ULTRASENSITIVE PROTEIN
(M-TP)

INTENDED USE

Vitro ultrasensitive protein reagent is intended for the in vitro quantitative determination of protein in urine and cerebrospinal fluid (CSF) on both automated and manual systems.

METHOD

Colorimetric endpoint pyrogallol red / SDS method[1]

BACKGROUND

Urine is formed by ultrafiltration of plasma across the glomerular capillary wall. Proteins with a relative molecular mass >40 000 are almost completely retained, while smaller substances easily enter the glomerular filtrate. Most CSF protein originates by diffusion from plasma across the blood-CSF barrier. Elevated levels occur as a result of increased permeability of the blood-CSF barrier or with increased local synthesis of immunoglobulins[2]. Measurement of urine protein is becoming increasingly important in the detection of renal pathology[3]. Proteinuria can occur in increased glomerular permeability, defective tubular reabsorption and abnormal secretion of protein into the urinary tract[4]. Albuminuria has been recognized as an early indicator of renal damage in diabetes that can be reversed if detected and treated sufficiently early[5]. The measurement of CSF total protein and specific protein is used to detect increased permeability of blood/brain barrier (the capillary endothelium of vessels of the central nervous system) to plasma proteins or to detect increased intrathecal secretion of immunoglobins[3]. Protein measurements in urine are used in the diagnosis and treatment of disease conditions such as renal or heart diseases, or thyroid disorders, which are characterized by proteinuria or albuminuria. CSF protein measurements are used in the diagnosis and treatment of conditions such as meningitis, brain tumors, and infections of the central nervous systems[2].

ASSAY PRINCIPLE

Turbidimetric methods using trichloroacetic acid (TCA) or sulfosalicylic acid (SSA) require precipitation of the protein in the sample; the resulting turbidity may be unstable and flocculate. Vitro ultrasensitive protein reagent is based on the procedure developed by Watanabe et al[1] which is a dye-binding colorimetric method utilizing pyrogallol red-molybdate complex, and modified[6] to equalize the reactivity of albumin and g-globulin, and provide good precision and linearity.

The pyrogallol red molybdenum acid, forming a red complex, when this complex is combined with protein in acidic conditions, forms a blue-purple color complex. Sodium dodecyl sulphate is added to increase accuracy in measuring proteins other than albumin.

Protein+ pyrogallol red molybdate $\xrightarrow{\text{Acidic medium}}$ **Blue-purple color complex**

The intensity of color measured photometrically at 600 nm, and its intensity is directly proportional to protein concentration in the specimen.

EXPECTED VALUES

CSF′	
Adults	15 – 45 mg/dl
Urine	
Random specimen	< 10 mg/dl
24 hrs specimen	28 – 141 mg/day

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference range.

REAGENTS

R_1	Standard	100	mg/dl
R_2	Succinate buffer	50	mmol/l
	Pyrogallol red	0.06	mmol/l
	Sodium molybdate	0.04	mmol/l
	SDS	0.1	mmol/l

- **Reagent Preparation & Stability**

All reagents are stable up to the expiry date given on label when stored at 2 – 8^0 C.

SPECIMEN

- Urine and cerebrospinal fluid (CSF).

Specimen Preparation & Stability

Urine

- 24 hours specimens should be collected (keep specimen on ice during collection). No preservatives are required[7].
- Random urine may be used, first morning specimen is preferred for random specimens.
- Stored urine specimen must be mixed well prior analysis.

CSF

- CSF specimen must be free from hemolysis. Centrifuge before analysis.
- CSF may be stored at 4^{0}C for 72 hours. Stable at -20^{0}C for 6 months or more at -80^{0}C, specimens should not contain blood[7].

PROCEDURE

- **Manual Procedure**

Wavelength	578 - 630 nm
Cuvette	1 cm light path
Temperature	20-25 ^{0}C
Zero adjustment	against reagent blank
Specimen	urine or CSF

	Blank	Standard	Specimen
R_2	1.0 ml	1.0 ml	1.0 ml
Standard	……	20 µl	……
Specimen	……	……	20 µl

Mix, and Incubate for 10 minutes at room temperature. Measure the absorbance of specimen ($A_{specimen}$) and standard ($A_{standard}$) against reagent blank.
The color is stable for 30 minutes.

- **Automated Procedure**
User defined parameters for different auto analyzers are available upon request.

