TURBIDIMETRIC BENCE JONES

Immunoturbidimetric reagent for qualitative in microplate and quantitative determination of the Bence Jones Proteinuria

TEST SUMMARY

The test consists in a protein-antibody reaction in an homogeneus phase, to form an antigen-antibody complex. The turbidity developed may be read by eyes (qualitative assay) or by a turbidimeter for the quantitative assay.

SAMPLES

Urine.
Stability: 7 days at 4°C or 2 months at -18°C.

REAGENTS

Antiserum: Buffered and stabilised solution of goat anti-Kappa and anti-Lambda andibodies, that contains PEG 4%.

Calibrator: Human base stabilised solution with a known titre of Kappa and Lambda chains.

Blank reagent: Buffered solution to determinate the colour of urine that contains PEG 4%.

Diluent: Physiologic solution to dilute the samples and calibrators.

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. Automatic micropipette. Optical glass cuvette or Optical polystyrene monouse cuvette. Physiologic solution.

PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

REAGENTS PREPARATION

Reagents are ready for the use.
The antibody reagent may develop a slight turbidity or opalescence that do not affect the reagent performances. This turbidity is due to the presence of aspecific impurities in the antibody. The reagent can be cleared by filtration at 0.2 micron or by centrifugation.
Running the quantitative assay the operator has to dilute the calibrator 1:5, 2:5, 3:5, 4:5 with diluent. These solutions have to be used with undiluted calibrator in order to perform the calibration curve.
For qualitative assay the calibrator must be diluted at cut-off value.
Store the reagents at 2-8°C.

SAMPLES PREPARATION

Turbid urine has to be filtered or centrifuged.

PROCEDURE

Qualitative assay: in microplate add with a pipette
Quantitative assay: in a cuvette series add with a pipette:

Reagents	Blank	Calibrator	Sample
Antiserum	250 µl	250 µl	250 µl
Physiologic	10 µl	-	-
Calibrator	-	10 µl	-
Sample	-	-	10 µl

Qualitative assay
Wait for 20 minutes and observe the turbidity formation.
At the same time pipette in another series of test tubes using the blank reagent instead antibody reagent.

Quantitative assay
Wait for 20 minutes and read the absorbance at a wavelength within 340-405 nm.
At the same time pipette in another series of test tubes using the blank reagent instead antibody reagent.

Reagents	Blank	Calibrator	Sample
Blank reagent	250 µl	250 µl	250 µl
Physiologic	10 µl	-	-
Calibrators	-	10 µl	-
Samples	-	-	10 µl

Qualitative assay
Wait for 20 minutes and observe the turbidity formation.

Quantitative assay
Wait for 20 minutes and read the absorbance at the same wavelength used before.

If the photometer requires bigger volumes, these volumes can be changed proportionally.

CALCULATION

The calibrators and samples turbidy values are obtained from difference between relative readings. The concentrations of sample are obtained from interpolation of calibration curve.

EXPECTED VALUES

Kappa and Lambda chains must be absent or present at a concentration lower than assay cut-off. Just for contrastografy we suggest a Bence Jones cut-off value of 50 mg/dl, but it's strongly advised that each laboratory establishes its own cut-off value.

NOTES

- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- In case an excess antigen is suspected repeat the reading after 10 - 20 minutes.
  If the variation observed between the different readings is small (few digits) the sample is not in antigen excess. If the difference is elevated it is opportune dilute 10 times the sample with diluent (1+9) and repeats the assay; this dilution has to be considered in the calculation of the results.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL

The kit is supplied with a calibrator that has a concentration of 100 mg/dl of Bence Jones. This can be used for internal quality control.

TEST PERFORMANCE

Precision

In series (n = 10)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	44.7	2.31	5.17
Sample 2	141.9	2.60	1.83

Among series (n = 20)	Mean (mg/dl)	SD (mg/dl)	CV%
Sample 1	44.4	2.11	4.76
Sample 2	145.2	2.35	1.62

Sensitivity/cut-off
Cut-off as from 4 mg/dl.

Methods comparison
A comparison with an available commercial method gave following results on 60 samples compared.

Bence Jones LTA = x
Bence Jones competitors = y
n = 60

y = 1,00267x - 0,142 mg/dl r = 0,999

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE UK00120	**(25 TESTS)**
Antisiera	1 x 6,25 ml
Calibrator	1 x 0,5 ml
Blank reagent	1 x 20 ml
Diluent	1 x 25 ml

CODE UK00100	**(80 TESTS)**
Antisiera	1 x 20 ml
Calibrator	1 x 0,5 ml
Blank reagent	1 x 20 ml
Diluent	1 x 25 ml

CODE UK00140	**(50 TESTS)**
Antisiera	1 x 12,5 ml
Calibrator	1 x 0,5 ml
Blank reagent	1 x 20 ml
Diluent	1 x 25 ml

REFERENCES

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MANUFACTURER

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SYMBOLS

IVD Only for IVD use
LOT Lot of manufacturing
REF Code number
Storage temperature interval
Expiration date
Warning, read enclosed documents
Read the directions
Biological risk

Mod. 01.06 (ver. 3.5 - 15/10/2010)

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