MICROPROTEIN

(Pyrogallol Red Method)

DIATEK

Diagnostic reagent for quantitative in vitro determination of total protein in urine and cerebrospinal fluid.

Presentation

Pack size	Microprotein Reagent (R1)	Standard (S)
2 x 30ml	2 x 30ml	3.0ml

The presence of protein in urine is a very sensitive indicator of renal disorders. There are four ways by which increased amounts of protein can occur: increased glomerular permeability: defective tubular re-absorption: increased plasma concentration of an abnormal, low molecular weight protein; and abnormal secretion of protein into the urinary tract.1 Albuminuria, increased amounts of albumin in urine, has been recognized as an early indicator of renal damage in diabetes that can be reversed if detected and treated early.2 The measurement of CSF total protein provides an indication of either increased permeability of the blood / brain barrier to plasma proteins, or of increased intrathecal secretion of immunoglobulins.

Method

Various methods have been described for the determination of protein concentrations in biological fluids. These methods are based on colorimetric, turbidimetric, electrophoretic or immunologic procedures." The dye binding methods are characterized as having good precision and sensitivity. This method is based on the procedure developed by Fujita6 and Watanabe.7 It is a sensitive dye binding, colorimetric method employing Pyrogallol Red. The method seldom stains cuvettes or plastic tubing, and can be automated.

Principle

Pyogallol Red is combined with molybdenum acid at a low pH. When the complex is combined with protein, a blue-purple colour is formed. The increase in absorbance at 600nm is directly proportional to the protein concentration in the sample.

$$\text{Proteins} + \text{Pyrogallol Red} + \text{Molybdate} \xrightarrow[\text{Medium}]{\text{Acidic}} \text{Blue Purple Coloured Complex}$$

Reagent Composition

1. Pyrogallol Red - 0.07%
2. Sodium Azide - 0.05 rnmol/L
3. Activator & Stabilizer

STANDARD - 1000mg/L

Storage Instructions and Reagent Stability

The reagents are stable up to the end of the indicated month of expiry, if stored at 2-8°C.

Warnings and Precaution

1. Microprotein Reagent is for in vitro diagnostic use only.
2. Normal precautions exercised in handling laboratory reagents should be followed.
3. Protein standard contains sodium azide. Do not ingest. May react with lead or copper plumbing to from highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build up.

Waste Management

Please refer to local legal requirements.

Reagent Preparation

The reagents are ready to use.

Specimen

It is recommended that specimen collection be carried out in accordance with NCCLS Document M29-T2. Specimens containing visible particulate matter should be clarified by centrifugation priorto testing.
URINE: Tests are performed on 24-hour samples. The urine should not be collected during periods of exercise because of its effect on albumin concentration. Protein determinations should be performed with fresh specimens. If test cannot be performed with fresh urine, specimens may be stored at -20°C for up to one year.
CSF: Blood contamination should be avoided during CSF collection. If test cannot be performed immediately, specimen may be stored at 2-8°C for up to 72 hours, or -20°C for six months.

Assay Procedure

Wavelength	600 nm
Optical path	1 cm
Temperature	37°C
Measurement	Against reagent blank

	Blank	Sample / Standard
Sample	-	20 l
Reagent	1000 l	1000 l

Mix, incubate 3min. At 37°C and read absorbance.

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Calculation

$$\text{Protein (mg/L)} = \frac{\text{Abs Sample}}{\text{AbsStd}} \times \text{Conc. of Std.}$$

Cone. Of Std. = Concentration of standard (1000 mg/L)

Quality Control

To ensure adequate quality control each run should include assayed normal abnormal controls.

Performance Characteristics

Measuring range

The microprotein procedure is linear to 2000 mg/L. Samples that exceed the linearity limit should be diluted with an equal volume of isotonic saline and re-assayed. Multiply the result by 2 to compensate for the dilution.

Specificity /Interferences

It is recommended not to use urine specimens with added preservatives since some added preservatives such as HCL and benzoic acid have been shown to interfere in the protein assay, giving false low results.7 Some drugs and medications may interfere, see Fujita.[6]

Reference Range [7,9]

Urine : 20 -140 mg/24 hours
CSF : 100-450 mg/L
It is recommended that each laboratory establish its own normal range representing its patient population

Literature

1. Tietz, N.W., Textbook of Clinical Chemistry. W.B. Saunders, Philadelphia p. 608,1986.
2. Viberti, G.C., Pickup, J.C., Jarrett, R.J., Keon, H., N Engl JMed. 300:638-41 1979.
3. Grant, G.H., Kachmer, J.F.: Fundamentals of Clinical Chemistry. N.W. Tietz, Editor, W.B. Saunders, Philadelphia, pp.358-374,1976,
4. Cannon, D.C., Olitzky, I,, Inkpen, J.a,: Clinical Chemistry- Principles and Technics, 2na Ed., R.J. Henry, D.C. Cannon, J.W., Winkleman, Editors, Harper & Row, New York, pp. 442-431, 1974.
5. Pesce, MA, Strande, C.S., Clin Chem 19:1265-1267,1973.
6. Fujita, Y, Mori, I., Kitano, S., BunsekiKagaku 32;E379-386,1983.
7. Watanable, N., Kamel, S., Ohkubo, A., Yamakna, M., ClinChem 32:1551-1554,1986.
8. Tietz, N.W.;ClinicalGuidetolaboratorytests, W.B. Saunders, Phil. P.470, 1990.
9. Tietz, N.W.; Textbook of Clinical Chemistry, W.B. Saunders, Phil. P.608,1986.

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