Liquick Cor-URINE PROTEINS

DIAGNOSTIC KIT FOR DETERMINATION OF TOTAL PROTEINS IN URINE AND CEREBROSPINAL FLUID

Cat. No

2 - 199

Kit name Liquick Cor-URINE PROTEINS "bulk"

INTRODUCTION

In healthy people with properly functioning kidneys proteins are actively reabsorbed in the proximal tubules and only small amounts proteins (several mg per day) are excreted in urine. The measurement of total proteins concentration 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.

The measurement of total proteins in cerebrospinal fluid (CSF) is especially useful in detecting increased permeability of the bloodbrain barrier and to detection increased intrathecal synthesis of immunoglobulins. Increase the concentration of protein in CSF may indicate brain tumors. Intracerebral hemorrhage, brain injury, bacterial and viralencephalitis and multiple sclerosis.

METHOD PRINCIPLE

Direct, colorimetric method with pyrogallol red.

At an acidic pH the protein aminoacid groups, with the pyrogallol red-molibdate complex, form a coloured compound which shows a maximum absorbance at a wavelength of 600 nm. Colour intensity is proportional to the concentration of proteins in the sample.

REAGENTS Package

8	Liquick Cor-URINE PROTEIN	
	"bulk"	
1-URINE PROTEINS	$1 \times 5000 \text{ ml}$	

Reagent preparation and stability

Reagent ready to use. The reagent when stored at $15-25^{\circ}$ C is stable up to expiry date printed on the package. The reagents are stable for 12 weeks on board of the analyser at 2-10°C.

Concentrations in the test

Succinate buffer	50 mmol/l
Pyrogallol red	0.06 mmol/l
Sodium molibdate	0.04 mmol/l

Warnings and notes

- Product for in vitro diagnostic use only.
- The reagents must be used only for intended purpose by suitably qualified laboratory personnel, under appropriate laboratory conditions.
- Do not use after expiry date.
- Protect from light evaporation and avoid contamination!
- Immediately after use, recap bottle and store at 15-25°C!
- Do not interchange caps.
- Do not freeze reagent.
- Reagent should be mixed before use by gentle inverting the bottle several times.
- The appearance of turbidity or control urine values outside the manufacturer's acceptable range may indicate of reagent instability.

ADDITIONAL EQUIPMENT

- automatic analyzer or photometer able to read at 600 nm
- thermostat at 37°C;
- general laboratory equipment;



SPECIMEN

Urine: Urine used for analysis may come from the first morning sample, random sample or timed collection sample according to the classification by ECLM ^[2]. In order to collect and prepare the samples only dedicated tubes and containers should be used. Do not use preservatives.

Freshly collected urine should be kept at room temperature for about an hour and then cooled to 4°C. Direct reduction of the temperature on freshly collected urine can cause precipitation of minerals. Samples with visible turbidity should be centrifuged before analysis. Determination of uncentrifuged samples may give increased results.

Cerebrospinal fluid: CSF should be centrifuged before analysis. The presence of blood in samples of cerebrospinal fluid may result in false results of protein determination. For proper interpretation of the results, cerebrospinal fluid must be determined simultaneously with a sample of blood taken from a patient at the same time.

Urine samples are stable for 2 days at 2-8°C^[6].

The stability of the cerebrospinal fluid is 3 days at 2-8°C, 6 months at the temperature -20°C ^[6].

Nevertheless it is recommended to perform the assay with freshly collected samples!

PROCEDURE

These reagents may be used for manual assay and in several automatic analysers. Applications for them are available on request.

Manual procedure

wavelength	600 nm
temperature	37°C
cuvette	1 cm

Pipette into the cuvette:

	blank	test	standard
	(B)	(T)	(S)
1-URINE PROTEINS	1000 µl	1000 µl	1000 µl

Bring up to the temperature of determination about 10 minutes. Then add:

calibrator	-	-	50 µl	
sample	-	50 µl	-	
deionised water	50 µl	-	-	
		~		

Mix thoroughly and incubate 5 minutes. Read the absorbance of test (T) and standard (S) against blank (B).

Calculation rule

Protein concentration = $A(T) / A(S) \times$ calibrator concentration

For the calculation of proteins excreted over 24 hours, multiply the concentration (mg/dl) by the volume (dl) of the 24 hours urine.

REFERENCE VALUES ^[7, 10]

urine (adults)	<15 mg/dl (0.15 g/l)		
urine 24-h (adults)	< 100 mg (0.10 g)		
cerebrospinal fluid	mg/dl	g/l	
0 - 4 weeks	20 - 80	0.20 - 0.80	
> 4 weeks, adults	15 - 45	0.15 - 0.45	

It is recommended for each laboratory to establish its own reference ranges for local population.

QUALITY CONTROL

For internal quality control it is recommended to use the CORMAY URINE CONTROL LEVEL 1 (Cat. No 5-161) and LEVEL 2 (Cat. No 5-162) with each batch of samples.

For calibration of manual method and automatic analysers CORMAY URINE PROTEINS CALIBRATORS (Cat. No 5-181) is recommended.

For automatic analysers 0.9% NaCl should be use as a calibrator 0. The calibration curve should be prepared every 12 weeks, with change of reagent lot number or as required e.g. urine control material assay values findings outside the specified range.

PERFORMANCE CHARACTERISTICS

These metrological characteristics have been obtained using Multi+ for manual assay and automatic analyser Biolis 24i Premium. Results may vary if a different instrument is used.

Sensitivity: 3.7 mg/dl (0.037 g/l).

Linearity: up to 121 mg/dl (1.21 g/l).

For higher concentration dilute sample with 0.9% NaCl and repeat the assay. Multiply the result by the dilution factor.

Specificity / Interferences

Hemoglobin up to 0.004 g/dl, ascorbic acid up to 20 mg/dl, creatinine up to 6 g/l, bilirubin up to 5 mg/dl, conjugated bilirubin up to 60 mg/dl, uric acid up to 85 mg/dl, glucose up to 35 g/l, citrates up to 250 mg/dl, oxalates up to 90 mg/dl, calcium ions up to 130 mg/dl, magnesium ions up to 1.8 g/l, phosphate ions up to 1.2 g/l, urea up to 50 g/l do not affect the results of the determination.

High concentration of iron(II) ions in the test sample may cause interferences $\ensuremath{^{[12]}}$.

Acetaminophen and some antibiotics from penicillins and aminoglycosides can interfere ^[4, 11-13].

Precision

Repeatability (run to run)	Mean	SD	CV
Multi $+ n = 10$	[mg/dl]	[mg/dl]	[%]
level 1	22.48	1.07	4.76
level 2	59.07	1.46	2.47
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Reproducibility (day to day)	Mean	SD	CV
Biolis 24i Premium n = 10	[mg/dl]	[mg/dl]	[%]
level 1	20.21	0.90	4.44
level 2	64.09	2.30	3.59

Method comparison

A comparison between total proteins values determined at Multi+ (y) and at ADVIA 1650 (x) using 22 urine samples gave following results:

y = 1.0616 x - 0.0321 mg/dl;

R = 0.991 (R – correlation coefficient)

A comparison between total proteins values determined at Multi+ (y) and at ADVIA 1650 (x) using 25 CSF samples gave following results:

y = 0.9896 x + 4.9044 mg/dl;R = 0.989 (R - correlation coefficient)

WASTE MANAGEMENT

Please refer to local legal requirements.

LITERATURE

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