**Subject**  Total Protein (Urine/CSF) – Cobas c501

**Index Number** Lab-4385

**Section**  Laboratory

**Subsection** Chemistry

**Category** Departmental

**Contact** Benjamin Michel

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**References**

Required document for Laboratory Accreditation by the College of American Pathologists.

**Applicable To**

Employees of the Gundersen Health System clinical laboratory.

**Detail**

**Intended Use**

In vitro test for the quantitative determination of protein in human urine and cerebrospinal fluid on Roche/Hitachi cobas c systems.

**PRINCIPLE:**

Turbidimetric method. The sample is preincubated in an alkaline solution containing EDTA, which denatures the protein and eliminates interference from magnesium ions. Benzethonium chloride is then added, producing turbidity.

The Roche Diagnostics Urinary/CSF Protein assay is based on the method described by Iwata and Nishikaze, later modified by Luxton, Patel, Keir, and Thompson. In this method benzethonium chloride reacts with protein in a basic medium to produce a turbidity that is more stable and evenly distributed than that observed with the sulfosalicylic acid(SSA) or trichloroacetic acid (TCA) methodologies. Due to their reaction mechanisms, all methods, turbidimetric and colorimetric, exhibit different sensitivities to various proteins, especially to protein fragments such as Bence Jones proteins and small proteins such as α1-microglobulin.

**CLINICAL SIGNIFICANCE:**

Protein measurements 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. Urine is formed by ultrafiltration of plasma across the glomerular capillary wall. Proteins with a relative molecular mass > 40000 are almost completely retained, while smaller substances easily enter the glomerular filtrate.

Cerebrospinal fluid (CSF) protein measurements are used in the diagnosis and treatment of conditions such as meningitis, brain tumors and infections of the central nervous system. Most CSF protein originates by diffusion from plasma across the blood-CSF barrier. Elevated levels occur as a result of increased permeability of the blood-CSF barrier or with increased local synthesis of immunoglobulins.

**SPECIMEN:**

Urine and CSF. Reject samples that are contaminated with blood. Minimal hemolysis allowed (see limitations). Specimens for urinary/CSF protein should be collected before fluorescein is given or at least 24 hours later. Centrifuge all specimens before testing. Universal precautions apply.

**Urine**: 24-hour urine is the specimen of choice, although 12-hour and 18-hour specimens may also be used. Random urine specimens may be used for protein/creatinine ratios. No special additives or preservatives are required, but keep urine specimen chilled during collection. Urinary albumin excretion is increased by physiological factors like exercise, posture, or diuresis. Samples should therefore not be collected after exertion or following acute ingestion of a fluid load.

Stability in urine: 1 day at 15-25°C, 7 days at 2-8°C, 1 month at -20°C

**CSF:** If specimen is bloody, notify the provider. CSF specimens must be collected with care to avoid blood contamination due to a traumatic tap. Since protein concentration of whole blood is about 1000 times higher than of normal CSF, the elevated total protein value from a bloody CSF specimen will not represent the actual CSF total protein value. In addition, bloody CSF specimens due to very high protein content may yield false low and unflagged results.

Stability in CSF: 1 day at 15-25°C, Up to 6 days at 2-8°C, 1 year at -20°C.

**REAGENTS/MATERIALS:**

Total Protein Urine/CSF Gen.3, 150 tests – the reagent cassette is labeled as TPUC3. R1 is in position B and R2 is in position C. If the cassette is discarded from the analyzer with >4 tests remaining, it must be discarded as CORROSIVE - BASIC waste.

**R1 -** Sodium hydroxide: 677 mmol/L; EDTA-Na: 74 mmol/L

**R2** - Benzethonium chloride: 32 mmol/L

Diluent NaCl 9%, 50 mL – the diluent cassette is labeled as NACL.

**Precautions and warnings:** For in vitro diagnostic use. Exercise the normal precautions required for handling all laboratory reagents. Disposal of all waste material should be in accordance with local guidelines.

**Storage:**

Total Protein Urine/CSF Gen.3 – Store at 2-8°C.

Diluent NaCl 9% – Store at 2-8°C.

**Stability:**

Total Protein Urine/CSF Gen.3 – Unopened at 2-8°C – up to the stated expiration date. On-board in use and refrigerated on the c501 – 6 weeks.

Diluent NaCl 9% – Unopened at 2-8°C – up to the stated expiration date. On-board in use and refrigerated on the c501 – 12 weeks.

**EQUIPMENT/INSTRUMENTATION:**

Roche c501 analyzer - Refer to the Operator’s Manual for operating instructions, maintenance, and troubleshooting.

