Manual Procedure

MEDICHEM MIDDLE EAST
Clinical Chemistry Reagents
Liquid Stable Reagents

Cat. No. 12741	R1	1 x		10	ml
For 90 tests	R2	6 x	Powder for	1.5	ml
	R3	1 x		7	ml

Copper in Urine

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	mmol/L
Detergent		
Reagent R2		
Ascorbic acid	50	mmol/L
Reagent R3		
4-(3,5-diBrom-2-pyridyl)-N-sulfopropylanilin (PAESA)	0.18	mmol/L
Standard: Concentration: As indicated on the bottle (5 µg/dl)		

Stability and preparation of working reagent

Reagent R1: *liquid.*
Reagent R2: *powder.*
Reagent R3: *liquid ready to use.*
All reagents are stable up to expiry date given on label when stored at + 20 ➔ + 25 °C.

Working Reagent:
Add 1.5 ml from bottle R1(Buffer) into one vial R2 (Powder). and mix.
Stability: 7 days at 20 - 25 °C.

Specimen collection and handling

Urine: 24 hours urine collected in plastic container, preferably polyethylene.
Centrifuge and use the supernatant for the test.
Stability: 24 hour at 2 – 8 °C. 15 days at -20°C.

Standard

Copper STD. Cat. No. 16081

Procedure

Wavelength	Hg 578 (570 - 590 nm)
Spectrophotometer	582nm
Cuvette	1 cm light path
Temperature	20 – 25 °C
Measurement	against reagent blank
Reaction	End point

Assay

	Blank	Standard	Sample Blank	Sample
Distilled water	1000 µl	--	100 µl	--
Standard	--	1000 µl	--	--
Sample	--	--	1000 µl	1000 µl
Reagent R3	100 µl	100 µl	--	100 µl
Working Reagent	100 µl	100 µl	100 µl	100 µl

Mix, incubate for 5 min. at 20 – 25 °C. Read the absorbance of sample blank (A_{SB}) against distilled water, and the absorbance of sample (A_S) and standard (A_{ST}) against reagent Blank. The final colour is stable for at lest 30 min.

Calculation

$$\text{Conc.}_{\text{Copper, Urine}}\ (\mu g/dl) = \frac{A_S - A_{SB}}{A_{ST}} \times \text{Conc.}_{\text{STD. /Urine}}\ (\mu g/dl)$$

$$\text{Conc.}_{\text{Copper, Urine/ 24 hr.}} = \frac{\text{Copper } \mu g/dl\ (\text{Urine}) \times (\text{vol./ ml})_{\text{Urine/ 24 hr.}}}{100}\ \mu g/24\ \text{hr.}$$

Linearity

Up to 20 µg/dl.
If the result exceeds 20 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

Urine/ 24 hr.	10 – 60	µg/24 hr.

References

1. Abe, A., Yamashita S., and al., Sensitive, Direct Colorimetric Assay for Copper in Serum, Clin. Chem., 35, (1989). 552.
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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.

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