Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12301	R1	1 x		20	ml
For 20 tests	R2	4 x	Powder for	5	ml

Copper in Serum

Without deproteinization

3,5-diBr.-PAESA Colorimetric method

Powder Reagents

Test principle

At pH 4.70, copper is released from the ceruloplasmin complex. Ascorbic acid acts to reduce the cupric copper to cuprous state which reacts with 3,5-diBr.-PAESA to produce a colored complex.

Concentrations in the test

Reagent R1		
Acetate buffer pH = 5.7	60.0	mmol/L
4-(3,5-diBrom-2-pyridyl)-N-sulfopropylanilin (PAESA)	18	µmol/L
Detergent		
Reagent R2		
Ascorbic acid	50	mmol/L
Standard : The concentration as indicated on vial.		

Stability and preparation of working reagent

Reagent R1: *liquid.*
Reagent R2: *powder*.
All reagents are stable up to expiry date given on label when stored at + 20 ➔ + 25 °C.
Working Reagent:
Add the required volume of bottle R1 liquid reagent into bottle R2 (powder) and mix.
Stability: 3 days at 20 - 25 °C.

Specimen collection and handling

Serum, heparinized plasma, free from hemolysis.
Stability: 7 days at 2 – 8 °C.

Standard

Copper STD. Cat. No. 16081

Quality control

Meditrol N Cat. No. 15171
Meditrol P Cat. No. 15181

Procedure

Wavelength	Hg 578 nm (570 - 590 nm)
Spectrophotometer	582 nm
Cuvette	1 cm light path.
Temperature	37°C / 20 - 25 °C
Measurement	against reagent blank
reaction	end point

Assay

	Blank	Standard	Sample
Double dist. water	50 µl	--	--
Standard	--	50 µl	--
Sample	--	--	50 µl
Working Reagent	1000 µl	1000 µl	1000 µl
Mix, incubate 15 min. at 37°C, or 25 min. at 20 - 25 °C. Read the absorbance (A). The final color is stable for 1 hour.			

Procedure notes:

1. For icteric or lipemic samples prepare sample blank as follow :
   - Add 50 µl sample to 1 ml dist. water and read absorbance against dist. water at 582 nm.
   - Subtract the serum blank absorbance from the test absorbance and use the corrected absorbance in the calculation.

Calculation

Serum:

$$\text{Conc.}_{\text{Copper}}\ (\mu g/dl) = \frac{A_{\text{Sample}}}{A_{\text{STD.}}} \times \text{Conc.}_{\text{STD.}}\ (\mu g/dl)$$

Linearity

Up to 500 µg/dl.
If the result exceeds 500 µg/dl, repeat the test using diluted sample (1+1) with sodium chloride solution (0.9 %) and multiply the result by 2.

Precautions

Please use copper free reaction tubes and cuvettes.

Interference

1. Bilirubin up to 5 mg/dl has no effect on this procedure. Use sample blank for higher bilirubin concentration.
2. Lipemic serum should have a sample blank for each test.

Reference range

< 4	mth.		8.9 – 46	µg/dl
4 – 6	mth.		25 – 108	µg/dl
7 – 12	mth.		51 – 133	µg/dl
1 – 5	yr.		83 – 152	µg/dl
6 – 9	yr.		83 – 133	µg/dl
10 – 13	yr.		83 – 121	µg/dl
14 – 19	yr.	women	70 – 159	µg/dl
		men	64 – 114	µg/dl
Adults		women	76 – 152	µg/dl
		men	70 - 140	µg/dl

References

1. Abe, A., Yamashita S., and al., Sensitive, Direct Colorimetric Assay for Copper in Serum, Clin. Chem., 35, (1989). 552.
2. Landers JW, Zak B. Determination of serum copper and iron in a single small sample. Amer J Clin Path 1958, 29: 590 - 2.
3. Houwen RHJ, Hattun van I, Hoogenraad TU. Wilson disease. Netherlands J. Med 1993, 43: 26 - 37.
4. Tanzi RE, et al. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. Nature Genetics 1993, 5: 344 - 50.
5. Aggett PT. Aspects of neonatal metabolism of trace elements. Acta Pediatr.