Mascia Brunelli S.p.a. Instructions for use

COPPER

For in *Vitro* diagnostics use

Colorimetric determination of Copper in serum and plasma

INTENDED USE

Copper is a co-factor of different enzymes such as cytochrome oxidase, tyrosinase, uricase. It is involved in iron metabolism, promoting its intestinal uptake, and its mobility from storage tissue.
A decrease level of copper is correlated to a decreased level of proteins in the serum, therefore in case of inadequate nutrition or not correct uptake (celiac disease, sprue), loss of protein through faeces or urine (nephritic syndrome), or in Wilson disease. An increase of copper level occurs in pregnancy, acute or chronic infections, surgery, myocardium infarction, iperthyroidism, and haematological diseases.

PRINCIPLE

Copper (Cu++) reacts with the chromogen Di-Br-PAESA at room temperature yielding a coloured complex which intensity is proportional to the Copper concentration present in the sample.
The method does not require serum deproteinisation either sample blank.

COMPOSITION

REATTIVO A:
Acetate buffer pH 4.9 0.100 mmol/l
Reducing agents and preservatives

REATTIVO B:
3,5 Di-Br-PAESA 0.02 g/l
Preservatives

STANDARD: 1x5 ml
Sulphate Copper 200 µg/dl as Cu++ ion

PREPARATION OF REAGENTS

Bireagent procedure:
The reagents are liquids ready to use.
Monoreagent procedure:
Mix 1 part of Reagent A with 1 part of Reagent B to obtain the working reagent (ex. 10 ml of RA + 10 ml of RB).

Storage and stability
Store at 2-8 °C. Do not freeze the reagents! The reagents are stable up to the expiry date stated on the label if contamination and evaporation are avoided, protected from light. The above conditions are valid if the vials are opened just only for the time to take the reagent, closed immediately with their cap and stored at the indicated conservation temperature.

Working reagent is stable for 15 days at 2-8°C.

ANCILLARY EQUIPMENT

- Automatic pipettes
- Photometer
- Analysis cuvettes (optical path = 1 cm)
- NaCl solution (9 g/l)

SAMPLES

Serum, plasma with heparin, do not use chelating agents as anticoagulant or haemolysed samples.
Stable 8 days at 2-8 °C.

Specimen collection/Preanalytical factors
It is recommended that specimen collection should be carried out in accordance with NCCLS Document H11-A3.

INTERNAL QUALITY CONTROL

It is recommended to use commercial Quality Control sera with known Copper concentration. Check that the values obtained are within the reference range provided.

ANALYTICAL PROCEDURE

Allow the reagents to reach working temperature before using.

Bireagent procedure:
Pipette into disposable or well clean cuvettes:

	Blank	Standard	Sample
Sample	-	-	100 µl
Distilled H_2O	100 µl	-	-
Standard	-	100 µl	-
Reagent A	750 µl	750 µl	750 µl
Mix and incubate **5 minutes** at **room temperature** (20-25 °C). Than add:			
Reagent B	750 µl	750 µl	750 µl
Mix and incubate 10 **minutes** at **room temperature** (20-25 °C). Read the absorbance A for all cuvettes at **580 (570-590) nm** against blank. Colour is stable for 30 minutes.			

Monoreagent procedure:
Pipettare in cuvette a perdere o ben pulite:

	Blank	Standard	Sample
Sample	-	-	100 µl
Distilled H_2O	100 µl	-	-
Standard	-	100 µl	-
Working reagent	1500 µl	1500 µl	1500 µl
Mix and incubate 10 **minutes** at **room temperature** (20-25 °C). Read the absorbance A for all cuvettes at **580 (570-590) nm** against blank. Colour is stable for 30 minutes.			

Note: reaction volumes may be proportionally changed.

It is possible to read the absorbance at 600 nm. In this case the values will be 30% lower than the ones obtained at the 570-590 nm range.

CALCULATION OF RESULTS

Utilize the following formula:

$$\text{Copper } \mu g/dl = \frac{\text{A sample}}{\text{A standard}} \times 200$$

REFERENCE VALUES

Men:	80 ÷ 140 µg/dl
Women:	80 ÷ 155 µg/dl
Newborn:	12 ÷ 67 µg/dl
Children up to 10 years:	30 ÷ 150 µg/dl

Each laboratory should establish reference ranges for its own patients population.

ANALYTICAL PERFORMANCES

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 M.B. = x
Copper competitor = y
n = 31

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

Sensivity/limit of detection

The method is able to discriminate until 8 µg/dl.

Linearity

The method is linear up to 500 µg/dl.

Interferences

Highly lipemic sera may interfere in the assay; it is recommended to centrifuge or filter (with membranes of 0.2 µm) the sample.
Do not use haemolysed serum since haemoglobin interferes.
Bilirubin does not interfere up to 20 mg/dl.

PRECAUTIONS IN USE

The reagents contain inactive components such as preservatives (Sodium azide or others), surfactants etc. The total concentration of these components is lower than the limits reported by 67/548/ECC and 88/379/EEC directives about classification, packaging and labelling of dangerous substances. However, the reagents should be handled with caution, avoiding swallowing and contact with skin, eyes and mucous membranes.
The use of the laboratory reagents according to good laboratory practice is recommended.

WASTE MANAGEMENT

Please refer to local legal requirements.

BIBLIOGRAPHY

PASQUINELLI F., Diagnostica e Tecniche di Laboratorio, (pag.:1099-1102) Rossini Ed. (1984)
2. AKITA ABE, SUMICO YIAMASHITA, Clin. Chem. 35(4): 197, 552-554 (1989)
3. CIUTI R., GALLI A., Giorn. It. Chim. Clin. 12 (2): 91-100 (1987)
4. CIUTI R., GALLI A., Giorn. It. Chim. Clin. 12 (2): 101-111 (1987)
5. NCCLS Document, "Procedures for the collection of arterial blood specimens", Appr. Std., 3rd Ed. (1999).
6. EU-Dir 1999/11 Commission Directive of 8 March 1999 adapting to technical progress the principles of good laboratory practice as specified in Council Directive 87/18/EEC

***CONTENTS* (66 tests)**	**REF. NB12000**
Reagent A	2 x 25 ml
Reagent B	2 x 25 ml
Standard	1 x 5 ml
Instruction for use	1 item

SYMBOLS

F	Only for IVD use
C	Lot of manufacturing
B	Code number
I	Storage temperature interval
K	Expiration date
J	Warning, read enclosed documents
L	Read the directions
A	Biological risk

CE IVD

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