BECKMAN COULTER

CE

AU/DxC AU

Instructions For Use

UP
Urinary/CSF Protein

REF OSR6170 4 x 19 mL R1, 1 x 3 mL Calibrator
OSR6270 4 x 52 mL R1, 1 x 3 mL Calibrator

For *in vitro* diagnostic use only.

PRINCIPLE

INTENDED USE

Photometric colour test for the quantitative determination of total protein in human urine and cerebrospinal fluid (CSF) on Beckman Coulter analysers.

SUMMARY AND EXPLANATION

Reference[1]

Measurement of total protein in urine is important in the diagnosis and treatment of diseases associated with renal, cardiac and thyroid function. These diseases are often characterised by proteinuria of which there are four main types: (a) increased glomerular permeability (glomerular proteinuria) (b) defective tubular reabsorption (tubular proteinuria) (c) increased concentration of low molecular weight protein (overload proteinuria) (d) abnormal secretion of protein into the urinary tract (postrenal proteinuria). Increased levels of urinary protein may also be present following strenuous exercise or in the following conditions: monoclonal gammopathies, nephritis, diabetic nephropathy or urinary tract infections.

The measurement of total protein in CSF is important in detecting increased permeability of the blood/brain barrier to plasma proteins or to detect increased intrathecal production of immunoglobulins. Increased permeability of the blood brain barrier may result from conditions such as brain tumour, intracerebral haemmorhage or by inflammation caused by bacterial or viral meningitis, encephalitis or poliomyelitis. Determination of increased intrathecal synthesis of immunoglobulins is important in the diagnosis of demyelinating diseases such as multiple sclerosis.

METHODOLOGY

Reference[1,2,3]

Pyrogallol red is combined with molybdate to form a red complex with a maximum absorbance at 470nm. The assay is based on the shift in absorption that occurs when the pyrogallol red-molybdate complex binds basic amino groups of protein molecules. A blue-purple complex is formed with a maximum absorbance at 600 nm. The absorbance of this complex is directly proportional to the protein concentration in the sample.

SPECIMEN

TYPE OF SPECIMEN

Urine or cerebrospinal fluid.

Urine: A 24 hour or 12 hour urine specimen with no preservative is preferred.[4,5,6]

Sample Stability:[7] Analyse fresh otherwise stable stored at 2…8°C for up to 48 hours.

Urine samples contaminated by haemoglobin will result in a falsely elevated value.

CSF: Beckman Coulter recommends that CSF samples be collected in plain collection devices.

In the case of CSF samples care should be taken to avoid blood contamination during collection.

Sample Stability:[4] Analyse fresh, otherwise stable stored at 4°C for up to 72 hours.

As with all dye based methods, analysis of urine samples containing immunoglobulin light chains (i.e. Bence-Jones Protein) may result in the underestimation of protein. Where such samples are suspected it is recommended that the sample be concentrated and further analysed via electrophoresis.[1]

Discrepancies may arise when analysing total urine protein in samples from patients who have been treated with polypeptide-based plasma substitutes.[8] The polypeptides from the plasma substitute may be excreted into the urine and result in an elevated total urine protein result. Where such samples are suspected it is recommended that the sample be concentrated and further analysed via electrophoresis.

Specimen storage and stability information provides guidance to the laboratory. Based on specific needs, each laboratory may establish alternative storage and stability information according to good laboratory practice or from alternative reference documentation.

REAGENTS

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents.

Calibrator: Biological materials of human origin contained in the calibrator were tested for anti-HCV, HbsAg and Anti-HIV 1/2 on a single donor basis using FDA approved methods and were found to be non-reactive. As there is no known test method that can offer complete assurance that products derived from human blood will not transmit infectious agents, this product should be handled as a potentially infectious material.

Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

Final concentration of reactive ingredients:

R1		Calibrator	
Pyrogallol Red	47 µmol/L	Human Serum Albumin	0.50 g/L
Sodium Molybdate	320 µmol/L	Also contains preservatives	
Succinic Acid	50 mmol/L		
Sodium Benzoate	3.5 mmol/L		
Sodium Oxalate	1.0 mmol/L		
Methanol	0.8% w/v		

The concentrations of the reactive components of the reagents shown on the kit label are the actual concentrations in the individual R1/R2 vials. The reagent composition which is shown in the Instructions For Use is the final concentration of these components in the reaction cuvette after addition of R1, Sample, and R2.

