Protein in urine and CSF

Pyrogallol red. Colorimetric

Quantitative determination of total urinary and CSF protein

CE IVD

PACKAGING

Ref.: 101-0459	Cont.: 2 x 100 mL

Store at 2 - 8º C

CLINICAL SIGNIFICANCE

In healthy persons, the urine contains no protein or only a trace amount of protein; normally the glomeruli prevent passage of protein from the blood to the glomerular filtrate. Glomerular injury causes increased permeability to plasma proteins, resulting in proteinuria, which refers to the presence of protein in the urine. A persistent finding of proteinuria is the single most important indication of renal disease. Elevated concentration of protein in cerebro-spinal fluid (CSF) can be cause by infections and intracranial pressure[1,5,6].

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Protein react in acid solution with pirogallol red and molybdate to form a colored complex.

The intensity of the color formed is proportional to the protein concentration in the sample[1,2].

REAGENTS

R	Pyrogallol red Sodium molybdate	50 mmol/L 0.04 mmol/L
PROTEIN U & CSF CAL	Albumin/Globulin aqueous primary standard 1000 mg/L	

PREPARATION

The reagents and standard provided are ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2 - 8º C protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.
- Blank absorbance (A) at 598 nm ≥ 0.70.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 598 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment.

SAMPLES

- Urine 24 h: Stability 8 days at 2 - 8º C.
- Cerebrospinal fluid (CSF): Stable 4 days at 2 - 8º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

PROTEIN U & CSF CAL: Proceed carefully with this product because due its nature it can get contamined easily.

Use clean disposable pipette tips for its dispensation.

Assay conditions:

Wavelength: . . . . . .598 nm
Cuvette: . . . . . 1 cm light path
Temperature . . . . . .37º C / 15 - 25º C

1. Adjust the instrument to zero with distilled water.
2. Pipette into a cuvette:

	Blank	Standard	Sample
R (mL)	1.0	1.0	1.0
Standard(Note 1-2) (µL)	--	20	--
Sample (µL)	--	--	20

3. Mix and incubate for 5 min at 37º C or 10 min at room temperature (15 - 25º C).
4. Read the absorbance (A) of the samples and Standard, against the Blank. The color is stable for at least 30 minutes.

CALCULATIONS

Urine 24 h

$$\frac{(A)Sample-(A)Blank}{(A)Standard-(A)Blank} \times 1000 \times \text{vol. (L) urine 24 h} = \text{mg protein/24 h}$$

CSF

$$\frac{(A)Sample-(A)Blank}{(A)Standard-(A)Blank} \times 1000\ (\text{Standard conc.}) = \text{mg/L protein in the sample}$$

QUALITY CONTROL

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[5]

Urine:	< 100 mg/24 h (< 150 mg/24 h in pregnancy)
CSF:	Children 300 - 1000 mg/L Adults 150 - 450 mg/L

These values are for orientation purpose; each laboratory should establish its own reference range.

PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* 9.44 mg/L up to *linearity limit* of 4000 mg/L.

If the concentration is greater than linearity limit, dilute the sample 1/2 with NaCl (9 g/L) and multiply the result by 2.

Precision:

	Intra-assay (n= 20)			Inter-assay (n= 20)		
Mean (mg/L)	220	536	1014	216	499	1018
SD	3.7	4.0	5.2	18.3	26.1	166.1
CV (%)	2.28	0.75	0.51	7.35	5.22	16.43

Sensitivity: 1mg/L = 0.00026 (A).

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 50 samples were the following:

Correlation coefficient $(r)^2$: 0.9338

Regression equation: y = 0.4294x – 5.4159

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

Hemolysis[1,2].

A list of drugs and other interfering substances with protein determination has been reported.

BIBLIOGRAPHY

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