

COPPER

CU 0100 CH

4 x 25 ml

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. A number of pathological conditions have been attributed to the loss of cuproenzyme activity. Failure of pigmentation has been attributed to the depressed tyrosinase activity required in the first step in the biosynthesis of melanin. A variety of connective tissue cross-linking defects are believed to be caused by a loss of amine oxidase activity, particularly that of lysyl oxidase. Ataxia may result from depressed cytochrome *c* oxidase activity in motor neurons. Depressed dopamine- β -hydroxylase activity can result in abnormal catecholamine conversions.

Copper plays an important role in iron metabolism. Copper deficiency impairs iron absorption, and anemia accompanies severe copper deficiency. Ceruloplasmin, the major copper-containing protein in plasma, has a ferroxidase activity that oxidizes ferrous iron to the ferric state prior to its binding by plasma transferrin. A variety of human copper deficiency conditions are recognized. Copper deficiency in infants has been observed in prematurity, malnutrition, malabsorption, chronic diarrhea, hyperalimentation, and prolonged feeding with low-copper, total-milk diets. Signs of copper deficiency include neutropenia and hypochromic anemia in the early stages; osteoporosis and various bone and joint abnormalities; decreased pigmentation of the skin and general pallor; and in the later stages, possible neurological abnormalities (hypotonia, apnea, psychomotor retardation), probably caused by a cytochrome *c* oxidase deficit. The concentrations of plasma copper and ceruloplasmin reflect the degree of copper deficiency and replete states.

An extreme form of copper deficiency is seen in Menkes' steely hair syndrome, an X-linked genetic defect in copper transport and storage. Clinical manifestations occur early in life and include kinky or twisted, brittle hair (steely), depigmentation of the skin and hair, hypothermia, seizures, cerebral degeneration, and vascular defects.

Wilson's disease (hepatolenticular degeneration) is a genetically determined copper accumulation disease that usually presents between 6 and 40 years of age. Disease symptoms include neurological disorders, cirrhosis of the liver, and Kayser-fleischer rings (green-brown discoloration) in the cornea caused by copper deposits. Copper accumulates in the liver, brain, kidney, and cornea.

Copper toxicity is characterized by nausea, vomiting, epigastric burning, and diarrhea. It can occur subsequent to ingestion of copper-contaminated solutions, the use of copper-containing intrauterine devices, the use of copper salts in animal feeds, and exposure to copper-containing fungicides. Systemic toxic effects include hemolysis, hepatic necrosis, gastrointestinal bleeding, oliguria, azotemia, hemoglobinuria, hematuria, proteinuria, hypotension, tachycardia, convulsions, coma, and death. Estrogens increase serum copper concentrations, probably by increasing hepatic ceruloplasmin synthesis. Serum copper is normally higher in women than in men. This difference increases further in women taking estrogenic oral contraceptives. Serum copper during pregnancy is increased two- to three-fold over normal during the last trimester. Testosterone and progesterone administration has also been reported to increase plasma copper. Because the liver is an important organ for copper storage and homeostatic regulation, abnormal copper metabolism is seen in liver disease. Elevated serum copper concentrations are seen in portal cirrhosis, biliary tract disease, and hepatitis, probably because excess copper that would normally be excreted in the bile is retained in the circulation. Hypocupremia has been observed in hemolytic jaundice, hemochromatosis, and some types of hepatic cirrhosis because of the inability of the damaged liver to synthesize ceruloplasmin.

AAS is the method of choice for copper determination, but laboratories without AAS instruments have used for long time a batocuproine method. However, this method is affected by a deproteinizing step. It is now available a fast, spectrophotometric method in which the deproteinization step is no more necessary.

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.

Reagent A: 2 x 25 ml (liquid) blue cap

Composition: acetate buffer 100 mM pH 4.90, surfactants and preservatives.

Reagent B: 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 equal quantities of both reagents A and B.

Warning! The reagent A could precipitate during refrigerate storage. It is suggested to let it redissolve at room temperature before use. Mix well after redissolving.

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: \geq 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

Wavelength:	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 = $A_x/A_s \times 200$ (standard value)

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:

QN 0050 CH QUANTINORM CHEMA 10 x 5 ml
with normal or close to normal control values

QP 0050 CH QUANTIPATH CHEMA 10 x 5 ml
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 5 µg/dl.

Interferences

no interference was observed by the presence of:

hemoglobin	\leq 120 mg/dl
bilirubin	\leq 50 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:

$$\begin{aligned} \text{Copper Chema} &= x \\ \text{Copper competitor} &= y \\ n &= 82 \end{aligned}$$

$$y = 1.046x - 6.67 \mu\text{g/dl} \quad r = 0.984$$

WASTE DISPOSAL

This product is made to be used in professional laboratories. Please consult local regulations for a correct waste disposal.

S56: dispose of this material and its container at hazardous or special waste collection point.

S57: use appropriate container to avoid environmental contamination.

S61: avoid release in environment. Refer to special instructions/safety data sheets.

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, Bur-tis-Ashwood (1994).

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SYMBOLS

	for in vitro diagnostic use only
	lot of manufacturing
	code number
	storage at temperature interval
	expiration date (year/month)
	warning, read enclosed documents
	read the directions