⚠ CAUTION

Sodium azide preservative may form explosive compounds in metal drain lines. See NIOSH Bulletin: Explosive Azide Hazard (8/16/76).
To avoid the possible build-up of azide compounds, flush wastepipes with water after the disposal of undiluted reagent. Sodium azide disposal must be in accordance with appropriate local regulations.

GHS HAZARD CLASSIFICATION

Urinary/CSF Protein R1	DANGER	
		
	H370	Causes damage to organs.
	P260	Do not breathe vapours.
	P308+P311	If exposed or concerned: Call a doctor/physician.
		Methanol 1 - 2%
Urinary/CSF Protein Calibrator	WARNING	
		
	H317	May cause an allergic skin reaction.
	H412	Harmful to aquatic life with long lasting effects.
	P273	Avoid release to the environment.
	P280	Wear protective gloves, protective clothing and eye/face protection.
	P333+P313	If skin irritation or rash occurs: Get medical advice/attention.
	P362+P364	Take off contaminated clothing and wash it before use.
		reaction mass of: 5-chloro-2-methyl-4-isothiazolin -3-one [EC# 247-500-7] and 2-methyl-4-isothiazolin-3-one [EC# 220-239-6](3:1) < 0.05%

SDS	Safety Data Sheet is available at beckmancoulter.com/techdocs

REAGENT PREPARATION

R1 is ready for use and can be placed directly on board the instrument. Protect R1 from direct sunlight. The Calibrator is ready for use.

STORAGE AND STABILITY

1. The unopened reagent and calibrator are stable until the expiration date printed on the label when stored at 2…8°C.
2. Once open, reagent stored on board the instrument is stable for 90 days. The opened calibrator is stable until the expiration date on the label, provided that the stopper and cap are replaced immediately after each use to avoid contamination and the calibrator is stored at 2…8°C.

CALIBRATION

CALIBRATOR REQUIRED

Use Calibrator provided in the kit. For value assigned to the calibrator please refer to bottle label. The calibrator is traceable to a primary standard which is prepared gravimetrically using reagent grade human serum albumin.

Recalibrate the assay when the following occur:

Change in reagent lot or significant shift in control values;

Major preventative maintenance was performed on the analyser or a critical part was replaced.

QUALITY CONTROL

Control material with values determined by this Beckman Coulter system may be used.

Each laboratory should establish its own control frequency however good laboratory practice suggests that controls be tested each day patient samples are tested and each time calibration/blanking is performed.

The results obtained by any individual laboratory may vary from the given mean value. It is therefore recommended that each laboratory generates analyte specific control target values and intervals based on multiple runs according to their requirements. These target values should fall within the corresponding acceptable ranges given in the relevant product literature.

If any trends or sudden shifts in values are detected, review all operating parameters.

Each laboratory should establish guidelines for corrective action to be taken if controls do not recover within the specified limits.

TESTING PROCEDURE(S)

Refer to the appropriate Beckman Coulter AU analyser User Guide/Instructions For Use (IFU) for analyser-specific assay instructions for the sample type as listed in the Intended Use statement.

CALCULATIONS

The Beckman Coulter analysers automatically compute the total protein concentration of each sample.

REPORTING RESULTS

REFERENCE INTERVALS

Urine[1]	0.05 – 0.08 g/day at rest
Value may increase to up to 0.30 g/day following exercise.	
CSF (Adults)[1]	0.15 – 0.45 g/L
CSF (newborn <1month)[1]	0.15 – 1.30 g/L

Expected values may vary with age, sex, sample type, diet and geographical location. Each laboratory should verify the transferability of the expected values to its own population, and if necessary determine its own reference interval according to good laboratory practice. For diagnostic purposes, results should always be assessed in conjunction with the patient's medical history, clinical examinations and other findings.

