Reactivos GPL

Barcelona, España

CE

- Cooper -

COPPER
Color 3,5-DiBr-PAESA

Store at: +2+8ºC.

Presentation:
Cod. SU045 CONT: *5 x 10 mL*

Procedure

Quantitative determination of copper.

Only for *in vitro use in clinical laboratory* (IVD)

TEST SUMMARY
Colorimetric test without deproteinization of the sample. End point increase. At pH 4.70, in a buffered media, Cooper 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 COMPOSITION

R.1 (Buffer)	Acetate pH 4.7	≥ 1 mol/L
R.2 (Color)	3,5-DiBr-PAESA Acetic acid Sodium hydroxide	0.4 mmol/L 1400 mmol/L 500 mmol/L
R.3 (Reducing agent)	Ascorbic acid (powder)	
COOPER CAL	Cooper aqueous primary standard	100 µg/dL

PRECAUTIONS
R1: H315-Causes skin irritation. H319-Causes serious eye irritation.
R2: H314-Causes severe skin burns and eye damage.
Follow the precautionary statements given in MSDS and label of the product.

CALIBRATION
The Cooper cal value is verified using NIST (National Institute of Standards and Technology) traceable reference standard.

REAGENT PREPARATION AND STABILITY
Working reagent (WR): Add (→) one small spoon contents of R3 to one vial of R1. Cap and mix gently to dissolve contents.
(WR) is stable after reconstitution 15 days at 2-8ºC 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 2-8ºC, if contamination avoided and vial recapped immediately after use.
Cooper Cal: Proceed carefully with this product because due its nature it can get contaminated easily.
Signs of reagent deterioration:
- Presence of particles and turbidity.

All the reagents of the kit are stable up to the end of the indicated month and year of expiry. Store tightly closed at 2-8º C. Do not use reagents over the expiration date.

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

MATERIAL REQUIRED BUT NOT PROVIDED
- Spectrophotometer or colorimeter measuring at 582 nm.
- Matched cuvettes 1.0 cm. light path.

General laboratory equipment *(note 1)*

TEST PROCEDURE
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 37ºC
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 37ºC.
7. Read the absorbance (A_2) of sample and standard against Blank. The colour is stable for at least 1 hour.

CALCULATIONS

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

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

QUALITY CONTROL
Control sera are recommended to monitor the performance of the procedure, Control H Normal Ref. QC003 and Control H Pathological Ref. QC004. If control values are found outside the defined range, check the instrument, reagents and calibrator for problems.

Serum controls are recommended for internal quality control. Each laboratory should establish its own Quality Control scheme and corrective actions.

REFERENCE VALUES

Male	80 - 155 µg/dL ≅ 12,6-24,4 µmol/L (Note 4)
Female	70 - 140 µg/dL ≅ 11,0-22,0 µmol/L (Note 4)

(These values are for orientation purpose).

It is suggested that each laboratory establish its own reference range.

CLINICAL SIGNIFICANCE
A variety of human Cooper deficiency conditions are recognized. Specific diseases associated with Cooper include head disease, bone and joint osteoarthritis and osteoporosis and Menkes' syndrome, Wilson's disease and others. Elevated levels of Cooper can also be toxic.
Clinical diagnosis should not be made on a single test result; it should integrate clinical and other laboratory data.

REAGENT PERFORMANCE
– *Measuring Range*:
From detection limit of 3 µg/dL. to linearity limit of 500 µg/dL., under the described assay conditions.
If results obtained were greater than linearity limit, dilute the sample 1/10 with NaCl 9 g/L. and multiply 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 GPL reagents did not show systematic differences when compared with other commercial reagents.
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.

INTERFERING SUBSTANCES
- 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 Cooper determination has been reported by Young et. al[3,4].

NOTES
1. It is recommended to use disposable material. If glassware is used the material should be soaking for 6 h in diluted HCl (20% v/v) and then thoroughly rinsed with distilled water and dried before use.
2. Calibration with the aqueous standard may cause a systematic error in automatic procedures. In these cases, it is recommended to use a serum Calibrator.
3. Use clean disposable pipette tips for its dispensation.

BIBLIOGRAPHY

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