SUMMARY
Ceruloplasmin is a α2-glycoprotein synthesized in the liver. Its major role is to transport plasmatic copper to the copper-containing enzymes. Ceruloplasmin is essential in the regulation of redox potential, transport and utilization of iron. Decreased ceruloplasmin levels are present in Wilson’s disease and in connective tissue diseases. Acquired ceruloplasmin deficiency may be due to hepatic insufficiency, protein-losing enteropathy, nephrotic syndrome and malabsorption syndrome. Increased ceruloplasmin levels are present during acute and chronic inflammatory process, cholestasis, systemic lupus erythematosus (SLE) and rheumatoid arthritis.

PRINCIPLE
The ceruloplasmin reacts to the specific antibody forming insoluble immune complexes. The turbidity caused by these immune complexes is proportional to the ceruloplasmin concentration in the sample and may be spectrophotometrically measured.

PROVIDED REAGENTS
A. Reagent A: phosphate buffer, pH 7.4.
B. Reagent B: polyclonal antibodies anti-human ceruloplasmin (goat) in phosphate buffer, pH 7.4.

NON-PROVIDED REAGENTS
- Saline solution
- Wiener lab.’s Ceruloplasmin Calibrator Turbitest AA

INSTRUCTIONS FOR USE
Provided Reagents: ready to use.

WARNINGS
The reagents are for “in vitro” diagnostic use. All patient samples should be handled as though capable of transmitting infectious diseases. 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: stable at 2-10°C until the expiration date stated on the box. Do not freeze.

SAMPLE
Serum
a) Collection: obtain in the usual way.
b) Additives: not required.
c) Known interfering substances: do not use hemolyzed, lipemic or contaminated samples. Before testing, particles in samples should be removed by centrifugation. No interferences are observed with hemoglobin up to 1000 mg/dl, triglycerides up to 2500 mg/dl, bilirubin up to 20 mg/dl, heparin up to 50 mg/dl and sodium citrate up to 1000 mg/dl. See Young, D.S. in References for effect of drugs on the present method.
d) Stability and storage instructions: sample should be preferably fresh. In case it cannot be processed immediately, the sample can be kept for up to 48 hours at 2-10°C or for longer period store at -20°C.

REQUIRED MATERIAL (non-provided)
- Spectrophotometer.
- Square spectrophotometric cuvettes.
- Micropipettes and pipettes for measuring the stated volumes.
- Kahn or hemolysis tubes.
- Stopwatch.

ASSAY CONDITIONS
- Wavelength: 340 nm
- Reaction temperature: room temperature (25°C). Temperature control is not critical, it can range between 22 and 30°C.
- Reaction time: 15 minutes
- Sample volume: 10 ul
- Final reaction volume: 1810 ul
Sample and reagents volumes may be proportionally changed without affecting the calculation factors.

PROCEDURE
CALIBRATION CURVE
In Kahn tubes dilute the Ceruloplasmin Calibrator Turbitest AA with saline solution 1:1, 1:2, 1:4, 1:8 and 1:16, using saline solution as the zero point.

<table>
<thead>
<tr>
<th>Diluted Calibrator</th>
<th>10 ul</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent A</td>
<td>1500 ul</td>
</tr>
</tbody>
</table>

Homogenize and measure the absorbance of each dilution at 340 nm (OD), setting the instrument to zero with
distilled water. Then, add:

| Reagent B | 300 ul |

Mix and incubate 15 minutes at room temperature. Measure the absorbance at 340 nm (OD$_2$), setting the instrument to zero with distilled water. Calculate the absorbance difference ($\Delta A = \text{OD}_2 - \text{OD}_1$) for each Ceruloplasmin Calibrator Turbitest AA, including the zero point. Draw on graph paper the $\Delta A$ absorbance differences based on the Ceruloplasmin Calibrator Turbitest AA concentration in mg/dl (g/l).

### SAMPLES PROCEDURE

| Sample | 10 ul |

Reagent A 1500 ul

Homogenize and measure the absorbance at 340 nm (OD$_1$), setting the instrument to zero with distilled water. Then add:

| Reagent B | 300 ul |

Mix and incubate 15 minutes at room temperature. Measure the absorbance at 340 nm (OD$_2$), setting the instrument to zero with distilled water.

### CALCULATIONS

Calculate the absorbance difference ($\Delta A = \text{OD}_2 - \text{OD}_1$) for each sample tested. Interpolate this $\Delta A$ in the calibration curve to determine the concentration in mg/dl (g/l) corresponding to the sample under study. Samples with an absorbance above that of the Ceruloplasmin Calibrator Turbitest AA must be diluted with saline solution and processed again. Multiply the obtained result by the dilution factor.

### QUALITY CONTROL METHOD

Wiener lab.’s Control Inmunológico nivel 1 Turbitest AA or Control Inmunológico nivel 2 Turbitest AA. The Control is processed in the same manner as samples.

### REFERENCE VALUES

20-60 mg/dl (0.20-0.60 g/l)

Each laboratory should set its own reference values.

### PROCEDURE LIMITATIONS

See Known interfering substances under SAMPLE. It is recommended to perform a complete recalibration when changing reagent lot or when suggested by Quality Control. Avoid contamination to preserve the integrity of the reagents. Only use thoroughly clean and dry micropipettes for measurement.

### PERFORMANCE

**a) Reproducibility:** replicates of samples containing different ceruloplasmin levels were assayed and the following results were obtained:

### Intra-assay precision

<table>
<thead>
<tr>
<th>Level</th>
<th>S.D.</th>
<th>C.V.</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.2 mg/dl</td>
<td>± 0.4 mg/dl</td>
<td>3.9%</td>
</tr>
<tr>
<td>35.6 mg/dl</td>
<td>± 0.6 mg/dl</td>
<td>1.6%</td>
</tr>
<tr>
<td>75.8 mg/dl</td>
<td>± 2.3 mg/dl</td>
<td>3.0%</td>
</tr>
</tbody>
</table>

**b) Detection limit:** 5 mg/dl

**c) Measuring range:** 5 - 110 mg/dl

**d) Prozone effect:** not noted until 150 mg/dl ceruloplasmin.

### WIENER LAB. PROVIDES

60 ml: - 1 x 50 ml Reagent A 
- 1 x 10 ml Reagent B 
(Cat. Nº 1009357)

### REFERENCES

- Dati, F et al. - Proteins-Laboratory testing and clinical use, 2005.
Symbols

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

- **CE**
  - Manufactured by:
  - Authorized representative in the European Community

- **EC REP**
  - "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**

- **Harmful**

- **Corrosive / Caustic**

- **Irritant**

- **Consult instructions for use**

- **Calibrator**

- **Control**

- **Positive Control**

- **Negative Control**

- **Catalog number**