SUMMARY
Proteins in urine
Small quantities of plasmatic proteins of low molecular weight are normally filtered through the renal glomeruli and then, in part, are reabsorbed by the renal tubules. There are physiologic or benign conditions where an increase of protein urinary excretion may be found such as vigorous exercise, fever, hypothermia, pregnancy. The measurement of urinary proteins is important in the renal pathology's detection. Proteinuria in renal disease may result in glomerular or tubular dysfunction. Glomerular dysfunction is due to an increase in the passage through glomerular capillaries and is characterized by a loss in plasmatic proteins with similar or larger size. Tubular dysfunction is due to a decrease of the tubule's protein absorption capacity. Among the pathologies that show an increase in the urinary protein excretion are found: nephritic syndrome, nephrotic syndrome, monoclonal hypergammaglobulinemia, diabetic nephropathy and infections in the urinary tract.

Cerebrospinal fluid (CSF) Proteins
Protein determination in CSF is useful to assess the permeability of the hematooencephalic barrier in many inflammatory or infectious diseases of the Central Nervous System (CNS), as it is occur in bacterial meningitis, viral meningitis or meningitis from other origins, encephalitis, poliomyelitis, neurosyphilis, multiple sclerosis, brain hemorrhage, cerebral or spinal tumors. Other disorders, as demyelinazation diseases, cause an abnormal protein production within the CNS. The present method's sensitivity makes it suitable to be used in biological fluids such as urine and cerebrospinal fluid, where protein concentration in relation to plasma is too low to be determined using the conventional methods for protein test in serum.

INSTRUCTIONS FOR USE
Provided Reagents: ready to use.

WARNINGS
Reagents are for "in vitro" diagnostic use. Use the reagents according to the working procedures for clinical laboratories. The reagents and samples should be discarded according to the local regulations in force.

STABILITY AND STORAGE INSTRUCTIONS
Provided Reagents: are stable in refrigerator at 2-10℃ until the expiration date stated on the box. The Reagent A should be protected from light.

INSTABILITY OR DETERIORATION OF REAGENTS
When the spectrophotometer has been set to zero with distilled water, Reagent A absorbance readings (at 600 nm) above 0.250 O.D. or bellow 0.030 O.D. indicate its deterioracion.

SAMPLE
Urine or cerebrospinal fluid
a) Collection: obtain occasional or 24-hour urine. Measure diuresis. If samples are turbid, it is convenient to centrifuge them.
b) Additives: not required.
c) Known Interfering Substances:
   - hemolysis may cause falsely increased results in urine as well as in CSF.
   - urine preservatives such as hydrochloric acid, benzoic acid or thymol may cause falsely decreased results;
   - some drugs or medications may interfere in the reaction. See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: urine may be stored at 2-10℃ for up to 8 days or at -20℃ for up to 3 months. CSF may be stored for up to 3 days at 2-10℃ or for up to 3 months frozen at -20℃.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer for readings at 600 nm (580-620 nm).
- Water bath at 37℃.
- Pipettes and micropipettes for measuring the stated volumes

PROCEDURE
Bring reagents and samples to room temperature before use. In three tests tubes or spectrophotometric cuvettes
labeled B (Blank), S (Standard), and U (Unknown), place:

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<tr>
<th></th>
<th>B</th>
<th>S</th>
<th>U</th>
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</thead>
<tbody>
<tr>
<td>Standard</td>
<td>-</td>
<td>20 ul</td>
<td>-</td>
</tr>
<tr>
<td>Sample</td>
<td>-</td>
<td>-</td>
<td>20 ul</td>
</tr>
<tr>
<td>Reagent A</td>
<td>1 ml</td>
<td>1 ml</td>
<td>1 ml</td>
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Mix and incubate the tubes for 10 minutes at 37°C. Measure in photocolorimeter between 580-620 nm or in spectrophotometer at 600 nm, setting the instrument to zero with the Blank.

STABILITY OF FINAL REACTION
Reaction color is stable for 30 minutes. Therefore, read the absorbance within that period.

CALCULATIONS
1) 24-hour urine Proteins

\[
\text{mg of Proteins/24 hours} = \frac{U}{S} \times V \times 1000
\]

where:

\[V = \text{diuresis volume expressed in liters/24 hours}\]
\[1000 = \text{mg/l Standard}\]

2) Proteins in occasional urine

\[
\text{mg/dl proteins} = \frac{U}{S} \times 100
\]

3) Proteins in CFS

\[
\text{mg/dl proteins} = \frac{U}{S} \times 100
\]

QUALITY CONTROL METHOD
Each time the test is performed, analyze two levels of a quality control material (Proti U/LCR Control 2 niveles) with known proteins concentration.

REFERENCE VALUES
24-hour urine: 30-140 mg/24 hours (up to 160 mg/24 hours in pregnant women)
Occasional urine: 25 mg/dl
CSF: 15-45 mg/dl in healthy individuals. In persons over 60 years old, this range is extended up to 60 mg/dl.
These values should be considered as an orientation. Each laboratory should establish its own values, since they may vary according to the patients’ population and laboratory conditions.

SI SYSTEM UNITS CONVERSION
Proteins (g/dl) x 10 = Proteins (g/l)

PROCEDURE LIMITATIONS
See Known interfering substances under SAMPLE. Protect the Reagent A from the light. The material used should be free from surfactants. Otherwise, discordant values may be obtained.

PERFORMANCE
a) Reproducibility: processing replicates of the same samples in one day, the following results were obtained:

<table>
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<th>Level</th>
<th>S.D.</th>
<th>C.V.</th>
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<tbody>
<tr>
<td>14 mg/dl</td>
<td>± 0.66 mg/dl</td>
<td>4.7 %</td>
</tr>
<tr>
<td>100 mg/dl</td>
<td>± 2.30 mg/dl</td>
<td>2.3 %</td>
</tr>
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b) Sensitivity: in spectrophotometer at 600 nm, a 100 mg/dl Standard gives a reading of approximately 0.200 O.D. Thus, for 0.001 O.D. the smallest detectable activity change will be of 0.5 mg/dl.

c) Linearity: reaction is linear up to 150 mg/dl proteins. For higher values, dilute sample 1:2 or 1:4 with saline solution and repeat determination. Correct calculations multiplying by the dilution factor used. To increase the analytic sensitivity in normal or lightly increased samples, 50 ul sample may be used. In that case, it is advisable to dilute the Standard 1:2 (1+1) with distilled water and use this 50 mg/dl standard in the test to adjust the calibration to low normal values.

PARAMETERS FOR AUTOANALYZERS
For programming instructions check the user manual of the autoanalyzer in use.

WIENER LAB PROVIDES
- 1 x 100 ml (Cat. Nr. 1690007).
- 4 x 20 ml (Cat. Nr. 1009317).
- 4 x 20 ml (Cat. Nr. 1009282).
- 2 x 60 ml (Cat. Nr. 1009631).

REFERENCES
Symbols
The following symbols are used in packaging for Wiener lab. diagnostic reagent kits.

- CE: This product fulfills the requirements of the European Directive 98/79 EC for "in vitro" diagnostic medical devices
- EC REP: Authorized representative in the European Community
- IVD: "In vitro" diagnostic medical device
- Σ: Contains sufficient for <n> tests
- Use by
- Temperature limitation (store at)
- Do not freeze
- Biological risks
- Volume after reconstitution
- Contents
- Batch code

Manufactured by:

Harmful
Corrosive / Caustic
Irritant
Consult instructions for use

Calibrator
Control
Positive Control
Negative Control
Catalog number