BECKMAN COULTER

AU/DxC AU US

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

For Rx use only

PRINCIPLE

INTENDED USE

System reagent for the quantitative determination of total protein in human urine and cerebrospinal fluid (CSF) on Beckman Coulter AU/DxC AU analyzers.

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 tumor, intracerebral hemorrhage 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]

Many methods are available for the determination of urinary/CSF protein. These are based on colorimetric, turbidimetric, electrophoretic or immunological principles. Of the colorimetric methods the Biuret method lacks sensitivity, the Coomassie Brilliant Blue method has a limited linear range and also has the disadvantage of staining glassware and cuvettes. Results with the turbidimetric methodologies may vary depending on the type of precipitant and type of protein.

The Urinary/CSF Protein reagent is a colorimetric method. 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 absorbance that occurs when the pyrogallol red-molybdate complex binds basic amino groups of protein molecules. Under the conditions of the test in the presence of protein, a blue-purple complex is formed with a maximum absorbance at 600nm. The absorbance of this complex is directly proportional to the protein concentration in the sample.

SPECIMEN

SPECIMEN STORAGE AND STABILITY

Urine: Analyze fresh otherwise stable stored at 2 - 8°C for up to 48 hours.[4]

CSF: Analyze fresh otherwise stable stored at 4°C for up to 72 hours.[5]

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.

Additional handling conditions as designated by this laboratory:

SPECIMEN COLLECTION AND PREPARATION

Urine or cerebrospinal fluid.

Urine: A 24 hour or 12 hour urine specimen with no preservative is preferred.[5,6,7]

Random collections may also be appropriate if the laboratory has established its own performance characteristics.

Use of urine samples contaminated by hemoglobin will result in a falsely elevated value.

CSF: Beckman Coulter recommends that CSF samples be collected in plain collection devices. Care should be taken to avoid blood contamination during collection.

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 analyzed via electrophoresis.[1]

Discrepancies may arise when analyzing 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 analyzed via electrophoresis.

Additional instructions for patient sample preparation as designated by this laboratory:

Additional type conditions as designated by this laboratory:

REAGENTS

CONTENTS

Urinary/CSF Protein Reagent

Urinary/CSF Protein Calibrator

Reagent storage location in this laboratory:

WARNING AND PRECAUTIONS

1. Exercise the normal precautions required for handling all laboratory reagents.
2. 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.
3. Dispose of all waste material in accordance with local guidelines.

REACTIVE INGREDIENTS

R1	
Pyrogallol Red	47 µM
Sodium Molybdate	320 µM
Succinic Acid	50 mM
Sodium Benzoate	3.5 mM
Sodium Oxalate	1.0 mM
Methanol	0.8% w/v
Also contains detergent.	

Calibrator	
Human Serum Albumin	50 mg/dL
Also contains preservatives.	

⚠ 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

EQUIPMENT AND MATERIALS

For use on the AU480, AU680, AU5800, DxC 500 AU, DxC 500i and DxC 700 AU Beckman Coulter Analyzers.

Storage location of the Calibrator in this laboratory:

Storage location of test tubes or sample cups in this laboratory:

REAGENT PREPARATION

The reagent is ready for use and may be placed directly on board the instrument. The calibrator is ready to use. Protect R1 from direct sunlight.

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. Opened bottles of reagent are stable for 90 days when stored in the refrigerated compartment of the analyzers.
3. Opened bottles of reagent are stable for 30 days when stored in the refrigerated compartment of the AU5800 analyzers.

   The opened calibrator is stable until the expiration date printed on the label providing that the stopper and cap are replaced immediately after each use to avoid contamination and the calibrator is stored at 2-8°C.

INDICATIONS OF DETERIORATION

Visible signs of microbial growth, gross turbidity, precipitate or change in color in the Urinary/CSF Protein reagent or calibrator may indicate degradation and warrant discontinuation of use.

Additional storage requirements as designated by this laboratory:

STABILITY OF FINAL REACTION MIXTURE

The Beckman Coulter AU/DxC AU analyzer automatically computes every determination at the same time interval.

CALIBRATION

CALIBRATOR REQUIRED

The frequency of calibration for the Urinary/CSF protein procedure is every 90 days. The frequency of calibration for the Urinary/CSF protein procedure is every 30 days for the AU5800.

Calibration of this procedure is accomplished by use of the Urinary/CSF Protein Calibrator included in the reagent kit. The calibrator is traceable to a primary standard, which is prepared gravimetrically using reagent grade human serum albumin.

Recalibration of this test is required when any of these conditions exist:

1. A reagent lot number has changed or there is an observed shift in control values.
2. Major preventative maintenance was performed on the analyzer.

3. A critical part was replaced.

QUALITY CONTROL

During operation of the Beckman Coulter AU/DxC AU analyzer, at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, controls should be performed after calibration with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate Beckman Coulter AU/DxC AU analyzer Instructions For Use (IFU) and Reference Manual. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Location of controls used at this laboratory.

