Copper

Color 3,5-DiBr-PAESA

Quantitative determination of copper

CE IVD

PACKAGING

Ref.: 101-0457	Cont.: 5 x 10 mL

Store at 2-8º C

CLINICAL SIGNIFICANCE

A variety of human copper deficiency conditions are recognized. Specific diseases associated with copper include head disease, bone and joint osteoarthritis and osteoporosis and Menkes' syndrome, Wilson's disease and others. Elevated levels of copper can also be toxic.

Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

PRINCIPLE OF THE METHOD

Direct colorimetric test without deproteinization of the sample. End point increase. At pH 4.70, in a buffered media, copper is released from ceruloplasmine complex and forms with the specific complexant 3-5 Di Br-PAESA a stable coloured complex.

The color intensity is proportional to the amount of cooper present in the sample.

REAGENTS

R 1 Buffer	Acetate. pH 4.7	$\geq$ 1 mol/L
R 2 Color	3,5-DiBr-PAESA	0.4 mmol/L
R 3 Reducing acid	Ascorbic acid (powder)	
COPPER CAL	Copper primary standard 100 µg/dL	

Optional (not included in the kit)

Contro-N	Ref.: 101-0252	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0083	20 x 5 mL	
Contro-P	Ref.: 101-0253	4 x 5 mL	Lyophilized human control serum
	Ref.: 101-0084	20 x 5 mL	

PRECAUTIONS

R2: Corrosive (C): R35: Causes severe burns.

CALIBRATION

The COPPER CAL value is verified using NIST (National Institute of Standards and Technology) traceable reference Standard.

PREPARATION AND STABILITY

- Working reagent (WR): Add (→) one dose (dispense using the enclosed spoon) of R 3 to one vial of R1. Cap and mix gently to dissolve contents. (WR) is stable after reconstitution 15 days at 2-8º C when stored tightly closed and contaminations prevented during their use. Do not use if appears turbid.
- R2: Ready to use. After opening, is stable 90 days 2-8º C, if contamination avoided and vial recapped immediately after use.
- COPPER CAL: Ready to use.

STORAGE AND STABILITY

All the components of the kit are stable until the expiration date on the label when stored tightly closed at 2-8º C, protected from light and contaminations prevented during their use.

Do not use reagents over the expiration date.

Signs of reagent deterioration:

- Presence of particles and turbidity.

ADDITIONAL EQUIPMENT

- Spectrophotometer or colorimeter measuring at 582 nm.
- Matched cuvettes 1.0 cm light path.
- General laboratory equipment[(Note 1)].

SAMPLES

- Serum or plasma[1]: Not hemolyzed. Use only heparin salts as anticoagulants. Stability: 24 hours at 2-8º C or 15 day at –20º C.

PROCEDURE

Notes: CHRONOLAB SYSTEMS has instruction sheets for several automatic analyzers. Instructions for many of them are available on request.

COPPER CAL: Proceed carefully with this product because due its nature it can get contamined easily.

It is recommended to use disposable material. If glassware is used the material should be scrupulously cleaned with hydrochloric acid 1 N and then thoroughly rinsed it with distilled water.

Use clean disposable pipette tips for its dispensation.

1. Allow reagents to reach working temperature before using. A proportional variation of the reaction volumes indicated does not change the result.
2. Assay conditions:
   Wavelength: . . . . . .582 nm (570 - 590)
   Cuvette: . . . . . 1 cm light path
   Temperature . . . . . 37º C
3. Adjust the instrument to zero with distilled water.
4. Pipette into a cuvette:

	Blank	Standard	Sample
WR (mL)	1.0	1.0	1.0
Distilled water	50	-	-
Standard[(Note 2)] (µL)	--	50	--
Sample (µL)	--	--	50

5. Mix and read the absorbance (A_1) of the sample against the Blank. Add:

	Blank	Standard	Sample
R2 (µL)	50	50	50

6. Mix and incubate for 4-5 min at 37º C.
7. Read the absorbance (A_2) of sample and standard against Blank. The colour is stable for at least 1 hour.

CALCULATIONS

$$\frac{(A_2 - A_1)\ \text{Sample} - (A_2 - A_1)\ \text{Blank}}{(A_2 - A_1)\ \text{Standard} - (A_2 - A_1)\ \text{Blank}} \times 100\ (\text{Standard conc.}) = \mu\text{g/dL copper in the sample}$$

Conversion factor: µg/dL x 0.1573 = µmol/L.

QUALITY CONTROL

Control sera are recommended to monitor the performance of assay procedures.

If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

REFERENCE VALUES[1]

Male	70 - 140 µg/dL	≅ 11.0 – 22.0	µmol/L
Female	80 - 155 µg/dL	≅ 12.6 – 24.4	µmol/L

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

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PERFORMANCE CHARACTERISTICS

Measuring range: From *detection limit* of 3 µg/dL to *linearity limit* of 500 µg/dL.

If the results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl (9 g/L) and multiply the result by 10.

Precision:

	Intra-assay (n=20)			Inter-assay (n=20)		
Mean (µg/dL)	71.8	120	170	72.6	121	170
SD	2.19	2.64	2.68	2.41	2.98	1.97
CV (%)	3.05	2.19	1.57	3.32	2.46	1.16

Accuracy: Results obtained using CHRONOLAB reagents (y) did not show systematic differences when compared with other commercial reagents (x).

The results obtained using 60 samples were the following:

Correlation coefficient (r): 0.96.

Regression equation: y= 0.9774x + 2.5776.

The results of the performance characteristics depend on the analyzer used.

INTERFERENCES

No interferences were observed to bilirubin up to 15 mg/dL, hemoglobin up to 0.5 g/dL and triglycerides up to 1000 mg/dL.

A list of drugs and other interfering substances with copper determination has been reported by Young et. al[3,4].

BIBLIOGRAPHY

1. Burtis A et al. Tietz Textbook of Clinical Chemistry, 3rd ed AACC 1999.
2. Kaplan A et al. Clin Chem The C.V. Mosby Co.1984.
3. Young DS. Effects of drugs on Clinical Lab. Tests, 4th ed AACC Press, 1995.
4. Young DS. Effects of disease on Clinical Lab. Tests, 4th ed AACC 2001.

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