



Proti U/LCR

Quantitative colorimetric method for protein determination
in urine and cerebrospinal fluid

SUMMARY

Proteins in urine

Small quantities of plasmatic proteins of low molecular weight are normally filtered through the renal glomeruli and then, in part, are reabsorbed by the renal tubules.

There are physiologic or benign conditions where an increase of protein urinary excretion may be found such as vigorous exercise, fever, hypothermia, pregnancy.

The measurement of urinary proteins is important in the renal pathology's detection. Proteinuria in renal disease may result in glomerular or tubular dysfunction. Glomerular dysfunction is due to an increase in the passage through glomerular capillaries and is characterized by a loss in plasmatic proteins with similar or larger size. Tubular dysfunction is due to a decrease of the tubule's protein absorption capacity. Among the pathologies that show an increase in the urinary protein excretion are found: nephritic syndrome, nephrotic syndrome, monoclonal hypergammaglobulinemia, diabetic nephropathy and infections in the urinary tract.

Cerebrospinal fluid (CSF) Proteins

Protein determination in CSF is useful to assess the permeability of the hematoencephalic barrier in many inflammatory or infectious diseases of the Central Nervous System (CNS), as it is occur in bacterial meningitis, viral meningitis or meningitis from other origins, encephalitis, poliomyelitis, neurosyphilis, multiple sclerosis, brain hemorrhage, cerebral or spinal tumors. Other disorders, as demyelination diseases, cause an abnormal protein production within the CNS. The present method's sensitivity makes it suitable to be used in biological fluids such as urine and cerebrospinal fluid, where protein concentration in relation to plasma is too low to be determined using the conventional methods for protein test in serum.

PRINCIPLE

Proteins present in sample react in acid media with the pyrogallol red-molybdate complex originating a new colored complex spectrophotometrically quantifiable at 600 nm.

PROVIDED REAGENTS

A. Reagent A: 0.1 mmol/l Pyrogallol Red stabilized solution, 0.1 mmol/l sodium molybdate in 50 mmol/l succinate buffer.

S. Standard: 100 mg/dl (1.0 g/l) albumin solution.

NON-PROVIDED REAGENTS

- Distilled water.
- Saline solution.

INSTRUCTIONS FOR USE

Provided Reagents: ready to use.

WARNINGS

Reagents are for "in vitro" diagnostic use.

Use the reagents according to the working procedures for clinical laboratories.

The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS

Provided Reagents: are stable in refrigerator at 2-10°C until the expiration date stated on the box. The Reagent A should be protected from light.

INSTABILITY OR DETERIORATION OF REAGENTS

When the spectrophotometer has been set to zero with distilled water, Reagent A absorbance readings (at 600 nm) above 0.250 O.D. or below 0.030 O.D. indicate its deterioration.

SAMPLE

Urine or cerebrospinal fluid

a) Collection: obtain occasional or 24-hour urine. Measure diuresis. If samples are turbid, it is convenient to centrifuge them.

b) Additives: not required.

c) Known Interfering Substances:

- hemolysis may cause falsely increased results in urine as well as in CSF.
- urine preservatives such as hydrochloric acid, benzoic acid or thymol may cause falsely decreased results;
- some drugs or medications may interfere in the reaction. See Young, D.S. in References for effect of drugs on the present method.

d) Stability and storage instructions: urine may be stored at 2-10°C for up to 8 days or at -20°C for up to 3 months. CSF may be stored for up to 3 days at 2-10°C or for up to 3 months frozen at -20°C.

REQUIRED MATERIAL (non-provided)

- Spectrophotometer for readings at 600 nm (580-620 nm).
- Water bath at 37°C.
- Pipettes and micropipettes for measuring the stated volumes

PROCEDURE

Bring reagents and samples to room temperature before use. In three tests tubes or spectrophotometric cuvettes

labeled B (Blank), S (Standard), and U (Unknown), place:

	B	S	U
Standard	-	20 ul	-
Sample	-	-	20 ul
Reagent A	1 ml	1 ml	1 ml

Mix and incubate the tubes for 10 minutes at 37°C. Measure in photocolormeter between 580-620 nm or in spectrophotometer at 600 nm, setting the instrument to zero with the Blank.

