Copper
(Colorimetric Test with Dibrom-PAESA)

Intended Use
Spectrum Diagnostics Copper reagent is intended for in-vitro quantitative, diagnostic determination of Copper in human serum, plasma or urine on both manual and automated systems.

Background
Copper (Cu) is an important trace element and is associated with a number of metalloproteins and it is a catalytic component of numerous enzymes and also a structural component of other important proteins. Copper is involved in many vital processes in the body, Energy Production, connective tissue formation, iron metabolism, melanin synthesis, Normal function of CNS, Regulation of gene expression and has Antioxidant function. Excess Cu ingestion interfere with absorption of zinc and can lead to zinc deficiency, which is frequently characterized by slow healing. The classical presentation of Cu toxicosis is represented by the genetic disease of Cu accumulation known as Wilson’s disease. This disease is typified by hepatocellular damage (increase transferase) and/or changes in mood and behavior because of accumulation of Cu in Central Neurons.

Method
Colorimetric with Dibrom-PAESA

Assay Principle
Copper forms with 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-sulfopropylamine a chelate complex. The increase of absorbance of this complex can be measured and is proportional to the concentration of total copper in the sample.

Reagents
Standard (ST) 100 µg/dL 15.7 µmol/L
R (Monoreagent)
Acetate buffer pH 5.0 0.2 mol/L
4-(3,5-dibromo-2-pyridylazo)-N-ethyl-sulfopropylamine 0.02 mmol/L

For further information, refer to the Copper reagent material safety data sheet.

Precautions and Warnings
Do not ingest or inhale. In case of contact with eyes or skin; rinse immediately with plenty of soap and water. In case of severe injuries; seek medical advice immediately.
Avoid contamination by using clean laboratory material (pipette, plastic vial for analyzer,....)

Reagent Preparation, Storage and Stability
Warning: The reagent could precipitate during refrigerate storage. It suggested to let it to redissolve at room temperature before use (15 minutes). Mix well after redissolving.

Spectrum Copper reagent is supplied ready-to-use and stable up to the expiry date labeled on the bottles. Once opened, the opened vial is stable for 3 months at 2-8 °C.

Specimen collection and preparation
Serum, Plasma (free from haemolysis)
24 hours Urine: Refrigerate or add 10 ml of 3 mol/L HCl to the container before collection.

System Parameters
Wavelength 580 nm (Hg 578)
Optical path 1 cm
Assay type End-point
Direction Increase
Temperature 37°C
Linearity 500 µg/dl (78.65 µmol/l)

Procedure
I- Determination of copper in serum

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>.......</td>
<td>50 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>.......</td>
<td>.......</td>
</tr>
</tbody>
</table>

Mix and incubate for 5 minutes at 37°C. Measure the absorbance of the sample As and of the standard Asi against the reagent blank Ablank.

ΔAs = As - Ablank.
ΔAstd = Astd - Ablank.

Calculation
Serum Copper conc. (µg/dl) = ΔAs
                        ΔAstd
                        x 100

Serum Copper conc. (µmol/l) = ΔAs
                          ΔAstd
                          x 15.7

II- Determination of copper in urine

Dilute Standard 20 Times (Example: 50 µl standard + 950 µl normal saline), then follow the method below:

<table>
<thead>
<tr>
<th>Blank</th>
<th>Standard</th>
<th>Urine Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reagent</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
</tr>
<tr>
<td>Standard</td>
<td>.......</td>
<td>500 µl</td>
</tr>
<tr>
<td>Sample</td>
<td>.......</td>
<td>.......</td>
</tr>
<tr>
<td>Dist.H2O</td>
<td>500 µl</td>
<td>.......</td>
</tr>
</tbody>
</table>

Mix and incubate for 5 minutes at 37°C. Measure the absorbance of the sample As and of the standard Asi against the reagent blank Ablank.

ΔAs = As - Ablank.
ΔAstd = Astd - Ablank.
Calculation

Urine Copper conc. (μg/dL) = \( \frac{\Delta A_s}{\Delta A_{std}} \times 5 \)

Urine Copper conc. (μmol/l) = \( \frac{\Delta A_s}{\Delta A_{std}} \times 0.785 \)

Quality Control

Normal & abnormal commercial control serum of known concentrations should be analyzed with each run.

Linearity

The reaction is linear up to a Copper concentration of 500 μg/dl (78.65 μmol/l)
Specimens showing higher concentration should be diluted 1+1 using physiological saline and repeat the assay (result × 2).

Interfering Substances:

Interferences are found according to the literatures.

Expected Values

In Serum

<table>
<thead>
<tr>
<th></th>
<th>Range</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult males</td>
<td>70 - 140 μg/dl</td>
<td>(11 - 22 μmol/l)</td>
</tr>
<tr>
<td>Adult females</td>
<td>80 - 155 μg/dl</td>
<td>(12.5 - 24.3 μmol/l)</td>
</tr>
<tr>
<td>Females in pregnancy</td>
<td>120 - 300 μg/dl</td>
<td>(18.8 - 47 μmol/l)</td>
</tr>
<tr>
<td>Children (6-12 years)</td>
<td>80 - 190 μg/dl</td>
<td>(12.5 - 29.8 μmol/l)</td>
</tr>
<tr>
<td>Infants</td>
<td>20 - 70 μg/dl</td>
<td>(3.14 - 11 μmol/l)</td>
</tr>
</tbody>
</table>

In 24hours Urine | 10 - 30 μg/24hours |

Spectrum Diagnostics does not interpret the results of a clinical laboratory procedure; interpretation of the results is considered the responsibility of qualified medical personnel. All indications of clinical significance are supported by literature references.

Waste Disposal

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. refer to special instructions/safety data sheets.

References


ORDERING INFORMATION

<table>
<thead>
<tr>
<th>CATALOG NO.</th>
<th>QUANTITY</th>
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<tbody>
<tr>
<td>232 001</td>
<td>50 Test</td>
</tr>
<tr>
<td>232 002</td>
<td>100 Test</td>
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</tbody>
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