Stanbio Total Protein LiquiColor® (CSF/Urine) Procedure No. 0345

Quantitative Colorimetric Determination of Total Protein in Urine or Cerebrospinal Fluid

Summary and Principle

Measurement of urinary proteins is becoming increasingly important in the detection of renal pathology[1]. Proteinuria (increased amounts of protein in urine) can occur in increased glomerular permeability, defective tubular reabsorption and abnormal secretion of protein into the urinary tract[2]. Albuminuria (increased amounts of albumin in urine) has been recognized as an early indicator of renal damage in diabetes, that can be reversed if detected and treated sufficiently early[3].

The measurement of CSF total protein and specific proteins is used to detect increased permeability of the blood/brain barrier (the capillary endothelium of vessels of the central nervous system) to plasma proteins or to detect increased intrathecal secretion of immunoglobulins[1].

The Stanbio Total Protein test for urine and CSF is based on the procedure developed by Watanabe et al[4] which is a dye-binding colorimetric method utilizing pyrogallol red-molybdate complex and modified[5] to equalize the reactivities of albumin and gamma globulin and provide good precision and linearity.

The pyrogallol red is combined with molybdenum acid, forming a red complex with maximum absorbance at 470 nm. When this complex is combined with protein in acidic conditions, a blue-purple color develops with an increase in absorption to 610 nm.

Reagents

Total Protein (CSF/Urine)Reagent, Cat. No. 0346
Buffered solution of pyrogallol red (2.6 mg/dL), sodium molybdate (19.4 mg/dL) and surfactants, pH 2.3.

Total Protein (CSF/Urine) Standard, Cat. No. 0347 (100 mg/dL)
Aqueous solution of serum albumin with sodium azide added as a preservative.

Precautions: *For in Vitro Diagnostic Use.*
Standard contains sodium azide. May react with copper or lead plumbing to form explosive metal azide build up. On disposal, flush with large volumes of water.

Reagent Preparation: Reagent and Standard are ready to use.

Reagent Storage and Stability: Reagent and standard are both stable stored at 2-8°C until their respective expiration dates. Bring reagent and standard to room temperature before use. Reagent absorbance less than 0.250 at 600 nm when read against water, indicates deterioration and should not be used.

Materials Required But Not Provided

Spectrophotometer capable of absorbance readings at 600 nm. (600-620nm)
Accurate pipetting devices to deliver 0.05 mL (50 uL), and 3.0 mL
Cuvets
Heat block or water bath, 37°C (optional)
Test Tubes
Interval timer

Specimen Collection and Preparation

Urine samples collected randomly or 24 hour samples may be used. Store at 2-8°C or freeze the specimen until assayed. No special additives or preservatives are required.

CSF should be free from hemolysis. Centrifuge any specimen containing red blood cells or particulate matter. CSF may be stored at 2-8°C for several days until assayed.

Interfering Substances: It is recommended not to use urine specimens with added preservatives since some preservatives such as HCl, Benzoic acid and Thymol have shown to interfere in the protein assay, giving false low results. Hemolyzed specimens must not be used. CSF or urine specimens containing red cells may give elevated protein values. Some drugs and medications may interfere, see fujita et al[7].

Automated Analyzer

Parameters:

Wavelength	600 nm
Reaction Type	Endpoint
Reaction Direction	Increasing
Reaction Temperature	37°C
Sample/Reagent Ratio	1:60
Equilibration Time	3 Seconds
Read Time	4 Seconds
Lag Time	300 Seconds
Blank Absorbance Limit	>0.250
High Absorbance	2.000A
Standard	(see note) * 100 mg/dL
Linearity	150 mg/dL

Above parameters should be employed in programming automated analyzers for Total Protein (CSF/Urine). Consult your instrument manual for programming instructions. Specific programming applications for most automated analyzers are available from Stanbio Customer Service Department.

Manual Procedure

1. Pipet into cuvets the following volumes (mL) and mix well:

	Reagent Blank (RB)	Standard (S)	Sample (U)
Reagent	3.0	3.0	3.0
Standard	–	0.05	–
Sample	–	–	0.05

2. Incubate all cuvets at 37°C for 5 minutes or for 10 minutes at room temperature (15-25°C).
3. Read S and U vs RB at 600 nm within 30 minutes.

*** NOTE:** If greater sensitivity is desired for normal or marginally elevated specimens, a 100 uL sample may be used. In this case, dilute the standard by ½ with distilled/deionized water and use the 50 mg/dL standard in the test.

Also, 50 mg/dL standard may be used with, or instead of, the 100 mg/dL standard when testing CSF and random urine samples, to adjust the calibration curve closer to the low normal values.

Quality Control: Use of commercial CSF or urine controls with known protein content, should be included with each run.

Results

Values are derived by the following equations:

$$\text{CSF Total Protein (mg/dL)} = \frac{Au}{As} \times 100$$

$$\text{Urine Total Protein (mg/dL)} = \frac{Au}{As} \times 100 \times VdL$$

Where Au and As are the absorbance values of unknown and standard, respectively, 100 the concentration of the standard (mg/dL), and VdL is the 24 hour urine volume in dL.

Example:

The following readings were obtained using 1 cm cuvets:

$$\text{Urine Total Protein (mg/24 hours)} = \frac{0.095}{0.189} \times 100 \times 12 = 603$$

NOTE: Samples having Total Protein values greater than 150 mg/dL are diluted 2-fold (1 + 1) with distilled/deionized water, the assay repeated and results multiplied by the dilution factor of 2.

Expected Values

CSF (Lumbar):	8 - 43 mg/dL
Urine:	22 - 120 mg/24 hrs. (up to 160 in pregnancy)
Random Urine:	1 - 14 mg/dL

Performance Characteristics[6]

Precision: Each of 3 urine pools, having mean Total Protein levels of 116.0, 70.9 and 8.2 mg/dL, were subjected to 20 replicate assays over a 5-day period. Standard deviations (SD) were calculated to be 1.8, 4.17 and 1.08 mg/dL respectively, with corresponding coefficients of variations (CV) of 1.56, 5.87 and 13.2%.

Correlation: Urine Total Protein determinations were performed on 20 specimens by the procedure described (y) and by a similar established technique (x) on a Gilford Impact 400E. The results showed a correlation coefficient (R) of 0.999 and a regression equation of y = 1.07x - 0.90.
20 spinal fluid specimens were assayed in the same manner and showed a correlation coefficient (R) of 0.999 and a regression equation of y = 0.969x + 2.10.

Sensitivity: The sensitivity of this test defined as two standard deviation of between-run at the low range is 1.4 mg/dL.

Linearity: When performed as directed this method is linear to 150 mg/dL.

References

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4. Watanabe N, Kamel S, Ohkubo A, et al. Urinary protein as measured with pyrogallol red-molybdate complex, manually and in a Hitachi 726 automated analyzer. Clin Chem 1986; 32: 1551-1554.
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6. Stanbio Laboratory data
7. Fujita Y, Mori I, Kitano S. Color Reaction Between Pyrogallol Red - Molybdenum (VI) Complex and Protein. Benseki Kagaku 1983; 32: 379-386.

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