absolutely vital characteristic for which maximum intra and inter-laboratory reproducibility is consequently required: the "sensitivity" and "precision" of each method should be defined.

b) *no "false positive" – Specificness of the Positivity Marker*
Efficacy gradually decreases while the number of "false positives" increases.

c) Good quantitative information
As well as "precision", "accuracy" should also be evaluated.
The value of this element depends on the degree of need for absolute quantitative determination.

Objectives of the Multi-Centre Experimentation

This experimentation means verifying, on the "reference samples" as defined in the previous Multicentre 1999 (2), the efficacy of the methods in use in the participating Laboratories in terms of "sensitivity" and "precision".
Evaluation of "accuracy" and of "general efficacy" will be the subject of future experimentation already programmed for 2001-2002.

Samples and Methods

The Samples were obtained by diluting the original urine pre-selected for the experimentation, so as to have the approximate concentrations of BJP agreed upon by the participants.
New Scientific Company dealt with preparation of the samples and their distribution to participants.
The dilutions were put in PBS and distributed in 5 ml bottles, clearly labelled with the conventional name of the sample, the type of BJP present and the dilution.

Result charts were included with the identified samples. This table shows the samples and dilutions tested.

Sample	BJP kappa (Lavagna) (2)			BJP lambda (Bellaria)		
Dilution	***A1***	***A***	***B***	***A1***	***A***	***B***
Indicative Concentration(1) mg/dl	1,8	0,7	0,3	1,8	0,7	0,2

N.B.

1) The indicative concentration is shown here for clarity; it was obtained subsequently, and is the average of the measurements obtained with the quantitative methods.
2) On IFE the Lavagna BJ-kappa sample highlights polyclonal FLC as well as BJP.
3) The "B" samples were only tested by the "Forlì" and "Liguria" Groups; the "Lazio" and "Toscana-Umbria" Groups were not included in the Multi-centre, as they were retained excessively low.

Question

If the samples had arrived at the participating laboratories with the request for "Detection of Bence Jones Protein", what would have been the result?

Operating Procedure

The samples were analysed by the participating laboratories with both screening and second stage methods in use for the detection of BJP routine.
Some laboratories carried out more repeats in various analysis sessions.

Expected Result

All the samples should have shown a positive result for the type of BJP indicated on the sample identification label.

Positive Result

The "abnormality signal" typical of each method was regarded as a "positive result".

Stability of the Dilutions Distributed

The time between preparation of the dilutions distributed and the laboratory testing was very varied.

The stability of the dilutions distributed was thus controlled, determining the FLC by the manual immunoturbidimetric method with specific anti FLC reagent.
Both the "B" kappa and lambda samples deteriorated somewhat and then stabilised, whereas the others showed no appreciable variations during the observation period.
Stability was, furthermore, confirmed by inter-laboratory reproducibility of the nephelometric and turbidimetric determinations.

Results and Discussion

Samples from **52 Hospital Laboratories** were analysed, and all in all **over 2000 determinations** were carried out.

It had emerged that, from previous multicentre evaluation work (1) & (2) for the detection of BJP, few methods are reliable, and the following should not **currently** be used.:

- The dosage of Total Proteins in urine
- The stick for the dosage of Total Proteins in urine

Therefore, these methods were not adopted by the participating Laboratories.

Summary and Comparison of the Methods

Table 1 shows a synthetic overall comparison of the results on the six samples with five techniques used by the different Laboratories.

The samples were analysed by 52 Laboratories who altogether carried out over 2,000 tests (many Laboratories did, in fact, check intra-laboratory repeatability with several repeats) and, with the various techniques, 750 "final results" were attained.

The overall comparison of the techniques demonstrates that the best performances were on Nephelometry/Turbidimetry with reagent for the Light Chains immediately followed by that with reagent for Total Light Chains (B&F); Immunofixation and Electrophoresis, which also gave some negative results on the "A1" samples with BJP concentration of approx. 2 mg/dl, clearly stand out.

Electrophoresis (EF)

Table 2 shows the results of various commercial electrophoretic methods.

The colloidal gold used by one laboratory did not produce better results than the other methods with "traditional" dye, which re-confirms (1) that the commercial version of this dye does not give the performance of the "homemade" one described in literature (3).

