COD 11837 1 x 50 mL
Only for *in vitro* use in the clinical laboratory

COPPER-PAESA

BioSystems

CE

COPPER-PAESA
3,5-DiBr-PAESA

INTENDED USE

Reagent for the measurement of copper concentration in human serum, plasma and urine for the assessment of its imbalance.

CLINICAL BENEFIT

The most significant clinical application of copper determination is its association with hepatolenticular degeneration or Wilson's disease, and the decrease in the synthesis of ceruloplasmin that results in low serum cooper levels. Other diseases associated with decreased serum copper are bone and joint osteoarthritis and osteoporosis or Menkes' syndrome[1].

Serum copper concentration can also be elevated in acute and chronic diseases such as leukemia, hemochromatosis and biliary cirrhosis.

Based on clinical guidelines and textbooks, and when used in conjunction with other diagnostic technologies and options, this medical information is useful for the assessment of copper imbalance. Clinical diagnosis should not be made on the findings of a single test result, but should integrate both clinical and laboratory data.

PRINCIPLE OF THE METHOD

Ceruloplasmin-bound copper ions in the sample are released at pH 4.7. Copper ions react with 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylaniline monosodium salt (PAESA) forming a coloured complex that can be measured by spectrophotometry[2].

$$\text{COPPER (Cu)} + 2\text{PAESA} \xrightarrow{\text{pH = 4.7}} [\text{Cu(PAESA)}_2]$$

CONTENTS AND COMPOSITION

A1. Reagent. 1 x 32 mL. Sodium acetate 200 mmol/L, pH = 4.7.

WARNING: H317: May cause an allergic skin reaction. H319: Causes serious eye irritation. P261: Avoid breathing vapours.P280: Wear protective gloves/protective clothing/eye protection/face protection.P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P362: Take off contaminated clothing and wash before reuse.

A2. Reagent. 1 x 8 mL. Ascorbic acid 500 mmol/L.

DANGER: H314: Causes severe skin burns and eye damage. H317: May cause an allergic skin reaction P260: Do not breathe vapours. P280: Wear protective gloves/protective clothing/eye protection/face protection. P303+P361+P353: If on skin (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. P305+P351+P338: If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

B. Reagent. 1 x 10 mL. Sodium acetate 200 mmol/L, 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylaniline monosodium salt (PAESA) 0.200 mmol/L.

WARNING: H317: May cause an allergic skin reaction. P261: Avoid breathing vapours. P280: Wear protective gloves/protective clothing/eye protection/face protection.P333+P313: If skin irritation or rash occurs: Get medical advice/attention. P362: Take off contaminated clothing and wash before reuse.

S. Copper Standard. 1 x 3 mL. Copper 200 µg/dL (31.4 µmol/L). For the Urine Procedure dilute the Standard 1:20 with physiological solution, Copper 10 µg/dL (1.57 umol/L). Aqueous primary standard.

WARNING: H317: May cause an allergic skin reaction. P261: Avoid breathing vapours. P280: Wear protective gloves/protective clothing/eye protection/face protection. P333+P313: If skin irritation or rash occurs: Get medical advice/attention

STORAGE AND STABILITY

Store at 2-8ºC.

Components are stable once opened until the expiry date marked in the label if they are stored at the recommended temperature, well closed and care is taken to prevent contamination during their use.

Indications of deterioration:

- Reagents: Presence of particulate material, turbidity, or absorbance of the blank over 0.200 at 580 nm (1 cm cuvette).
- Standard: Presence of particulate material, turbidity.

WARNING AND PRECAUTIONS

Exercise the normal precautions required for handling all laboratory reagents. Safety data sheet available for professional user on request. Disposal of all waste material should be in accordance with local guidelines. Any serious incident that might occur in relation to the device shall be reported to BioSystems S.A.

ADDITIONAL MATERIALS REQUIRED (NOT PROVIDED)

- Analyzer, spectrophotometer or photometer able to read at 580 nm ± 20 nm.
- Cuvettes with 1 cm light path.
- Biochemistry Calibrator (BioSystems cod. 18011) or Biochemistry Calibrator Human (BioSystems cod. 18044) (Note 1).

REAGENT PREPARATION

Reagent A: pour the contents of the Reagent A2 vial into the Reagent A1 bottle. Mix gently. Other volumes can be prepared in the proportion: 4 mL Reagent A1 + 1 mL Reagent A2. Stable for 2 months at 2-8ºC.

Reagent B and Standard (S): Ready to use.

SAMPLES

Serum, heparinized plasma or urine collected by standard procedures.

Copper in serum or heparinized plasma is stable for 2 weeks at 2-8ºC or 1 year at -20ºC. Use heparin as anticoagulant[3].

Collect 24-hour urine in a bottle. Centrifuge or filter the sample before measurement. Stable for 1 week at 2-8ºC[3].

