COPPER – Liquid

Colorimetric method with 3,5-Di-Br-PAESA

4 x 25 ml + 2 x 10 ml CL52-125S

INTENDED USE

Kit for quantitative determination of copper in serum.

CLINICAL MEANING

Traces of copper can be found in serum, mainly linked to its vector protein (ceruloplasmin). Plasmatic copper and ceruloplasmin diminish with Wilson's disease (hepatolenticular degeneration) because of a slow hepatic synthesis of ceruloplasmin. This causes an increase of urinary excretion and of copper deposits in liver.
During estrogen therapy (and pregnancy), usage of contraceptives or anti-epileptics ceruloplasmin synthesis and plasmatic copper increase. An increase may also be noticed in different inflammatory states and in Hodgking's disease.

PRINCIPLE

Copper, liberated at pH 4.9 by the vector protein ceruloplasmin, forms a colored compound with 3,5-Di Br- PAESA, whose intensity is proportional to the copper concentration in the sample.

SAMPLE

Fresh, non hemolyzed serum. Do not use hemolyzed samples.
STABILITY: 7 days at 2-8°C, one month at -20°C. Separate serum from the clot as soon as possible. Shake and bring the samples to room temperature before use.

REAGENTS

Only for in Vitro diagnostics.
Liquid reagents ready to use.

Package contents:	**CL52-120S**
REAGENT 1 (Liquid) Acetic buffer pH 4.9 100 mmol/L, preservatives, surfactants.	4 x 25 ml
REAGENT 2 (Liquid) 3.5-DiBr-PAESA 0.1 mmol/L, Preservatives.	2 x 10 ml
STANDARD (Liquid) Copper 100 µg/dl.	1 x 4 ml

STABILITY: stored at 2-8°C in a closed bottle, all kit components are stable up to the expiration date on the label. The reagent's stability after opening is 60 days (stored at 2-8°C).

***WARNING**: Reagent 1 may precipitate during conservation in the fridge. It's advisable to dissolve at >25°C before use. Stir carefully after dissolution.*

NECESSARY ITEMS – NOT PROVIDED

Usual laboratory equipment: UV/VIS Spectrophotometer with temperature control; automatic micropipettes; Optical glass cuvettes or, alternatively, disposable ones in optical polystyrene; Saline solution.

PREPARATION OF THE REAGENTS

(for mono-reagent procedure only)
Mix 5 volumes of Reagent 1 with 1 volume of Reagent 2.
STABILITY: 7 days at room temperature, 30 days at 2-8°C if stored in a closed bottle away from light.

MANUAL ASSAY PROCEDURE

Wavelength: 580 nm (578-600)
Optical path: 1 cm
Temperature: 37°C
Reaction: Increasing endpoint
Sample/Reagent Ratio (mono): 1/20

Monoreagent procedure

Pipette	Blank	Sample	Standard
Working reagent	1000 µL	1000 µL	1000 µL
Distilled water	50 µL		
Sample		50µL	
Standard			50 µL

Mix accurately and incubate at 37°C for 5 minutes. Read the sample (As) and the standard (Astd) absorbencies against blank reagent.

Bireagent Procedure

Pipette	Blank	Sample	Standard
Reagent 1	1000 µL	1000 µL	1000 µL
Distilled water	60 µL		
Sample		60µL	
Standard			60 µL

Mix accurately and incubate for 5 minutes. Read the blank sample (Abc) and the blank standard (Abs) absorbencies against blank reagent.

Reagent 2	200 µL	200 µL	200 µL

Mix and incubate at 37°C for 5 minutes.
Read the sample (As) and the standard (Astd) absorbencies against blank reagent.
The produced color is stable for at least 15 minutes at room temperature. Reaction volumes can be varied proportionally. This sheet describes the utilization of the kit in manual. The calibration with aqueous standard can cause a systematic error in the usage with automatic analyzers. It's recommended to use a proteic human calibrator.

CALCULATION

Monoregent procedure
Copper µg/dL = (Ac/As) x 100 (Standard Value)
Biregent procedure

$$\text{Copper µg/dL} = \frac{(Ac - Abc)}{As - Abs)} \times 100 \text{ (Standard Value)}$$

Conversion factor: [µg/dL] x 0.1573 = Cu [µmol/L]

REFERENCE VALUES

Male: 70-140 µg /dl
Female: 80-155 µg/dl
These values are only for reference. Each laboratory should define their own values.

QUALITY CONTROL – CALIBRATION

All Clinical Chemistry laboratories should implement a quality control program. Control serums of human origin are available for this purpose on request:
PRE-NORM serums with normal values
PRE-PATH serums with pathological values
If the method requires it, a multiparameter calibrator of human origin is available.

PERFORMANCE CHARACTERISTICS

Sensitivity: 1 µg/dL a 580 nm
Measuring range/Linearity: 3 – 500 µg/dL
Measurable limit: 3 µg/dL

Precision:

	Within run (n=30)			**Between run (n=30)**		
Average (µg/dL)	55.5	121.3	180.3	55.7	122.4	179.3
CV (%)	2.1	1.4	1.1	1.5	1.8	1.8

Correlation: r = 0.9375
Linear regression: y = 1.03 x + 9.02
Interference
Bilirubin (40 mg/dl) does not interfere.
Triglycerides (1500 mg/dl) do not interfere.
Hemoglobin (300 mg/dl) does not interfere
Method limits
5 µg/dl, repeat the analysis on a diluted sample (1:2) and multiply the result by two.

DISPOSAL

The product must be used for professional analysis only. The product must be disposed of according to national/international laws.

WARNINGS AND PRECAUTIONS

The compound is not classified as dangerous according to current laws. Total concentration of non active components (preservatives, detergents, stabilizers) is inferior to required limits. Nevertheless, handle the product with care, using the normal precautions expected with correct behavior in laboratory. Avoid ingestion, contact with skin, eyes and mucous membranes. Samples should be handled as potentially infected by HIV or hepatitis.

REFERENCE

1. Hinggins, T., et al. Clin. Chem., 27, 1619, (1981)
2. Vassault, A.et al. Ann. Biol. Clin., 44,686, (1986)
3. Young D.S., et. al., Clin. Chem. 21 :1D (1975).

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KEY SYMBOLS

IVD	In Vitro diagnostic medical device
LOT	batch number
REF	catalog number
	temperature limits
	use by
	caution
	consult accompanying documents

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