If the sensitivity of EF is virtually as expected to be, the lack of inter-laboratory reproducibility even within the same method should, on the other hand, be underlined, as is demonstrated on both samples, including dilution "A1" (≈ 1.8 mg/dl), with Hydragel for both black and violet dye. This shows that the method worked to the limit of its analytical sensitivity and that on the samples of higher concentration the results would be homogeneous. However this does not alter the fact that on at least two samples examined, out of 7 laboratories using the same method – Violet Hydragel – 3 resulted BJ positive and 4 BJ negative.

This factor may not be surprising where the number of variables affecting the electrophoretic technique is not considered: gel hydration, environment temperature, humidity and ventilation, sample quantity actually applied and actually absorbed by the gel, "age" of the migration buffer and dye, migration times, voltage, amperage, staining times and quality of de-staining etc. It thus follows that electrophoresis, amongst its many qualities, does not have and cannot have that of good repeatability.

Immunofixation (IFE)

The IFE results with anti Total Light Chain (Free & Bound) and anti Free Light Chain antisera are in Table 3.

Considerations on the IFE reproducibility, even within the same method, are similar to those for Electrophoresis, which is, in any case, the first step for IFE. We should add the other variables connected to ImmunoPrecipitation, which are: antiserum quality, incubation period, washing, etc.

As regards sensitivity, compared to EF, IFE did not show the significant improvement which would have been expected; neither was there a notable difference between the use of anti Total Light Chain and anti Free Light Chain antisera.

Nephelometry/Turbidimetry with anti Total Light Chain (TLC) Reagents

The qualitative results, in positive/negative terms, are shown in Table 4 and in overall quantitative terms in Table 5.

The Dade-Behring nephelometer produced very good results both for sensitivity – with only 6 negative results on all the samples examined – and for precision, with acceptable inter-laboratory imprecision.

The Beckman nephelometer showed less sensitivity, on account of the minimum measurement limit set by the manufacturer.

The results from the two manufacturers' instruments seemingly vary by a factor of 3.33. Therefore, the Beckman results were divided by this factor so as to give the data better comparability to that of the nephelometry and turbidimetry with anti Free Light Chain reagent.

Nephelometry/Turbidimetry with anti Free Light Chain (FLC) Reagents

The qualitative results, in positive/negative terms, are given in Table 4 and in overall quantitative terms in Table 5.

On the Dade-Behring BNA/BNII nephelometers and on Beckman's Immage, the method "notched up" 100% with no negative on a total of **152** results and as many as **724** repeats.

The APS nephelometer result with turbidimetry on Cobas was also quite good.

From the quantitative point of view, precision was quite good on the whole, considering the rather low values of the samples.

Conclusions

Methods

In order to obtain better reproducibility on Electrophoresis and Immunofixation, at least for the same commercial method, an improved standardisation of methods would be required, alongside the collaboration of the manufacturers.
Regarding the Total Light Chain nephelometry, Beckman would need to adapt sensitivity and agree, with Dade-Behring, to uniformity in expressing the result, with the elimination of the 3.33 factor.

The use of EF and IFE with the materials and methods on the market for routine as the only method for the detection of BJP is somewhat perplexing, given the results obtained and the insufficient reproducibility demonstrated on the low concentrations, yet which appear to be of clinical importance.

With the current situation, the use of a protocol for the detection of BJP would seem appropriate, made up of: a nephelometric/turbidimetric method for Total or Free Light Chains, and Immunofixation (or also, in the majority of cases, Electrophoresis only); the first would ensure sensitivity, reproducibility and automation and the second, always to be used as confirmation of the first, would ensure specificness in identifying monoclonality. The ideal answer would be to carry out both method types simultaneously and, in the case of discrepancy, to carry out further opportune tests; this would cost both time and money however.

On the basis of the results of this work, the hypothesis of a "two-part" protocol – nephelometry as first level method and IFE on the positive samples – could be the best choice, provided that it is demonstrated with experimentation by both methods on a significant number of samples that nephelometry is comparable to IFE in detecting BJ-positive subjects.

Reference Samples

From the multicentre evaluation it was possible to choose the two highest concentrations of samples utilised as "reference samples" in "positive/negative" terms.
For both samples, the concentration of BJP in the two dilutions was estimated at approx. 2 mg/dl e 0.5 mg/dl.

The aforementioned "reference samples" are available from New Scientific Company.