STABILITY OF FINAL REACTION

Reaction color is stable for 30 minutes. Therefore, read the absorbance within that period.

CALCULATIONS

1) 24-hour urine Proteins

$$\text{mg of Proteins/24 hours} = \frac{U}{S} \times V \times 1000$$

where:

V = diuresis volume expressed in liters/24 hours

1000 = mg/l Standard

2) Proteins in occasional urine

$$\text{mg/dl proteins} = \frac{U}{S} \times 100$$

3) Proteins in CFS

$$\text{mg/dl proteins} = \frac{U}{S} \times 100$$

QUALITY CONTROL METHOD

Each time the test is performed, analyze two levels of a quality control material (**Proti U/LCR Control 2 niveles**) with known proteins concentration.

REFERENCE VALUES

24-hour urine: 30-140 mg/24 hours (up to 160 mg/24 hours in pregnant women)

Occasional urine: 25 mg/dl

CSF: 15-45 mg/dl in healthy individuals. In persons over 60 years old, this range is extended up to 60 mg/dl.

These values should be considered as an orientation. Each laboratory should establish its own values, since they may vary according to the patients' population and laboratory conditions.

SI SYSTEM UNITS CONVERSION

Proteins (g/dl) x 10 = Proteins (g/l)

PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE. Protect the Reagent A from the light. The material used should be

free from surfactants. Otherwise, discordant values may be obtained.

PERFORMANCE

a) Reproducibility: processing replicates of the same samples in one day, the following results were obtained:

Level	S.D.	C.V.
14 mg/dl	± 0.66 mg/dl	4.7 %
100 mg/dl	± 2.30 mg/dl	2.3 %

b) Sensitivity: in spectrophotometer at 600 nm, a 100 mg/dl Standard gives a reading of approximately 0.200 O.D. Thus, for 0.001 O.D. the smallest detectable activity change will be of 0.5 mg/dl.

c) Linearity: reaction is linear up to 150 mg/dl proteins. For higher values, dilute sample 1:2 or 1:4 with saline solution and repeat determination. Correct calculations multiplying by the dilution factor used. To increase the analytic sensitivity in normal or lightly increased samples, 50 ul sample may be used. In that case, it is advisable to dilute the Standard 1:2 (1+1) with distilled water and use this 50 mg/dl standard in the test to adjust the calibration to low normal values.

PARAMETERS FOR AUTOANALYZERS

For programming instructions check the user manual of the autoanalyzer in use.

WIENER LAB PROVIDES

- 1 x 100 ml (Cat. Nr. 1690007).
- 4 x 20 ml (Cat. Nr. 1009317).
- 4 x 20 ml (Cat. Nr. 1009282).
- 2 x 60 ml (Cat. Nr. 1009631).

REFERENCES

- Watanabe, N.; et.al. - Clin. Chem. 32:1551, 1986.
- Fujita, Y.; Mori, I.; Kitano, S. - Benseki Kagaku 32:379, 1983.
- Watson, M.; Scott, M. - Clin. Chem. 41/3:343, 1995.
- Killingsworth, L. - Clin. Chem. 28/5:1093, 1982.
- Orsonneau, J.; Douet, P.; Massoubre, C.; Lustenberger, P.; Bernard, S. - Clin. Chem. 35/11:2233, 1989.
- Young, D.S. - "Effects of Drugs on Clinical Laboratory Tests", AACC Press, 4th ed., 2001.

Symbols

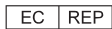
The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.



This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices



Manufactured by:



Authorized representative in the European Community



Harmful



"In vitro" diagnostic medical device



Corrosive / Caustic



Contains sufficient for <n> tests



Irritant



Use by



Consult instructions for use



Temperature limitation (store at)



Do not freeze



Calibrator



Biological risks



Control



Volume after reconstitution



Positive Control



Contents




Negative Control



Batch code



Catalog number

 Wiener Laboratorios S.A.I.C.
Riobamba 2944
2000 - Rosario - Argentina
<http://www.wiener-lab.com.ar>
Dir. Téc.: Viviana E. Cétola
Bioquímica
A.N.M.A.T. Registered product
Cert. N°: 1128/95



Wiener lab.

2000 Rosario - Argentina