COPPER

CU 0100 CH 4 x 25 ml

INTENDED USE

Reagent for quantitative in vitro determination of copper in biological fluids.

SUMMARY OF TEST

The major functions of copper metalloproteins involve oxidation-reduction reactions; most known copper-containing enzymes bind and react directly with molecular oxygen. Copper is an integral component of many metalloenzymes, including ceruloplasmin, cytochrome c oxidase, superoxide dismutase, dopamine-β-hydroxylase, ascorbate oxidase, lysyl oxidase, and tyrosinase.

PRINCIPLE OF THE METHOD

3,5-Di-Br-PAESA combines with Cu(II) to form a blue-violet complex, the absorbance of which is measured at 580 nm. The reaction has high specificity and interference from other cations is avoided, due to specific pH and environment.

KIT COMPONENTS

For in vitro diagnostic use only.
The components of the kit are stable until expiration date on the label at 2-8°C.
Keep away from direct light sources.

CU R1 2 x 25 ml (liquid) blue cap
Composition: acetate buffer 100 mM pH 4.90, surfactants and preservatives.

CU R2 2 x 25 ml (liquid) red cap
Composition: 3,5 Di-Br-PAESA 10 mM.

Standard: copper solution 200 μg/dl - 5 ml

Store all components at 2-8°C.

MATERIALS REQUIRED BUT NOT SUPPLIED

Current laboratory instrumentation. Spectrophotometer UV/VIS with thermostatic cuvette holder. Automatic micropipettes. Glass or high quality polystyrene cuvettes. Saline solution.

REAGENT PREPARATION

Mix reagents R1 and R2 in equal parts.
Caution! Reagent R1 may precipitate during refrigerated storage. It is recommended to let it redissolve at room temperature before use. Mix thoroughly after complete dissolution.
Reagent R1 may also precipitate after mixing with R2 if the mixture is stored refrigerated. If precipitation occurs, allow the reaction solution (R1 + R2) to reach room temperature and mix thoroughly until the precipitate is completely dissolved.

Stability of working reagent: 30 days at 2-8°C and 7 days at room temperature, well closed.
Stability of unopened vials: up to expiration date on labels at 2-8°C.
Stability since first opening of vials: preferably within 60 days at 2-8°C.

PRECAUTIONS

Reagent may contain some non-reactive and preservative components. It is suggested to handle carefully it, avoiding contact with skin and swallow.
Perform the test according to the general "Good Laboratory Practice" (GLP) guidelines.

SPECIMEN

Serum (preferred), plasma heparinate.
Copper is stable 7 days at 2-8°C and 1 month at -20°C.

TEST PROCEDURE

Wavelenght: 580 nm (allowed 570 ÷ 600 nm)
Lightpath: 1 cm
Temperature: 25, 30 or 37°C

dispense:	blank	standard	sample
reagent	1.5 ml	1.5 ml	1.5 ml
water	100 μl	-	-
standard	-	100 μl	-
sample	-	-	100 μl

Mix, incubate at 25, 30 or 37°C for 5 minutes.
Read absorbances of standard (As) and samples (Ax) against reagent blank.

RESULTS CALCULATION

serum/plasma sample:
copper μg/dl = Ax/As x 200 (standard value)

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EXPECTED VALUES

men:	70 - 140 μg/dl	(11.0 - 22.0 μmol/l)
women:	80 - 155 μg/dl	(12.6 - 24.4 μmol/l)
pregnant women:	118 - 302 μg/dl	(18.5 - 47.4 μmol/l)
children 6-12 y:	80 - 190 μg/dl	(12.6 - 29.9 μmol/l)
infants:	20 - 70 μg/dl	(3.1 - 11.0 μmol/l)

Each laboratory should establish appropriate reference intervals related to its population.

QUALITY CONTROL AND CALIBRATION

It is suggested to perform an internal quality control. For this purpose the following human based control sera are available:
QUANTINORM CHEMA
with normal or close to normal control values
QUANTIPATH CHEMA
with pathological control values.
Please contact Customer Care for further information.

TEST PERFORMANCE

Linearity
the method is linear up to 500 μg/dl.
If the limit value is exceeded, it is suggested to dilute sample 1+9 with distilled water and to repeat the test, multiplying the result by 10.

Sensitivity/limit of detection (LOD)
the limit of detection is 4 μg/dl.

Interferences
no interference was observed by the presence of:

hemoglobin	≤ 120 mg/dl
bilirubin	≤ 30 mg/dl

Lipids interfere.

Precision

intra-assay (n=10)	mean (μg/dl)	SD (μg/dl)	CV%
sample 1	120.00	3.06	2.50
sample 2	268.50	3.14	1.20

inter-assay (n=20)	mean (μg/dl)	SD (μg/dl)	CV%
sample 1	120.99	3.36	2.80
sample 2	265.19	5.73	2.22

Methods comparison
a comparison between Chema and a commercially available product gave the following results:

Copper Chema = x
Copper competitor = y
n = 82

$y = 1.046x - 6.67$ μg/dl $r^2 = 0.984$

WASTE DISPOSAL

This product is made to be used in professional laboratories.
P501: Dispose of contents according to national/international regulations.

REFERENCES

K.Ueno, T.Imamura, K.L.Cheng - Handbook of organic analytical reagents - CRC Press (1992).
Clin.Chem. 35/4, 552-554 (1989)
Tietz Textbook of Clinical Chemistry, Second Edition, Burtis-Ashwood (1994).

MANUFACTURER

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SYMBOLS

IVD	*in vitro* diagnostic medical device
LOT	batch code
REF	catalogue number
	temperature limit
	use-by date
	caution
	consult instructions for use

CE