**Clinical Significance**

Ceruloplasmin is a 
-globulin that contains approximately 95% of total serum copper. Each molecule of Ceruloplasmin contains six to eight copper atoms. The high content of copper ions gives Ceruloplasmin a blue color. Ceruloplasmins can also bind, and possible transport, other cations such as magnesium. The molecule of caeruloplasmin has a single polypeptide chain and carbohydrate, and results a molecular mass of 132 kD. Caeruloplasmin is synthesized primarily by the hepatic cells and small quantities by macrophages and lymphocytes.

Caeruloplasmin is most often quantified as a screening test for Wilson's disease. However, it is important to realize that several other factors, including diet, hormone levels, and other genetic disorders, can influence plasma levels. Synthesis of Ceruloplasmin is increased modestly in the acute-phase response, peaking at 4 to 20 days after a single, acute insult. Synthesis is also stimulated by estrogens, and during pregnancy.

Low plasma caeruloplasmin levels are due to lack of incorporation of Cu^{2+} into the molecule during synthesis. The causes are the dietary insufficiency (including malabsorption), inability to release Cu^{2+} from gastrointestinal epithelium into circulation, or inability to insert Cu^{2+} into developing caeruloplasmin molecule. Levels may also be low in blood loss or gastrointestinal or renal protein losing syndromes.

**Principle**

Anti-human Ceruloplasmin antibodies when mixed with samples containing Ceruloplasmin, form insoluble complexes. These complexes cause an absorbance change, dependent upon the Ceruloplasmin concentration of the patient sample that can be quantified by comparison from a calibrator of known Ceruloplasmin concentration.

**Reagents**

<table>
<thead>
<tr>
<th>Diluent (R1)</th>
<th>Tris buffer 20 mmol/L, PEG 8000, pH 8.3.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibody (R2)</td>
<td>Goat serum, anti-human Ceruloplasmin, pH 7.5. Sodium azide 0.95 g/L.</td>
</tr>
<tr>
<td>Optional</td>
<td>Cod Cod: 1102003 PROT CAL.</td>
</tr>
</tbody>
</table>

**Calibration**

The assay is calibrated to the Reference Material CRM 470/RPPHS (Institute for Reference Materials and Measurements). It must be used the PROT CAL to calibrate the reagent. The calibrator should not be diluted, as it is ready to use. The reagent, monoreagent and bireagent, should be recalibrated every three weeks and every month, respectively. It should also be recalibrated when the controls are out of specifications, and when changing the reagent lot or the instrument settings. For monoreagent, a reagent blank should be run daily before sample analysis.

**Reagent Preparation**

**Working reagent**: Swirl the latex vial gently before use. Prepare the necessary amount as follow:

- 1 mL Latex Reagent + 4 mL Diluent.

**Storage And Stability**

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8°C and contaminations are prevented during their use. Do not use reagents over the expiration date.

**Reagent deterioration**: The presence of particles and turbidity. Do not use.

**Do not freeze**: frozen Antibody or Diluent could change the functionality of the test.

**Materials required**

- Thermostatic bath at 37°C.
- Spectrophotometer or photometer thermostatable at 37°C with a 340 nm filter (320 - 360 nm).

**Specimen and Stability**

Fresh serum or plasma. EDTA or heparin should be used as anticoagulant. Stable 7 days at 2-8°C or 3 months at −20°C. The samples with presence of fibrin should be centrifuged. Do not use highly hemolyzed or lipemic samples.

**Automated Assay Procedure**

1. Bring the reagents and the photometer (cuvette holder) to 37°C.
2. Assay conditions:
   - Wavelength: 340 nm
   - Temperature: 37°C
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:
   - Reagent R1: 800 µL
   - Sample or Calibrator: 7 µL
5. Mix and read the absorbance (A1) after the sample addition.
6. Immediately, pipette into the cuvette:
   - Reagent R2: 200 µL
7. Mix and read the absorbance (A2) of calibrators and sample exactly 2 minutes after the R2 addition.