PROCEDURE (Notes 2,3)

1. Pipette into test tubes:

	Reagent Blank	Sample Blank	Standard	Sample
Distilled water (Ser/Uri)	50 µL / 950 µL	-	-	-
Copper Standard (S) (Ser/Uri)	-	-	50 µL / 950 µL	-
Serum/Plasma	-	50 µL	-	50 µL
Urine	-	950 µL	-	950 µL
Reagent A	800 µL	1000 µL	800 µL	800 µL
Reagent B	200 µL	-	200 µL	200 µL

2. Mix thoroughly and incubate for 5 minutes at room temperature.
3. Measure the absorbance (A) of the Sample Blanks at 580 nm against distilled water.
4. Measure the absorbance (A) of the Sample and Calibrator at 580 nm against Reagent Blank.

CALCULATIONS

The copper concentration in the sample is calculated using the following general formula:

$\frac{A_{Sample} - A_{Blank\ Sample}}{A_{Standard}}$	Serum/Plasma	Urine
	x 200 = µg/dL Copper x 31.4 = µmol/L Copper	x 10 = µg/dL Copper x 1.57 = µmol/L Copper

QUALITY CONTROL

It is recommended to use the Biochemistry Control Serum level I (cod. 18005, 18009 and 18042) and II (cod. 18007. 18010 and 18043) to verify the accuracy of the measurement procedure.

Each laboratory should establish its own internal Quality Control scheme and procedures for corrective action if control results are not within the acceptable limits.

REFERENCE VALUES[4]

Serum and plasma: 70 - 140 µg/dL = 10.9 - 21.9 µmol/L

Urine: < 60 µg/24-h = < 1.0 µmol/24-h.

These ranges are given for orientation only; each laboratory should establish its own reference ranges.

METROLOGICAL CHARACTERISTICS

The metrological characteristics for serum sample described below have been obtained using a BA400 analyzer and following the guidelines of the Clinical & Laboratory Standards Institute (CLSI). Results may vary if a different instrument or a manual procedure are used. The metrological characterisitics for urine sample have been ontained using an spectrophotometer an the manual procedure.

- Detection limit: 11.0 µg/dL=1.73 µmol/L (serum); 2.08 µg/dL=0.33 µmol/L (urine). Quantification limit: 60.6 µg/dL=9.53 µmol/L (serum); 5.74 µg/dL=0.90 µmol/L (urine).
- Linearity limit: 500 µg/dL=78.7 µmol/L (serum), 50 µg/dL=7.87 µmol/L (urine). For higher values dilute sample 1/2 with distilled water and repeat measurement. Measuring range: 60.6 - 500 µg/dL= 9.53 - 78.7 µmol/L (serum), 5.74 - 50.0 µg/dL=0.90 - 7.87 µmol/L (urine).
- Precision:

Serum. Mean concentration	Repeatability (CV)	Within-laboratory (CV)
133 µg/dL = 20.9 µmol/L	1.8 %	2.6 %
219 µg/dL = 34.5 µmol/L	1.2 %	1.7 %
307 µg/dL = 48.3 µmol/L	1.0 %	1.3 %

Urine. Mean concentration	Repeatability (CV)	Within-laboratory (CV)
12.2 µg/dL = 1.91 µmol/L	4.2 %	7.4 %
27.3 µg/dL = 4.30 µmol/L	3.6 %	4.8 %

- Trueness: Results obtained with this reagent did not show systematic differences when compared with reference reagents. Details of the comparison experiments are available on request.

LIMITATIONS OF THE PROCEDURE

- Interferences: bilirubin (up to 30 mg/dL=513 µmol/L), hemolysis (hemoglobin up to 500 mg/dL) and lipemia (triglycerides up to 363 mg/dL=4.10 mmol/L) do not interfere. Other drugs and substances may interfere[5].

NOTES

1. Calibration with the provided aqueous standard may cause a matrix related bias, specially in some analyzers. In these cases, it is recommended to calibrate using a serum based standard (Biochemistry Calibrator, cod. 18011 and 18044).
2. These reagents may be used in several automatic analysers. Instructions for many of them are available on request. Tap water may contain larger amounts of iron or copper. The use of such tap water introduces errors into the determination of copper in a specimen. If the variability between duplicate reagent blank measurements exceeds 5 % conta-mination should be suspected.
3. Contamination of glassware with copper will affect the test. Use acid-washed glassware or plastic tubes.

BIBLIOGRAPHY

1. Young DS. Effects of disease on clinical laboratory tests, 4th ed. AACC Press, 2001.
2. Abe A, Yamashita S, Noma A. Sensitive, Direct Colorimetric Assay for Cooper in Serum. Clin Chem 1989; 35: 552-554.
3. Word Health Organization (WHO). Use of anticoagulants in diagnostic laboratory investigations. Document WHO/DIL/LAB/99.1, Rev.2; 2002.
4. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics, 6th ed. Rifai N, Horvath AR, Wittwer CT, Elsevier, 2018.
5. Young DS. Effects of drugs on clinical laboratory tests, 5th ed. AACC Press, 2000.