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Tables

Table 1

Some laboratories used more than one method and/or several repetitions for each method

Overall comparison of the methods

Qualitative results expressed in no. of Laboratories which carried out each method

Method		Electrophoresis		Immunofixation (IFE) As LC (B&F)		Immunofixation (IFE) As FLC		Nephelometry - Turbidimetry LC (B&F)		Nephelometry - Turbidimetry FLC		**Totals**
Laboratories		26		39		28		23		32		25
No. of Tests		*240*		*344*		*316*		389		*750*		*2039*
Sample **kappa**												
Dil. A1	pos	7		28		16		23		32		106
1.9 mg/dl	neg	19	73%	11	28%	12	43%	0	0%	0	0%	42
Dil. A	pos	1		17		9		13		31		71
0.6 mg/dl	neg	25	96%	22	56%	19	68%	10	43%	1	3%	77
Dil. B	pos	0		3		2		3		20		28
0.3 mg/dl	neg	13	100%	15	83%	14	88%	6	67%	3	13%	51
Totals	*pos*	*8*		*48*		*27*		*39*		*83*		***205***
	neg	*57*	**88%**	*48*	**50%**	*45*	**63%**	*16*	**29%**	*4*	**5%**	***170***
Sample **lambda**												
Dil. A1	pos	8		30		23		22		32		115
1.7 mg/dl	neg	18	69%	9	23%	5	18%	1	4%	0	0%	33
Dil. A	pos	1		13		11		18		31		74
0.6 mg/dl	neg	25	96%	26	67%	17	61%	5	22%	1	3%	74
Dil. B	pos	0		2		1		5		20		28
0.2 mg/dl	neg	13	100%	16	89%	15	94%	4	44%	3	13%	51
Totals	*pos*	*9*		*45*		*35*		*45*		*83*		***217***
	neg	*56*	**86%**	*51*	**53%**	*37*	**51%**	*10*	**18%**	*4*	**5%**	***158***
Overall	pos	17		93		62		84		166		**422**
Totals	neg	113	**87%**	99	**52%**	82	**57%**	26	**24%**	8	**5%**	**328**
	test	130		192		144		110		174		**750**

Abbreviations As = antiserum
LT(B&F) = (Total) Light Chains (Bound&Free)
FLC = Free Light Chains

Some Laboratories carried out several repetitions

Electropheresis

Results expressend in no. of Laboratories

Method		Acetate	Paragon SPE Protur HiSi	Hydragel	Hydragel	Acetate Cell.	Rep Helena	Capillary	**Totals**
Dye		*red*	*blue*	*black*	*violet*	*gold*	*blue*		
Laboratories		**2**	**3**	**10**	**7**	**1**	**2**	**1**	**26**
No. of tests		***10***	***22***	***106***	***58***	***18***	***22***	***4***	***240***
Sample **kappa**									
Dil. A1	pos	0	2	1	3	0	1	0	7
1.9 mg/dl	neg	2	1	9	4	1	1	1	19
Dil. A	pos	0	1	0	0	0	0	0	1
0.6 mg/dl	neg	2	2	10	7	1	2	1	25
Dil. B	pos	0	0	0	0	0	0	0	0
0.3 mg/dl	neg	1	2	3	5	1	1	0	13
Totals	*pos*	*0*	*3*	*1*	*3*	*0*	*1*	*0*	8
	neg	***5***	***5***	***22***	***16***	***3***	***4***	***2***	57
Sample **lambda**									
Dil. A1	pos	0	2	1	3	1	1	0	8
1.7 mg/dl	neg	2	1	9	4	0	1	1	18
Dil. A	pos	0	1	0	0	0	0	0	1
0.6 mg/dl	neg	2	2	10	7	1	2	1	25
Dil. B	pos	0	0	0	0	0	0	0	0
0.2 mg/dl	neg	1	2	3	5	1	1	0	13
Totals	*pos*	*0*	*3*	*1*	*3*	*1*	*1*	*0*	9
	neg	*5*	*5*	*22*	*16*	*2*	*4*	*2*	56
Overall	***pos***	***0***	***6***	***2***	***6***	***1***	***2***	***0***	**17**
Totals	***neg***	***10***	***10***	***44***	***32***	***5***	***8***	***4***	***113***
									130