**Manual Procedure for Ceruloplasmin**

1. Bring the working reagent and the photometer (cuvette holder) to 37°C.
2. Assay conditions:
   - Wavelength: 340 nm
   - Temperature: 37°C
   - Cuvette light path: 1 cm
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:
   - Working Reagent (µL): 500
   - Calibrator or sample (µL): 3.5
5. Mix and read the absorbance immediately (A1) and after 2 minutes (A2) of the sample addition.

**Application sheets for automated systems are available on request.**

**Calculations**

\[
\frac{(A_1-A_2)}{A_1} \times 100 = \frac{Ceruloplasmin}{\text{mg/dL}}
\]

**Quality Control**

Control sera are recommended to monitor the performance of manual and automated assay procedures. Spinreact PROT CONTROL (Cod.: 1102004). Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.
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Reference Values
Between 15 – 60 mg/dL. Each laboratory should establish its own reference range.

These values are for orientation purpose. Each laboratory should establish its own reference range.

Performance Characteristics

Linearity limit: Up to 120 mg/dL, under the described assay conditions. Samples with higher concentrations should be diluted 1/5 in NaCl 9 g/L and retested again. The linearity limit depends on the sample / reagent ratio. It will be higher by decreasing the sample volume, although the sensitivity of the test will be proportionally decreased.

Detection limit: Values less than 3.27 mg/dL give non-reproducible results.

Precision: The reagent has been tested for 20 days, using three levels of serum in an EP5-based study (NCCLS).

<table>
<thead>
<tr>
<th>EPS</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>28.96 mg/dL</td>
<td>4%</td>
</tr>
<tr>
<td>55.47 mg/dL</td>
<td>2.3%</td>
</tr>
<tr>
<td>76.54 mg/dL</td>
<td>2%</td>
</tr>
</tbody>
</table>

Total 4%, Within Run 2.2%, Between Run 3.1%, Between Day 1.1%

Accuracy: Results obtained using this reagent (y) was compared to those obtained with a Bayer Immunoturbidimetry method. 45 samples ranging from 20 to 80 mg/dL of Ceruloplasmin were assayed. The correlation coefficient (r) was 0.96 and the regression equation y = 0.896x + 10.57. The results of the performance characteristics depend on the analyzer used.

Interferences
Haemoglobin (20 g/L), bilirubin (40 mg/dL), lipemia (< 2.5 g/L), and rheumatoid factor (800 IU/mL) do not interfere. Other substances may interfere.

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

Only for invitro use in Clinical laboratory (IVD)

Literature

Manufactured by: Spinreact, S.A.
Marketed By: Euro Diagnostic Systems Pvt. Ltd., Millennium House, M.K.Srinivasan Nagar Main Road, No. 144, Old Mahabalipuram Road, Perungudi, Chennai – 600 096; Email: eurods@vsnl.net, www.eurods.in

SYSTEM PARAMETERS

<table>
<thead>
<tr>
<th>Mode</th>
<th>Two Point/Fixed time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction</td>
<td>Ascending</td>
</tr>
<tr>
<td>Wavelength</td>
<td>340 nm</td>
</tr>
<tr>
<td>Blank with</td>
<td>Distilled water</td>
</tr>
<tr>
<td>Sample Volume</td>
<td>3.5 μL</td>
</tr>
<tr>
<td>Reagent Volume</td>
<td>500 μL</td>
</tr>
<tr>
<td>Delay Time</td>
<td>10 (Sec)</td>
</tr>
<tr>
<td>Read Time</td>
<td>120 (Sec)</td>
</tr>
<tr>
<td>Calibrator</td>
<td>stated on the vial</td>
</tr>
<tr>
<td>Normal ranges</td>
<td>15 – 60 mg/dL</td>
</tr>
<tr>
<td>Linearity limit</td>
<td>120 mg/dL</td>
</tr>
<tr>
<td>Unit</td>
<td>mg/dL</td>
</tr>
</tbody>
</table>