Infinite
Microprotein
Pyrogallol Red

INTRODUCTION

1. ***Infinite*** Microprotein is a reagent set for quantitative determination of protein in human urine & CSF based on Pyrogallol red method.
2. ***Infinite*** Microprotein is a ready-to-use, single liquid reagent system.
3. ***Infinite*** Microprotein, is linear upto 300mg/dL
4. ***Infinite*** Microprotein assay can be performed in 5 minutes at 37° C.

CLINICAL SIGNIFICANCE

Proteinuria occurs with (1) increased glomerular permeability (glomerular proteinuria), in which the urinary protein is mainly albumin; (2) defective tubular reabsorption (tubular proteinuria), in which the urinary proteins are mainly low molecular weight proteins; (3) increased concentration in the plasma of an abnormal Low molecular weight protein, such as immunoglobulin light chains (overload proteinuria); and (4) abnormal secretion of protein into the urinary tract (postrenal proteinuria). The last two are the least common.

Permeability of the blood-CSF barrier to plasma proteins is increased by high intracranial pressure resulting from (1) a brain tumour, (2) intracerebral haemorrhage, or (3) traumatic injury. In addition, increased permeability is seen with inflammation associated with (1) bacterial or viral meningitis, (2) encephalitis, or (3) poliomyelitis. The most striking elevations of CSF total protein are seen in bacterial meningitis. Lumbar CSF protein is increased when the CSF circulation is mechanically obstructed above the puncture site and plasma proteins equilibrate across the walls of meningeal capillaries into the stagnant CSF. The effect of any of these conditions is that the proportions of specific proteins in CSF increasingly resemble those in serum.

PRINCIPLE

Pyrogallol red is combined with molybdenum acid at a low pH. When the complex is combined with protein, a blue-purple colour is formed. The intensity of the colour developed is directly proportional to the protein concentration in the sample and is measured at 600nm.

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

REAGENT STORAGE, STABILITY & HANDLING

The reagent is ready-to-use.
The reagent kit should be stored at 2° - 8° C and is stable till the expiry date indicated on the label.
Keep the Reagent away from direct light sources. Do not freeze the reagent.
Contamination of the reagent should be strictly avoided.

COMPONENTS & CONCENTRATION OF REAGENT

Component	Concentration
Pyrogallol red	> 0.037 mmol/l
Sodium molybdate	> 0.029 mmol/l

SPECIMEN COLLECTION & PRESERVATION

24-hour or random/first morning midstream urine is the specimen for this assay.

Specimen should be tested immediately, preferably within 12 hours of collection. Specimen can be stored upto 2 days at 2-8°C provided they are not contaminated.

Specimen should be free from particulate matter. Turbid or particulate urine specimen must be clarified by centrifugation at 2000 rpm for 10 minutes. Use the clear supernatant for testing.

Blood contamination should be avoided during CSF collection. Ideal Collection procedures i.e. disinfecting the site & collecting with aseptic precaution, should be followed.

PROCEDURE

- **Reaction type…….....End-Point**
- **Wavelength…….…….600nm(578/630 nm)**
- **Flowcell temperature……...……...37° C**
- **Zero setting with…….....Reagent**
- **Sample volume……..……...20 µl**
- **Reagent volume……..…….....1000 µl**
- **Standard Concentration…….……100mg/dL**
- **Linearity…….…….....300mg/dL**

Manual assay procedure:
Prewarm at room temperature (25°C -30°C) the required amount of reagent before use. Perform the assay as given below:

	Blank	Standard	Sample
Sample	-	-	20µl
Standard	-	20µl	-
Reagent	1000µl	1000µl	1000µl
Mix well. Incubate at 37°C for 5 minutes. Measure absorbance of Standard and test against Blank at 600nm (578/630nm) within 30 minutes.			

Calculation:

$$\text{Protein (mg/dL)} = \frac{\text{Absorbance of Sample}}{\text{Absorbance of Standard}} \text{X } 100$$

EXPECTED VALUES

Urine

Random: 1-14 mg/dL
Normal condition: 10 - 100 mg/day
Pregnancy: <150 mg/day
CSF: < 45 mg/dL

Expected range varies with regards to the time of collection of the urine sample.
It is recommended that each laboratory should establish its own normal reference range.

NOTES

For in vitro diagnostic use only. The reagent to be handled by entitled and professionally educated person.
Reagents of the kit are not classified like dangerous.
High concentration of chelating agents and traces of detergents may interfere. Hence their presence should be avoided.
Small amounts of protein attached to the cuvette wall after measurement of certain other tests will cause an erroneously high measured value when the test solution is transferred to the cuvette. If this should occur, wash the cuvette completely and measure again. If the absorbed protein cannot be removed completely by washing with water, clean the cuvette with an alkaline solution containing hypochlorite and wash thoroughly with water.

PERFORMANCE CHARACTERISTICS

1. **Linearity limit:** If the protein concentration exceeds 300 mg/dL dilute the specimen with normal saline (0.9% NaCl) and repeat the assay. The result obtained should be then multiplied with dilution factor to obtain correct protein concentration.
2. **Detection limit:** Value less than 5 mg/dL gives non-reproducible results.
3. **Interferences:** Haemoglobin shows about one-half the color of albumin. If haematuria is present, a falsely high value will be measured.
4. **Precision Studies:**

	Within Run (n=10)			Between Run (n=20)		
	Mean (mg/dL)	SD (mg/dL)	CV%	Mean (mg/dL)	SD (mg/dL)	CV%
Sample 1	23.44	1.09	4.7	10.68	0.40	3.8
Sample 2	61.56	2.12	3.45	48.24	1.25	2.6

QUALITY CONTROL

To ensure adequate quality control, it is recommended that each laboratory should use a normal and an abnormal commercial reference control material. It should be realized that the use of quality control material checks both reagent and instrument functions together. Factors which might affect the performance of this test include instrument function, temperature control, cleanliness of glassware and accuracy of pipetting.

REFERENCES

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2. Tietz N. W., (Ed.),Textbook of Clinical Chemistry. Burtis CA and Ashwood ER, Sixth Edition (2015).
3. Yoshizaki, H., Osawa, S. and Furuya, S.: The Japanese Journal of Clinical Pathology, 32 suppl. 227 (1984).
4. Fujita, Y., Mori, I. and Kitano, S.: Bunseki Kagaku, 32, 379 (1983).
5. In-house data: Accurex Biomedical Pvt. Ltd. (2017)

IVD	*In vitro* diagnostic *use only*		Date of manufacturing
	Consult instructions for use		Use by (YYYY-MM-DD)
REF	Catalogue Number		Temperature Limitation
LOT	Batch Code		Manufacturer

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