Table 3

Some Laboratories carried out several repetitions

Immunofixation

Results expressend in no. of Laboratories

		As (Total) Light Chains (bound&free) (LC(b&f))							As Free Light Chains (FLC)							
System		Beckman Paragon Protur	Helena Immuno Fix	Helena Auto IFE	Helena Auto IFE	Sebia Hydragel	Acetate gold	**Totals**	Beckman Paragon	Helena Immuno Fix	Helena Auto IFE	Helena Auto IFE	Sebia Hydragel	Sebia Hydragel	Acetate gold	**Totals**
Antiserum		*Beckman*	*Helena*	*Helena*	*Sebia*	*Sebia*	*Dasit*		*N S C*	*Helena*	*Helena*	*N S C*	*Sebia*	*N S C*	*Dasit*	
Laboratories		**9**	**2**	**4**	**1**	**22**	**1**	**39**	**2**	**2**	**2**	**2**	**17**	**2**	**1**	**28**
No. of Tests		*80*	*10*	*40*	*18*	*192*	*4*	***344***	*42*	*22*	*22*	*30*	*180*	*16*	*4*	***316***
Sample **kappa**																
Dil. A1	pos	8	1	3	1	14	1	28	2	0	1	2	8	2	1	16
1.9 mg/dl	neg	1	1	1	0	8	0	11	0	2	1	0	9	0	0	12
Dil. A	pos	4	0	1	1	10	1	17	2	0	0	1	5	0	1	9
0.6 mg/dl	neg	5	2	3	0	12	0	22	0	2	2	1	12	2	0	19
Dil. B	pos	1	0	0	1	1	0	3	0	0	0	1	1	0	0	2
0.3 mg/dl	neg	6	1	1	0	7	0	15	2	1	1	1	8	1	0	14
Totals	*pos*	*13*	*1*	*4*	*3*	*25*	*2*	***48***	*4*	*0*	*1*	*4*	*14*	*2*	*2*	***27***
	neg	*12*	*4*	*5*	*0*	*27*	*0*	***48***	*2*	*5*	*4*	*2*	*29*	*3*	*0*	***45***
Sample **lambda**																
Dil. A1	pos	8	1	3	1	16	1	30	2	1	1	2	14	2	1	23
1.7 mg/dl	neg	1	1	1	0	6	0	9	0	1	1	0	3	0	0	5
Dil. A	pos	3	0	0	0	9	1	13	2	0	1	1	5	1	1	11
0.6 mg/dl	neg	6	2	4	1	13	0	26	0	2	1	1	12	1	0	17
Dil. B	pos	1	0	0	0	1	0	2	0	0	0	0	1	0	0	1
0.2 mg/dl	neg	6	1	1	1	7	0	16	2	1	1	2	8	1	0	15
Totals	*pos*	*12*	*1*	*3*	*1*	*26*	*2*	***45***	*4*	*1*	*2*	*3*	*20*	*3*	*2*	***35***
	neg	*13*	*4*	*6*	*2*	*26*	*0*	***51***	*2*	*4*	*3*	*3*	*23*	*2*	*0*	***37***
Overall	pos	25	2	7	4	51	4	**93**	8	1	3	7	34	5	4	**62**
Totals	neg	25	8	11	2	53	0	**99**	4	9	7	5	52	5	0	**82**
	test							**192**								**144**