CALCULATION

Calculate the protein concentration in urine or CSF by using the following formulae:

Protein Concentration=

$$\frac{\text{Absorbance of Specimen}}{\text{Absorbance of Standard}} \times \text{Standard value}$$

QUALITY CONTROL

It is recommended that controls (normal and abnormal) be included in:

- Each set of assays, or
- At least once a shift, or
- When a new bottle of reagent is used, or

Medical Device Safety Services
MDSS GmbH
Burckhardtstr. 1
30163 Hannover, Germany
Tel.: +49 511 6262 8630
Fax: +49 511 6262 8633

Article # : 130-EN
Date of Revision : 10/2012

CE

- After preventive maintenance is performed or a clinical component is replaced.

Commercially available control material with established protein values may be routinely used for quality control.

Failure to obtain the proper range of values in the assay of control material may indicate:

- Reagent deterioration,
- Instrument malfunction, or
- Procedure errors.

The following corrective actions are recommended in such situations:

- Repeat the same controls.
- If repeated control results are outside the limits, prepare fresh control serum and repeat the test.
- If results on fresh control material still remain outside the limits, then repeat the test with fresh reagent.
- If results are still out of control, contact Vitro Technical Services.

INTERFERING SUBSTANCES

- **Bilirubin:**
  No significant interference from free or conjugated bilirubin up to a level of 20 mg/dl. Interference may increase or decrease sample results.
- **Haemolysis:**
  Due to protein nature of haemoglobin hemolysis leads elevated values dependent on the degree of lysis of the erythrocytes.
- **Drugs:**
  Young[8] in 1990 has published a comprehensive list of drugs and substances, which may interfere with this assay.

- Vitro ultrasensitive protein reagent is for in vitro diagnostic use only. Normal precautions exercised in handling laboratory reagents should be followed.
- The reagent and specimen volumes may be altered proportionally to accommodate different spectro-photometer requirements.
- Valid results depend on an accurately calibrated instrument, timing, and temperature control.
- Don't use if the reagent appears turbid or has precipitation.

PERFORMANCE CHARACTERISTICS

Imprecision

Reproducibility was determined using in an internal protocol. The following results were obtained.

	Within Run	
Control	Level I	Level II
Number of samples	40	40
Mean (mg/dl)	2.8	4.4
SD (mg/dl)	0.47	0.6
CV (%)	1.7	1.4

	Between Day	
Control	Level I	Level II
Number of samples	40	40
Mean (mg/dl)	2.8	4.4
SD (mg/dl)	0.7	1
CV (%)	2.2	2.4

Method Comparison

A comparison of the VITRO Microprotein Reagent (y) with a commercial reagent of the same method (x) was performed on 102 urine samples in a range of 1.1 - 108.7 mg/dl.

A correlation coefficient of 0.999 was obtained; the linear regression equation was y =1.053x - 0.436.

Sensitivity

The sensitivity is defined as the change of analytical response per unit change in analyte concentration at a path length of 1 cm.

When run as recommended the sensitivity of this assay is 0.6 mg/dl.

LINEARITY

When run as recommended, the assay is linear up to 400 mg/dl.

Specimens with values above 400 mg/dl should be diluted with distilled water and reassayed. Multiply the result by the dilution factor.

BIBLIOGRAPHY

1. **Watanabe et al., (1986):** Clin. Chem. 32/8:1551-1544.
2. **Tietz, NW. (1987):** Fundamintals of Clinical Chemistry. 3rd ed. Philadelphia, Pa: WB Saundes co; 336, 339, 340, 341.
3. **Carone, F.A. et al., (1979):** Kidney Int. 16: 271-8.
4. **Teitz, NW. (1986):** Textbook of Clinical Chemistery. W.B. Saunders Co. pp. 602-613.
5. **Viberti, G.C, et al., (1979):** N. Engl. J. Med. 300: 638-41.
6. **Orsonneau, JL. (1989):** Clin. Chem.; 35: 2233-6.
7. **Tietz, NW, ed (1990):** Clinical Guide to laboratory Tests. 3rd ed. Philadelphia: WB Saunders; 470-473
8. **Young, DS (1990):** Effects of Drugs on Clinical Laboratory Tests. Third Edition. 1990: 3: 6-12.

SYMBOL DECLARATION

	Manufacturer
	Consult instructions for use
LOT	Batch code (Lot #)
REF	Catalog number
	Temperature limitation
IVD	In vitro diagnostic medical device
	Use by
	Caution. Consult instructions
	Keep away from light

ORDERING INFORMATION

REF	SIZE
1331	2 X 50 ml
1332	2 X 100 ml

Manufactured in Egypt by:
Vitro Scient
www. vitroscient.com

Technical Support:
+202 26439699
info@vitroscient.com

orders:
+202 26439698
order@vitroscient.com

Article # :130 -EN
Date of Revision : 10/2012

CE