PROCEDURAL NOTES

INTERFERENCES

Results of studies conducted on a representative analyzer show that the following substances interfere with this assay by < 10% or 0.030g/L up to the concentrations specified below:

Substance	Level Tested (mmol/L)	Level Tested (g/L)
Ammonia	139	
Ascorbate	1.1	
Bilirubin	0.3	
Citric Acid	10	
Creatinine	26	
Cu^{2+}	1.6	
Fe^{3+}	0.01	
Gentamycin		0.02
Glucose	277	
Oxalic acid	5.8	
Tartaric Acid	13	
Tobramycin		0.03
Uric Acid	18	

Refer to Young[9] for further information on interfering substances.

PERFORMANCE CHARACTERISTICS

PERFORMANCE CHARACTERISTICS

Data contained within this section is representative of performance on Beckman Coulter systems. Data obtained in your laboratory may differ from these values.

LINEARITY

The test is linear within a concentration range of 0.01 – 2.00 g/L.

SENSITIVITY

The lowest detectable level on a DxC 700 AU analyser was estimated at 0.005 g/L.

The lowest detectable level represents the lowest measurable level of total protein that can be distinguished from zero. It is calculated as the absolute mean plus three standard deviations of 20 replicates of an analyte-free sample.

METHODS COMPARISON

Patient urine samples were used to compare this Urinary/CSF Protein OSR6170 assay on the AU640 against another commercially available urinary/CSF protein assay. Results of linear regression analysis were as follows:

y = 0.957x + 0.009	r = 0.998	n = 108	Sample range = 0.01 – 1.99 g/L

PRECISION

Estimates of precision based on NCCLS recommendations[10] are consistent with typical performance. The following data was obtained on a DxC 700 AU using 3 urine pools analysed over 20 days.

n = 80	Within-run		Total	
Mean g/L	SD	CV%	SD	CV%
0.14	0.002	1.5	0.003	1.8
0.44	0.003	0.8	0.010	2.3
1.60	0.017	1.0	0.042	2.6

ADDITIONAL INFORMATION

DxC 700 AU requires that each reagent application has a standard format of abbreviated Closed Test Name. This Closed Test Name is required to allow automated loading of the calibrator information for each application as part of the DxC 700 AU Closed System. Refer to the table below for the Closed Test Name assigned to each application for this assay.

Test Name	Description
UCP1N	Urinary/CSF Protein (Urine)
UCP1N	Urinary/CSF Protein (CSF)

Setting Sheet Footnotes

User defined

† Beckman Coulter Urinary/CSF Protein Calibrator supplied with kit
* Values set for working in SI units g/L. To work in mg/dL multiply by 100.

§ Same setting for CSF application

REVISION HISTORY

Revised Interferences section.

Preceding version revision history

Added new languages

REFERENCES

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2. Fujita Y, Mori I, Kitano S. Color reaction between pyrogallol red molybdenum (VI) complex and protein. Bunseki Kagaku 1983;32:379-386.
3. Watanabe N, Kamei S, Ohkubo A, Yamanaka M, Ohsawa S,Makino K, Tokuda K. Urinary protein as measured with pyrogallol red-molybdate complex manually and in Hitachi 726 automated analyser. Clin Chem 1986;32:1551-1544.
4. Tietz NW. Clinical guide to laboratory tests, 3rd ed. Philadelphia: WB Saunders Company, 1995:518-520.
5. NCCLS. Urinalysis and collection, transportation and preservation of urine specimens; approved guideline - second edition. NCCLS document GP16-A2; 2001.
6. Young DS. Effects of preanalytical variables on clinical laboratory tests, 2nd ed. AACC Press, 1997.
7. First MR. Renal Function. In: Kaplan LA, Pesce, AJ, eds. Clinical chemistry: theory, analysis and correlation, 3rd ed. St. Louis: Mosby-Year Book, 1996:484-504.
8. Pena C, Martinez-Bru C, Homs R, Planella T, Cortes M. Effect of plasma replacement therapy on determinations of urine protein concentration [Letter]. Clin Chem 1998;44:359-360.
9. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, Washington D.C, 2000.
10. NCCLS. Evaluation of precision performance of clinical chemistry devices; approved guideline. NCCLS document EP5-A;1999.

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