**Calibration:** This method has been standardized against the National Bureau of Standards Reference Material SRM-927 using the biuret method for the quantitation of protein. Calibration mode: RCM, full calibration.

**Calibrators:** C.f.a.s PUC. Use deionized water as zero calibrator.

Preparation: Ready for use. Mix carefully, avoiding foam formation.

Stability: 4 weeks at 2-8°C.

**Calibration Frequency:** Calibration must be performed once per reagent lot using fresh reagent (i.e. not more than twenty-four hours since the reagent pack was registered on the analyzer). Renewed calibration is recommended as follows:

1. If necessary after instrument service or repair
2. If dictated by quality control results

**QUALITY CONTROL**:

BioRad Urine Chemistry controls, levels 1 and 2

Refer to Lab-4405 *Quality Control Criteria for Chemistry* for interpretation of QC. Each level ~~Two levels~~ of Quality Control should be performed at a minimum:

1. once every twenty-four hours
2. if a new pack of reagent is put in use
3. if a calibration is performed

**Implementation**

**PROCEDURAL NOTES:**

Refer to the Cobas 6000 Operator’s Manual Located in the chemistry department.

Results are reported to the nearest tenth in mg/dL.

**AMR (Analytical Measurement Range):** 4-200 mg/dL. The extended measuring range with Decrease mode (1:3) is 4-600 mg/dL.

The c501 will automatically rerun specimens greater than 200 mg/dL in a decreased mode. For non-bloody specimens, if the result is greater than 600 mg/dL, the specimen should be manually diluted with 0.9% NaCl. To obtain result, multiply by the dilution factor. The concentration of a manually diluted specimen must fall within the extended measuring range before applying the dilution factor.

If CSF is yellow, indicate xanthochromia.

**CALCULATIONS:**

The COBAS 6000 system automatically calculates the total protein concentration of each sample.

Timed urines are reported in g/24 hrs.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Urine Protein (mg/dL)  100,000 | X | Total Volume (mL)  Hours of Collection | X | 24 | = | Protein, Timed Urine (g/24hr) |

Protein/Creatinine Ratio (random urines only):

mg/dL Protein

mg/dL Creatinine

If the collection is less than 22 hours or greater than 26 hours, the LIS will automatically append a comment stating, “This estimated 24 hour result is calculated on a urine collection other than 24 hours. Please note actual collection period.” For a split collection (i.e. separate~~d~~ collections for upright and supine times), refer to Lab-4003 *Split Urine Collection*.

**INTERPRETATION:**

Expected range: CSF – 15-45 mg/dL

Timed urine – 0-0.15 g/24 hrs.

Studies have shown a close correlation between 24-hour urine protein excretion and the protein/ creatinine ratio in a random urine specimen. The normal ratio is near 0.1 (100-150 mg of protein per 24 hours and 1000-1500 mg of creatinine per 24 hours). A urine protein/creatinine ratio of 1.0 correlates well with quantitative proteinuria of approximately 1 g per 24 hours; a ratio of 2.0 equates with 2 g per 24 hours, and so forth. This test is extremely useful for clinical purposes; it provides the physician with enough information to classify proteinuria as mild (<1 g/24 hrs), moderate (1-3 g/24 hrs), or heavy (>3 g/24 hrs).

**LIMITATIONS:**

Hemolysis: No significant interference up to an H index of 30 (approximate hemoglobin concentration: 0.03 g/dL).

Icterus: No significant interference up to an I index of 20 (approximate total bilirubin concentration: 20 mg/dL).

See package insert for additional interference and cross-reactivity studies. The results should always be assessed in conjunction with the patient’s medical history, clinical examination and other findings.

Sample results with high total protein concentrations above the measuring range up to 10,000 mg/dL will be flagged by the instrument with >Abs. Determine these samples via the rerun function.

**Special Wash Requirements:**

The determination of certain analytes interferes with this assay requiring a special wash step. Refer to the NaOHD/SMS/SmpCln1+2/SCCS method sheet and the operator manual for further instructions.

**REVIEW AND CHANGES:**

This document and all attached forms should be reviewed optimally on an annual basis, with 2 years as the maximum review date. Review will be done by the Technical Leader, Medical Director or designated person. Changes require retyping document or form and review by the Medical Director.

**REFERENCES:**

1. Roche Total Protein Urine/CSF Gen.3 package insert
2. Roche C.f.a.s. PUC package insert
3. Roche Cobas 6000 Operator’s Manual