COPPER KIT

(Colorimetric method)

For the determination of Copper in serum.
(Laboratory Reagent for Professional Use Only)

Summary

Copper is widely distributed in the various organs of the body. The highest concentration is found in the liver followed by the brain and kidneys. It plays an important part in the iron metabolism by converting the ferrous ions to a ferric state. Over 90% of the copper in plasma is bound to the protein ceruloplasmin. Increased levels are found in chronic/malignant diseases e.g. leukaemia, cirrhosis, various infections and in patients on oral contraceptives and oestrogens. Decreased levels are found in Wilson's disease, decreased synthesis of ceruloplasmin, malabsorption, malnutrition, and nephrotic syndrome.

Principle

Copper, released from ceruloplasmin, in an acidic medium, reacts with Di-Br-PAESA to form a coloured complex. Intensity of the complex formed is directly proportional to the amount of Copper present in the sample.

$$\text{Copper} + \text{Di-Br-PAESA} \xrightarrow[\text{Medium}]{\text{Acidic}} \text{Coloured complex}$$

Normal reference values

Serum	(Males)	:	80 - 140 µg/dl
	(Females)	:	80 - 155 µg/dl
	(Newborns)	:	12 - 67 µg/dl
	(Children upto 10 yrs.)	:	30 - 150 µg/dl

It is recommended that each laboratory establish its own normal range representing its patient population.

Contents	25 ml	75 ml
L1 : Buffer Reagent	12.5 ml	37.5 ml
L2 : Colour Reagent	12.5 ml	37.5 ml
S : Copper Standard (200 µg/dl)	2 ml	2 ml

Storage / stability

Contents are stable at 2-8°C till the expiry mentioned on the labels.

Reagent Preparation

Reagents are ready to use. Protect from bright light.
The cold Buffer (L1) when retrieved from 2-8°C may have a particulate suspension. The suspension clears up once the Buffer attains a temperature over 25°C.
Working reagent : For larger assay series a working reagent may be prepared by mixing equal volumes of L1 (Buffer Reagent) and L2 (Colour Reagent). The Working reagent is stable at 2 - 8 °C for at least 3 weeks. Keep tightly closed.

Sample material

Serum, free from hemolysis. Copper is reported to be stable in the sample for 6 days when stored at 2-8°C.

Procedure

Wavelength / filter	:	580 nm (Hg 578 nm) / Yellow
Temperature	:	R.T.
Light path	:	1 cm

Pipette into clean dry test tubes labelled as Blank (B), Standard (S), and Test (T) :

Addition Sequence	B (ml)	S (ml)	T (ml)
Buffer Reagent (L1)	0.5	0.5	0.5
Colour Reagent (L2)	0.5	0.5	0.5
Distilled water	0.05	-	-
Copper Standard(S)	-	0.05	-
Sample	-	-	0.05

Mix well and incubate at R.T. (25°C) for 10 min. Measure the absorbance of the Standard (Abs.S), and Test Sample (Abs.T) against the Blank, within 30 Min.

Calculations

$$\text{Copper in } \mu g/dl = \frac{\text{Abs.T}}{\text{Abs.S}} \times 200$$

Linearity

This procedure is linear upto 500 µg/dl. If the value exceeds this limit, dilute the serum with normal saline (NaCl 0.9%) and repeat the assay. Calculate the value using the proper dilution factor.

Notes

Chelating agents such as EDTA, Oxalate and Citrate, present even in traces, prevent the formation of the colour complex, hence necessary care should be taken during the assay.
Highly lipemic samples could interfere and should be cleared by centrifugation or filtration before use.
The assay can be run at 600 nm however the absorbances would be approx. 30% lower as compared to 570 nm.

Reference

Akita Abe, Yiamashita, S., (1989) Clin. Chem. 35/4 : 552 - 554

System Parameters

Reaction	:	End Point
Wavelength	:	578 nm
Zero Setting	:	Reagent Blank
Incub. Temp.	:	R.T.
Incub. Time	:	10 min.
Delay Time	:	---
Read Time	:	---
No. of read.	:	---
Interval	:	---
Sample Vol.	:	0.05 ml
Reagent Vol.	:	1.00 ml
Standard	:	200 µg/dl
Factor	:	---
React. Slope	:	Increasing
Linearity	:	500 µg/dl
Units	:	µg/dl

CPR(Col):01(P)

Coral Clinical Systems

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