Table 4

Nephelometry and Turbidimetry Light Chains (1) - Unconcentrated Sample

Some Laboratories carried out several repetitions

Qualitative Results expressend in no. of Laboratories

		Reagent (Total) Light Chains (bound&free)					Reagent Free Light Chains (FLC)				
Instrument		BNA/BNII	Immage	APS	Cobas Mira	**Totals**	BNA/BNII	Immage	APS	Cobas Mira	**Totals**
Reagent		*Behring*	*Beckman*	*Beckman*	*Dako*		*N S C*	*N S C*	*N S C*	*N S C*	
Laboratories		17	3	2	1	**23**	22	6	3	1	**32**
No. of Tests		*278*	*70*	*11*	*30*	***389***	*628*	*96*	*20*	*6*	***750***
Sample **kappa**											
Dil. A1	pos	17	3	2	1	23	22	6	3	1	32
1.9 mg/dl	neg	0	0	0	0	0	0	0	0	0	0
Dil. A	pos	12	0	0	1	13	22	6	2	1	31
0.6 mg/dl	neg	5	3	2	0	10	0	0	**1**	0	1
Dil. B	pos	3	0	0	0	3	14	6	0	0	20
0.3 mg/dl	neg	1	2	2	1	6	0	0	2	1	3
Totals	*pos*	*32*	*3*	*2*	*2*	*39*	*58*	*18*	*5*	*2*	*83*
	neg	**6**	**5**	**4**	**1**	**16**	0	0	**3**	**1**	**4**
Sample **lambda**											
Dil. A1	pos	17	2	2	1	22	22	6	3	1	32
1.7 mg/dl	neg	0	1	0	0	1	0	0	0	0	0
Dil. A	pos	17	0	0	1	18	22	6	2	1	31
0.6 mg/dl	neg	0	3	2	0	5	0	0	1	0	1
Dil. B	pos	4	0	0	1	5	14	6	0	0	20
0.2 mg/dl	neg	0	2	2	0	4	0	0	2	1	3
Totals	*pos*	*38*	*2*	*2*	*3*	*45*	*58*	*18*	*5*	*2*	*83*
	neg	0	**6**	**4**	0	**10**	0	0	**3**	**1**	**4**
Overall	*pos*	*70*	*5*	*4*	*5*	*84*	*116*	*36*	*10*	*4*	*166*
Totals	neg	**6**	**11**	**8**	**1**	**26**	0	0	**6**	**2**	**8**
						110					**174**

Note (1) This method is considered by Laboratories as a "first level test" in BJP research protocol. Positive samples are followed by EF and/or IFE
Lack of intra-laboratory repeatability in terms of positive/negative: none.

Table 5

Nephelometry and Turbidimetry Light Chains (1) - Unconcentrated Sample

Quantitative Results - Average and CV inter-laboratory

Some Laboratories carried out up to 10 repeats on 10 different days

	Reagent (Total) Light Chains (bound&free)								Reagent Free Light Chains (FLC)							
	Overall tests: 389								Overall tests: 746							
Instrument	BNA/BNII		Immage (2)		APS (2)		Cobas Mira		BNA/BNII		Immage		APS		Cobas Mira	
Reagent	*Behring*		*Beckman*		*Beckman*		*Dako*		*N S C*		*N S C*		*N S C*		*N S C*	
Laboratories	9	8	3		2		1		6	16	6		2		1	
No. of Tests	278		70		11		30		628		96		16		6	
Samples	Media mg/dl	CV	Media mg/dl	CV	Media mg/dl	CV	Media mg/dl	CV	Media mg/dl	CV	Media mg/dl	CV	Media mg/dl	CV	Media mg/dl	CV
kappa																
Dil. A1	**1,52**	31%	**1,53**	19%	**2,01**	4%	**1,51**	n.c.	**1,84**	16%	**2,09**	9%	**n.s.**	n.c.	**2,36**	n.c.
Dil. A	**0,79**	13%	**0,61**	n.c.	**< 0.55**	n.c.	**0,66**	n.c.	**0,73**	22%	**0,62**	20%	**n.s.**	n.c.	**0,93**	n.c.
Dil. B	**0,80**	n.c.	**< 0.55**	n.c.	**< 0.55**	n.c.	**<**	n.c.	**0,34**	28%	**0,32**	29%	**n.s.**	n.c.	**neg**	n.c.
lambda																
Dil. A1	**1,97**	12%	**1,65**	12%	**1,84**	18%	**4,87**	n.c.	**1,75**	5%	**1,79**	6%	**n.s.**	n.c.	**1,96**	n.c.
Dil. A	**0,92**	13%	**< 1.5**	n.c.	**< 1.5**	n.c.	**1,22**	n.c.	**0,67**	17%	**0,57**	13%	**n.s.**	n.c.	**0,72**	n.c.
Dil. B	**0,40**	3%	**< 1.5**	n.c.	**< 1.5**	n.c.	**0,25**	n.c.	**0,18**	42%	**0,20**	28%	**n.s.**	n.c.	**neg**	n.c.

Legend: n.c. = not calculable; n.s. = not significant
(1) - This method is considered by Laboratories as a "first level test" in BJP research protocol. Positive samples are followed by EF and/or IFE
(2) - The instrument results are divided by a factor of 3.33