



URINARY/CSF PROTEIN

OSR6170

4 x 19 mL
1 x 3 mL

**R1
Calibrator**

OSR6270

4 x 52 mL
1 x 3 mL

**R1
Calibrator**

Intended Use

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

Summary¹

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 characterized 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^{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.

System Information

For AU400/400[®]/480, AU600/640/640[®]/680 and AU2700/5400 Beckman Coulter Analyzers.

Reagents

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.

Precautions

1. For *in vitro* diagnostic use.
2. Do not ingest. Harmful if swallowed.
3. 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.
4. To avoid the possible build-up of azide compounds, flush waste-pipes with water after the disposal of undiluted calibrator.
5. Dispose of all waste material in accordance with local guidelines.

Preparation of reagents

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. 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.

Urinary/CSF Protein

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.

Specimen Collection and Preparation

Urine or cerebrospinal fluid.

Urine: A 24 hour or 12 hour urine specimen with no preservative is preferred.^{4,5,6}

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.⁷ 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.

Sample Storage and Stability

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

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

Interfering Substances

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	300
Cu ²⁺	10
Fe ³⁺	6
Gentamycin	4
Glucose	5000
Oxalic Acid	70
Tartaric Acid	200
Tobramycin	4
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. For further information on interfering substances, refer to Young for a compilation of reported interferences with this test.⁹

Procedure

A complete list of test parameters and operational procedure can be found in the User's Guide appropriate to the analyzer.

Materials Provided

Urinary/CSF Protein Reagent
Urinary/CSF Protein Calibrator

Materials required but not provided

Urine Calibrator (Cat # DR0090)

Stability of Final Reaction Mixture

The Beckman Coulter AU analyzers automatically compute every determination at the same time interval.

Calibration

The frequency of calibration for the Urinary/CSF protein procedure is every 90 days. Calibration of this procedure is accomplished by use of the Urinary/CSF Protein Calibrator included in the reagent kit or with Urine Calibrator (Cat # DR0090). 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 analyzer at least two levels of an appropriate quality control material should be tested a minimum of once a day. In addition, these controls should be tested after calibration, with each new lot of reagent, and after specific maintenance or troubleshooting steps described in the appropriate User's Guide. Quality control testing should be performed in accordance with regulatory requirements and each laboratory's standard procedure.

Results

Automatically printed out for each sample in mg/dL at 37°C.

Urinary/CSF Protein

Dynamic 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.

Expected Values

Urine¹ 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

Expected values may vary with age, sex, diet and geographical location. Each laboratory should determine its own expected values as dictated by good laboratory practice.

Specific Performance Characteristics

The following data was obtained using the Urinary/CSF Protein reagent on Beckman Coulter AU analyzers according to established procedures. Results obtained in individual laboratories may differ.

Precision¹¹

Estimates of precision, based on CLSI recommendations,¹⁰ 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 Mean, mg/dL	Within run		Total	
	SD	CV%	SD	CV%
15	0.26	1.7	0.72	4.8
53	0.64	1.2	1.01	1.9
152	1.06	0.7	2.58	1.7

Method Comparison¹¹

Patient samples were used to compare this Urinary/CSF Protein Reagent. The table below demonstrates representative performance on the AU analyzers.

Y Method	AU640
X Method	Method 2
Slope	1.147
Intercept	6.7
Correlation Coeff. (r)	0.888
No. of Samples (n)	107
Range (mg/dL)	1-192

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. Tietz NW. Clinical guide to laboratory tests, 3rd ed. Philadelphia: WB Saunders Company, 1995:518-520.
5. CLSI/NCCLS. Urinalysis and collection, transportation and preservation of urine specimens; approved guideline - second edition. CLSI/NCCLS document GP16-A2; 2001.
6. Young DS. Effects of preanalytical variables on clinical laboratory tests, 2nd ed. AACC Press, 1997.
7. 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.
8. 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.
9. Young DS. Effects of drugs on clinical laboratory tests, AACC, 5th ed. AACC Press, 2000.
10. CLSI/NCCLS. Evaluation of precision performance of clinical chemistry devices; approved guideline. CLSI/NCCLS document EP5-A;1999.
11. Data is on file for specific AU analyzers.

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