COPPER

Quantitative colorimetric determination of copper in serum and plasma (Cupremia) with Di-Br-PAESA method

TEST SUMMARY

Copper (Cu) is an important component of many enzymes and is found mainly in plasma bound to ceruloplasmin.
Copper plays a fundamental role in iron metabolism, favoring intestinal absorption, mobilization and use by deposits.
Copper not absorbed and excess copper, derived from biliary excretion, is mainly eliminated by faeces.
The concentration of free copper in the blood (Cupremia) may register a reduction in correlation with a decrease of proteins in serum, then in the states of insufficient nutrition or malabsorption (celiac disease, sprue), loss of proteins in the stool or with the urine (nephrotic syndrome) and in Wilson's disease.
An increase in the Cupremia can occur in the case of pregnancy, in various acute or chronic infections, in liver diseases, after surgery, in myocardial infarction, in hyperthyroidism, Hodgkin's disease, in neoplasms and in numerous diseases of haematological interest.

PRINCIPLE OF TEST

the chromogen 3,5-Di-Br-PAESA react with cupric ions and forming a blue-violet compound, which intensity is proportional to the copper concentration in the sample.
The method does not require de-proteinization of the serum nor the blank sample.

SAMPLES

Serum or plasma unhemolyzed. Use heparin salt as anticoagulant.
Stability: 8 days at 2-8°C.

Strong lipemic serum can sometimes interfere with the analysis, is suggested to centrifuge or filtrate those samples with membrane 0.2 µm.

REAGENTS

Reagent A:	Acetate buffer 0.1 M pH 4.9; reducing agents and preservatives.
Reagent B:	3,5-Di-Br-PAESA.
Standard:	Ion copper 200 µg/dl; preservatives.

MATERIAL REQUIRED BUT NOT SUPPLIED

Normal laboratory equipment. Spectrophotometer UV/VIS with thermostatation. Automatic Micropipette. Cuvette in optical glass or monouse in optical polystyrene. Distilled water.

PRECAUTIONS

Reagent may contain not reactive and conservative components. It is opportune to avoid contacts with the skin and do not swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

REAGENTS PREPARATION

Prepare the Work Reagent mixing in equal quantity the Reagent A with Reagent B.
Reagents are stored at 2-8°C and are stable until expiration date on label.
Work Reagent is stable 20 days at room temperature.

PROCEDURE SERUM/PLASMA

Kind of analysis:	End point
Reading time:	10 minutes
Colour stability:	30 minutes
Wavelength:	580 nm (570-590)
Temperature:	20-25°C
Lightpath	1 cm
Zero:	Blank Reagent

REAGENTS	BLANK	STANDARD	SAMPLE
Work Reagent	1 ml	1 ml	1 ml
Distilled water	66 µl	--	--
Standard	--	66 µl	--
Sample	--	--	66 µl

Mix and wait for 10 minutes then read the absorbances against the blank at 580 nm.
The colour is stable for 30 minutes.

CALCULATION

SERUM/PLASMA

$$\text{Copper µg/dl} = \frac{\text{A (sample)}}{\text{A (standard)}} \times 200$$

$$\text{Copper µmol/l} = \frac{\text{A (sample)}}{\text{A (standard)}} \times 31.47$$

EXPECTED VALUES

SERUM/PLASMA

Men	80 - 140 µg/dl	(12.59 – 22.03 µmol/l)
Women	80 - 155 µg/dl	(12.59 – 24.39 µmol/l)
New-borns	12 - 67 µg/dl	(1.89 – 10.54 µmol/l)
Children up to 10 years	30 - 150 µg/dl	(4.72 – 23.60 µmol/l)

Every laboratory should establish own reference intervals in accordance with own population.

NOTES

- Is possible to make the reading even at 600 nm. In that case the reading gave absorbance's values that are about 30% lower than the ones obtained in the declared reading range.
- If the results are incompatible with clinical presentation, they have to be evaluated within a total clinical study.
- Only for IVD use.

CALIBRATION/QUALITY CONTROL

It is suggested to perform an internal quality control using control serum with known copper values.

TEST PERFORMANCE

Precision

Intra-assay (n = 25)	Media (µg/dl)	SD (µg/dl)	CV%
Sample 1	121.88	1.301	1.07
Sample 2	247.64	1.186	0.48

Inter-assay (n = 25)	Media (µg/dl)	SD (µg/dl)	CV%
Sample 1	124.96	1.485	1.19
Sample 2	248.08	2.308	0.93

Methods comparison
A comparison with a commercial available product gave the following results in a comparison on 31 samples of serum:

Copper Acid LTA = x
Copper competitor = y
n = 31

y = 0,98353x + 2,80806 r = 0,99741

Sensitivity/limit of detection
The method is able to discriminate until 8 µg/dl.

Linearity
The method is linear up to 500 µg/dl.

WASTE DISPOSAL

Product is intended for professional laboratories. Waste products must be handled as per relevant security cards and local regulations.

PACKAGING

CODE CC02150	(120 TESTS)	
Reagent A	3 x 20 ml	(liquid)
Reagent B	3 x 20 ml	(liquid)
Standard	1 x 3 ml	(liquid)

REFERENCES

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3. Ciuti R., Galli A., Giorn. It. Chim. Clin. 12 (2): 91-100 (1987).
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MANUFACTURER

LTA s.r.l.
Via Milano 15/F
20060 Bussero (Milan) ITALY
Tel: +39 02 95409034
Fax: +39 02 95334185
e-mail: info@LTAonline.it
Website: http://www.LTAonline.it

SYMBOLS

IVD	Only for IVD use
LOT	Lot of manufacturing
REF	Code number
	Storage temperature interval
	Expiration date
	Warning, read enclosed documents
	Read the directions
	Biological risk

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