Cromatest

LINEAR

PROTEIN (URINE AND CSF) CE

REF 1162005
2 x 50 mL
CONTENTS
R1. Reagent 2 x 50 mL
CAL. Standard 1 x 3 mL
For *in vitro* diagnostic use only

PROTEIN
URINE AND CSF
Colorimetric method
ENDPOINT

PRINCIPLE

The method[1] measures the shift in the absortion spectrum from 460 to 600 nm of the complex that occurs at acid pH between pyrogallol red-molibdate (PRM) and the basic amino groups of urine and cerebrospinal fluid (CSF) proteins. The intensity of the colored complex formed is proportional to the concentration of protein in the sample.

$$\text{PRM} + \text{Urine/CSF protein} \xrightarrow{\text{pH 2.5}} \text{PRM-protein complex}$$

REAGENT COMPOSITION

R1 **Pyrogallol reagent**. Succinate buffer 60 mmol/L pH 2.5, pyrogallol red 0.06 mmol/L, sodium molibdate 0.04 mmol/L, SDS 0.08 mmol/L. **X_n. R20/22 S:24/25**.

CAL **Urine protein standard**. Albumin/Globulin 200 mg/dL (2 g/L). Buffered mixture (80/20) on an artificial matrix. Biocides. Concentration value is traceable to Standard Reference Material 927.

STORAGE AND STABILITY

Store **R1** at 15-30ºC, and **CAL** at 2-8ºC once opened.
All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date.
Store the vials tightly closed, protected from light and prevented contaminations during the use.
Discard If appear signs of deterioration:
- Presence of particles and turbidity.
- Blank absorbance (A) at 600 nm > 0.200 in 1cm cuvette.

REAGENT PREPARATION

The reagents are ready-to-use.

SAMPLES

Urine collected without preservatives and CSF (see Notes).
Turbid specimens should be centrifuged before testing.
Urine proteins are stable up to 8 days at 2-8ºC, and for 3 months at –20ºC.
CSF proteins are stable for 3 days at 2-8ºC and for 3 months at –20ºC.

INTERFERENCES

- Bilirubin (< 5 mg/dL) does not interfere.
- Hemoglobin may affect the results.
- Other drugs and substances may interfere[3].
- Positive interferences in urine of patients under treatment with aminoglycosids-gentamicine or tobromycine-reported with other pyrogallol tests have been shown to have no influence with this specific formulation.[2]
- CSF contaminated by red cells from a traumatic lumbar puncture or intracerebral hemorrhage will increase protein concentrations by ≈ 10 mg/L for every 1000 erithrocytes.[5]

MATERIALS REQUIRED

- Photometer or colorimeter capable of measuring absorbance at 600 ± 20 nm.
- Constant temperature incubator set at 37ºC.
- Pipettes to measure reagent and samples.

PROCEDURE

1. Bring reagents and samples to room temperature.
2. Pipette into labelled tubes:

TUBES	Blank	Sample	CAL. Standard
R1. Reagent	1.0 mL	1.0 mL	1.0 mL
Sample	–	20 µL	–
CAL.Standard	–	–	20 µL

3. Mix and incubate the tubes 5 minutes at 37ºC or 10 minutes at room temperature.
4. Read the absorbance (A) of the samples and the standard at 600 nm against the reagent blank.

The color is stable for 30 minutes protected from light.

CALCULATIONS

Urine

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times V \times 2000 = \text{mg/24-h}$$

V = Liters urine/ 24-h
2000 = mg/L standard

Urine (single samples), CSF

$$\frac{A_{\text{Sample}}}{A_{\text{Standard}}} \times C_{\text{Standard}} = \text{mg/dL protein (see Notes)}$$

Samples with concentrations higher than 400 mg/dL should be diluted 1:2 with saline and assayed again. Multiply the results by 2.

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687

REFERENCE VALUES[4]

Urine

Adults	24-h samples : < 150 mg/ 24-h single samples: < 25 mg/dL

CSF

Adults	< 45 mg/dL
Children	< 100 mg/dL

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used.
To ensure adequate quality control (QC), each run should include urine controls with assayed values handled as unknowns.
If the values are found outside of the defined range, check the instrument, reagents and procedure.
Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

Total protein in the urine measurement is increasingly being replaced by the measurement of albumin, as this is the predominant urinary protein as this have demonstrated improved sensitivity and specificity for glomerular permeability changes.
The presence of increased urinary excretion signals an increase in the transcapillary escape rate, being usually a marker of microvascular disease eventhough it may be also altered by physiological factors (exercise, diuresis and posture) as a consequence of altered intrarenal hemodynamics.
The tubular reabsortive process is saturable and any increase in the glomerular permeability or in plasma concentration (e.g., of Bence-Jones protein), or decreases in reabsortive capacity due to proximal tubular damage (e.g., from nephrotoxic drugs) can result in proteinuria.
Persistent urinary albumin excretion preceeds and is highly predictive of diabetic nephropathy, end-stage renal disease, and proliferative retinopathy in type I diabetes.[5]
Measurement of CSF protein is used to distinguish septic from aseptic meningitis. Protein concentrations > 1 g/L are often viewed as diagnostic for bacterial, fungal, or tuberculous meningitis.[6]

NOTES

- Currently samples acceptable are : 24-h collection; overnight (8-12-h) collection; 1-2-h collection, or first morning sample. Because the high intraindividual and diurnal variation, at least three separate samples should be assayed.
- To increase the sensitivity in the normal range test 50 µL of sample, and dilute the standard 1:4 (1+3) with saline. Use the new concentration of 50 mg/dL for the calculations.
- This method may be used with different instruments. Any application to an instrument should be validated to demonstrate that results meets the performance characteristics of the method. It is recommended to validate periodically the instrument. Contact to the distributor for any question on the application method.
- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

ANALYTICAL PERFORMANCE

- ***Detection Limit :*** 8 mg/dL
- ***Linearity :*** Up to 400 mg/dL
- ***Precision:***

mg/dL	**Within-run**		**Between-run**	
Mean	73	279	73	279
SD	1.0	2.0	2.0	5.0
CV%	1.35	0.67	3.09	1.84
N	10	10	10	10

- ***Sensitivity :*** 2.3 mA / mg/dL proteins.
- ***Correlation.*** This assay (y) was compared with a similar commercial method (x). The results were:
  N = 50 r = 0.99 y = 0.95x - 0.01

The analytical performance have been generated using on authomatic instrument. Results may vary depending on the instrument.

REFERENCES

1. Orsonneau, J.L., Dovet, P., Massoubre, C., Lustenberger, P., and Bernard, S. Clin. Chem. 35: 2233 (1989).
2. Koerbin, G., Taylor, L., Dutton, J., Marshall, K, Low, P., and Potter, J.M. Clin. Chem. 47: 2183 (2001).
3. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.
4. Tietz. N.W. Clinical Guide to Laboratory Tests, 3rd Edition. W.B. Saunders Co. Philadelphia, PA. (1995).
5. Greenlee, S.E. Infect. Dis. Clin. North Am. 4: 583 (1990).
6. Viberti, G.C., Hill, R.D., and Jarret, R.J. Lancet, I : 1430 (1982). Bonadio, W.A. Pediatr. Infect. Dis. J. 11: 423 (1992).

B1162-2/0901
R1.ing

QUALITY SYSTEM CERTIFIED
ISO 9001 ISO 13485

LINEAR CHEMICALS, S.L.U. Joaquim Costa 18 2ª planta. 08390 Montgat (Barcelona) SPAIN
Telf. (+34) 934 694 990; E-mail: info@linear.es ; website: www.linear.es NIF-VAT:B60485687