CONTROL NAME	SAMPLE TYPE	STORAGE

TESTING PROCEDURE(S)

A complete list of test parameters and operational procedures are provided in the relevant AU/DxC AU analyzer IFU and Reference Manual.

RESULTS INTERPRETATION

The default unit of measure is mg/dL, for conversion to SI units (g/L) the result is divided by 100.

REPORTING RESULTS

EXPECTED RESULTS

Urine[1]	50 – 80 mg/24 h at rest

Value may increase to up to 300 mg/24 h following exercise.

CSF (Adults)[1]	15 – 45 mg/dL
CSF (newborn <1month)[1]	15 – 130 mg/dL

Reference Intervals shown above were taken from the literature.

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.

Expected reference ranges in this laboratory:

INTERVALS	SAMPLE TYPE	UNITS (mg/dL)

Additional reporting information as designated by this laboratory:

PROCEDURAL NOTES

INTERFERENCES

Reference[9]

Results of studies show that the following substances interfere with this Urinary/CSF protein procedure by <10%:

Substance	Level Tested (mg/dL)
Ammonia	250
Ascorbate	20
Bilirubin	20
Citric Acid	200
Creatinine	290
Cu^{2+}	10
Fe^{3+}	0.055
Gentamycin	2
Glucose	4990
Oxalic acid	52
Tartaric Acid	200
Tobramycin	3
Uric Acid	300

The information presented is based on results from Beckman Coulter studies and is current at the date of publication. Beckman Coulter Inc., makes no representation about the completeness or accuracy of results generated by future studies. Further information on interfering substances is available.[10]

Laboratory specific procedure notes:

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.

DYNAMIC RANGE / ANALYTICAL MEASURING RANGE

The Urinary/CSF protein procedure is linear from 4 to 200 mg/dL. Samples exceeding the upper limit of linearity should be diluted with water and repeated. The sample may be diluted, repeated and multiplied by the dilution factor automatically utilizing the AUTO REPEAT RUN.

METHODS COMPARISON

Reference[11]

Patient Urine samples were evaluated in method comparison studies.

Results of Deming regression analysis were as follows:

Y Method	DxC 500 AU
X Method	DxC 700 AU
Slope	1.030
Intercept	-2.039
Correlation Coeff. (r)	0.9995
No. of Samples (n)	119
Range (mg/dL)	10.94 - 179.93

PRECISION

Reference[11]

Estimates of precision, based on CLSI recommendations,[12] are consistent with typical performance. The within run precision is less than 4% CV and total precision is less than 5% CV. Assays of control material were carried out and data reduced following CLSI guidelines above.

N = 80	Within-run		Total	
Mean mg/dL	SD	CV%	SD	CV%
17	0.45	2.7	0.54	3.2
61	0.96	1.6	1.07	1.8
151	1.86	1.2	2.2	1.5

ADDITIONAL INFORMATION

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

Test Name	Description
UCP1U	Urinary/CSF Protein (Urine)
UCP1U	Urinary/CSF Protein (CSF)

Refer to the Beckman Coulter Chemistry Systems Reagent Guide (BAGUIDE) for specific chemistry information for the AU/DxC AU clinical chemistry systems and guidance on symbols used on all AU/DxC AU product labelling.

Setting Sheet Footnotes

User defined

Lot or Lot + Bottle
† Beckman Coulter Urinary/CSF Protein Calibrator supplied with kit

* Values set for working in mg/dL. To work in SI units (g/L) divide by 100

§ Same setting for CSF application

REVISION HISTORY

Add DxC 500i instrument to IFU

Preceding version revision history

Updated REPORTING RESULTS section

Updated PROCEDURAL NOTES section

Updated Performance Characteristics section

Updated References section

REFERENCES

1. Tietz NW. Textbook of clinical chemistry, 2nd ed. Philadelphia: WB Saunders Company, 1994.
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 analyzer. Clin Chem 1986;32:1551-1544.
4. 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.
5. Tietz NW. Clinical guide to laboratory tests, 3rd ed. Philadelphia: WB Saunders Company, 1995:518-520.
6. CLSI/NCCLS. Urinalysis and collection, transportation and preservation of urine specimens; approved guideline - third edition. CLSI/NCCLS document GP16-A3: 2009.
7. Young DS. Effects of preanalytical variables on clinical laboratory tests, 2nd ed. AACC Press, 1997.
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. CLSI Interference Testing in Clinical Chemistry EP07 ED3:2018.
10. AACC Effects on Clinical Laboratory Tests: Drugs, Disease, Herbs and Natural Products https://clinfx.wiley.com/aaccweb/aacc/
11. Data is on file for specific AU/DxC AU analyzers.
12. CLSI/NCCLS. Evaluation of precision performance of clinical chemistry devices; approved guideline. CLSI/NCCLS document EP5-A 3:2014

Beckman Coulter, Inc., 250 S. Kraemer Blvd., Brea, CA 92821 U.S.A.
+(1) 800-854-3633
www.beckmancoulter.com