**PRINCIPLE**

The method measures the shift in the absorption spectrum from 460 to 600 nm of the complex that occurs at acid pH between pyrogallol red-molibdate (PRM) and the basic amino groups of urine and cerebrospinal fluid (CSF) proteins. The intensity of the colored complex formed is proportional to the concentration of protein in the sample.

\[
\text{PRM} + \text{Urine/CSF protein} \rightarrow \text{PRM-protein complex}
\]

**REAGENT COMPOSITION**

**R1**

- Pyrogallol reagent. Succinate buffer 60 mmol/L pH 2.5, pyrogallol red 0.06 mmol/L, sodium molybdate 0.04 mmol/L, SDS 0.08 mmol/L.

**CAL**

- Urine protein standard. Albumin/Globulin 200 mg/dL (2 g/L).
- Buffered mixture (80/20) on an artificial matrix. Biocides.
- Concentration value is traceable to Standard Reference Material 927.

**STORAGE AND STABILITY**

Store R1 at 15-30°C, and CAL at 2-8°C once opened. All the kit compounds are stable until the expiry date stated on the label. Do not use reagents over the expiration date. Store the vials tightly closed, protected from light and prevented contaminations during the use.

- Discard if appear signs of deterioration:
  - Presence of particles and turbidity.
  - Blank absorbance (A) at 600 nm > 0.200 in 1cm cuvette.

**REAGENT PREPARATION**

The reagents are ready-to-use.

**SAMPLES**

Urine collected without preservatives and CSF (see Notes). Turbid specimens should be centrifuged before testing. Urine proteins are stable up to 8 days at 2-8°C, and for 3 months at −20°C. CSF proteins are stable for 3 days at 2-8°C and for 3 months at −20°C.

**INTERFERENCES**

- Bilirubin (< 5 mg/dL) does not interfere.
- Hemoglobin may affect the results.
- Other drugs and substances may interfere.
- Positive interferences in urine of patients under treatment with aminoglycosids-gentamicine or tobramycine-reported with other pyrogallol tests have been shown to have no influence with this specific formulation.
- CSF contaminated by red cells from a traumatic lumbar puncture or intracerebral hemorrhage will increase protein concentrations by ≈ 10 mg/L for every 1000 erythrocytes.

**MATERIALS REQUIRED**

- Photometer or colorimeter capable of measuring absorbance at 600 ± 20 nm.
- Constant temperature incubator set at 37°C.
- Pipettes to measure reagent and samples.

**PROCEDURE**

1. Bring reagents and samples to room temperature.
2. Pipette into labelled tubes:

<table>
<thead>
<tr>
<th>TUBES</th>
<th>Blank</th>
<th>Sample</th>
<th>CAL Standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1:Reagent</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
<td>1.0 mL</td>
</tr>
<tr>
<td>Sample</td>
<td>–</td>
<td>20 µL</td>
<td>–</td>
</tr>
<tr>
<td>CAL:Standard</td>
<td>–</td>
<td>–</td>
<td>20 µL</td>
</tr>
</tbody>
</table>

3. Mix and incubate the tubes 5 minutes at 37°C or 10 minutes at room temperature.
4. Read the absorbance (A) of the samples and the standard at 600 nm against the reagent blank.

The color is stable for 30 minutes protected from light.

**CALCULATIONS**

Urine

\[
\text{A Sample} \times \frac{\text{V}}{2000} = \text{mg/24-h}
\]

Urine (single samples), CSF

\[
\text{A Sample} \times \frac{\text{C Standard}}{\text{A Standard}} = \text{mg/dL protein (see Notes)}
\]

Samples with concentrations higher than 400 mg/dL should be diluted 1:2 with saline and assayed again. Multiply the results by 2.
REFERENCE VALUES

<table>
<thead>
<tr>
<th></th>
<th>Urine</th>
<th></th>
<th>CSF</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adults</td>
<td>24-h samples: &lt; 150 mg/24-h</td>
<td></td>
<td>Adults</td>
<td>&lt; 45 mg/dL</td>
</tr>
<tr>
<td></td>
<td>single samples: &lt; 25 mg/dL</td>
<td></td>
<td>Children</td>
<td>&lt; 100 mg/dL</td>
</tr>
</tbody>
</table>

It is recommended that each laboratory establishes its own reference range.

QUALITY CONTROL

The use of a standard to calculate results allows to obtain an accuracy independent of the system or instrument used. To ensure adequate quality control (QC), each run should include a set of urine controls (normal and abnormal) with assayed values handled as unknowns. If the values are found outside of the defined range, check the instrument, reagents and procedure. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

CLINICAL SIGNIFICANCE

Total protein in the urine measurement is increasingly being replaced by the measurement of albumin, as this is the predominant urinary protein as this have demonstrated improved sensitivity and specificity for glomerular permeability changes. The presence of increased urinary excretion signals an increase in the transcapillary escape rate, being usually a marker of microvascular disease eventhough it may be also altered by physiological factors (exercise, diuresis and posture) as a consequence of altered intrarenal hemodynamics. The tubular reabsorptive process is saturable and any increase in the glomerular permeability or in plasma concentration (e.g., of Bence-Jones protein), or decreases in reabsorptive capacity due to proximal tubular damage (e.g., from nephrotoxic drugs) can result in proteinuria. Persistent urinary albumin excretion precedes and is highly predictive of diabetic nephropathy, end-stage renal disease, and proliferative retinopathy in type I diabetes. Measurement of CSF protein is used to distinguish septic from aseptic meningitis. Protein concentrations > 1 g/L are often viewed as diagnostic for bacterial, fungal, or tuberculous meningitis.

NOTES

- Clinical diagnosis should not be made on findings of a single test result, but should integrate both clinical and laboratory data.

ANALYTICAL PERFORMANCE

- Detection Limit : 8 mg/dL
- Linearity : Up to 400 mg/dL
- Precision:

<table>
<thead>
<tr>
<th>mg/dL</th>
<th>Within-run</th>
<th>Between-run</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>73</td>
<td>73</td>
</tr>
<tr>
<td>SD</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>CV%</td>
<td>1.35</td>
<td>3.09</td>
</tr>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

- Sensitivity : 2.3 mA / mg/dL proteins.
- Correlation. This assay (y) was compared with a similar commercial method (x). The results were:

\[ N = 50 \quad r = 0.99 \quad y = 0.95x - 0.01 \]

The analytical performance have been generated using on automatic instrument. Results may vary depending on the instrument.

REFERENCES