MICRO PROTEIN KIT

(PYROGALLOL RED - END POINT)

LIQUI STAT

BIO LAB DIAGNOSTICS

ISO 9001:2008
ISO 13485:2012
CE

CLINICAL SIGNIFICANCE

Proteins are involved in the maintenace of the normal distribution of water between blood and the tissues and consist mainly of the albumin and globulin fractions. The measurement of low levels of urinary proteins is important in the detection of renal diseases. Proteinurea occurs in increased glomerular permeability and defective tubular reabsorption. Albuminuria is recognised as an early indicator or reversible renal damage in diabetics. The measurement of CSF proteins is used for the detection of increased permeabiliry of the blood/brain barrier in various diseases.

PRINCIPLE

Proteins in the sample (urine or cerebral spinal fluid) bind pyrogallol red in presence of molybdate and form a colored complex which can be read 600 nM.

REAGENTS COMPOSITION

Micro Protein Reagent 1 - R1

1. Pyrogallol red	60 µmol/L
2. Sodium molybdate	40 µmol/L
3. Sodium oxalate	1.04 mmol/L
4. Sodium benzoate	3.47 mmol/L
5. Methanol	1 mol/L
6. Succinic acid	50 mmol/L

Standard - Std. R2

1. Protein	100 mg/dL 1 g/L

Working Reagent Preparation

All reagents are ready to use.

STORAGE AND STABILITY

When stored at 2-8°C and protected from light, the reagent and standard are stable until the expiry date stated on the label.

SAMPLE

Sample can be urine or cerebral spinal fluid which has no sign of haemolysis. Specimen should be stored at 2-8°C till the test is being performed. No Prior patient preparation is needed. (All samples should be handled as potential infective agents as no laboratory methods make conclusive finding for its safety. Therefore, adequate protective laboratory measures should be taken while handling such materials).

PROCEDURE

All Lab wares should be free of contamination

Pipette in to 3 Test Tubes marked as	BLANK	STD	TEST
Reagent - R1 ..... µL	500	500	500
Distilled Water ..... µL	10	-	-
Standard - R2 ..... µL	-	10	-
Sample ..... µL	-	-	10

Mix and incubate for 3 minutes, read at 600 nM (Red filter) against blank. Record the absorbance of Standard as ΔA std and Test as ΔA test. The final color is stable for at least 1 hour. Reagent and Sample Volume can be altered proportionately.

SYSTEM PARAMETERS

Reaction	End Point
Temperature	RT
Wavelength	600 nM
Factor	Calculate
Standard Concentration	100 mgs/dL
Absorbance Range	0-2 Å
Cuvette Path Lenghth	1cm
Reagent Volume	**500 µL**
Sample Volume	**10 µL**
Reaction Time	2 mins.
Linearity	200 mgs/dL
Max. Limit of Bank Reagent	1.00 Å
Final Colour Stability	60 mins.

RESULT CALCULATION

Proteins (mg/dL) =

$\Delta A\ test$ = OD *Test* – OD Blank
$\Delta A\ std$ = OD *Standard* – OD Blank

$$\frac{\Delta A\ test}{\Delta A\ std} \times 100 = \text{Protein (mg/dL)}$$

Example :
OD Blank = 0.31 and
OD Test = 0.52
OD Standard = 0.61

$$\text{Proteins (mg/dL)} = \frac{0.52 - 0.31}{0.61 - 0.31} \times 100 = 70$$

Take dilution factor into account for the calculation of protein concentration if sample is diluted.

As with all diagnostic methods, the final diagnosis should not be made on the result of a single test as well as laboratory diagnosis must be confirmed with clinical manifestations.

EXPECTED VALUES

Urine :	0 - 140 mg/24 h
Cerebral Spinal Fluid :	10 - 40 mg/dL

Note : It is recommended for each laboratory to establish and maintain its own reference values. Values given here are only an indication.

LIMITATIONS

This assay is linear up to 200 mg/dL proteins. For values higher than 200 mg/dL dilute sample with 09% normal saline and multiply the results by dilution factor i.e. by 2 for 1 : 1 dilution.

Notes: Strong lipemic and hemolytic sera should not be used. Contaminated glassware is the greatest source of error. Disposable plastic ware is recommended for the test.

WARNING

This reagent system is for in vitro use only. This reagent system contains preservatives and components that have not established for safety if contacted on broken skin or eye or taken orally. In case of such incidents wash off with plenty of water, or consult a physician.

QUALITY CONTROL

To ensure adequate quality control, each kit should be tested against standard control sera. It should be realized that the use of quality control material checks both instrument and reagent function together. Factors which might affect the performance of this test include proper instrument function, temperature control, cleanliness of glass wares and accuracy of pipetting.
It is appropriate to establish each laboratory's accuracy constant and interpret values accordingly. Similarly, laboratory findings should be established by clinical manifestations.

BIBLIOGRAPHY

1. Friedenauer S., Berlet HH. Sensitivity and Variability of the Bradford Protein Assay in the presence of detergents. Anal bio. (1989), 178, 263-268.
2. Watanabe Nobuko, Kamei S., Ohkubo A. et Al. - Urinary Protein as Measured with a Pyrogallol Red -Molybdate Complex, Manually and a Hitachi 726 Automated Analyser., Clin. Chem. (1986), 32, 1551.
3. Vassault, A., et al., Protocole de validation de techniques, (Document B, stade 3.) Ann. Biol. Clin., (1986), 44, 686.
4. Young, D. S., Effects of preanalytical variables on clinical laboratory tests, 2nd Ed., AACC Press, (1997).
5. Young, D. S., Effects of drugs on clinical laboratory tests, 4th Ed., AACC Press, (1995).

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