COPPER

For the determination of Copper in serum (Colorimetric Method)

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 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. Decrease levels are found in Wilson's disease, decreased synthesis of ceruloplasmin, malabsorption, malnutrition, and nephritic syndrome.

PRINCIPAL

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

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

R1	:	Buffer Reagent
R2	:	Colour Reagent
Std.	:	Copper Standard (200 µg/dl)

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 R1) 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 R1 (Buffer Reagent) and R2 (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 : 578 nm
Temperature : R.T.
Light path : 1 cm

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

Addition Sequence	B (ml)	S (ml)	T (ml)
Buffer Reagent R1	0.5	0.5	0.5
Colour Reagent R2	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

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

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.

Reaction	End Point
Wavelength	578 nm
Zero Selting	Reagent Blank
Incub. Temp.	R.T.
Incub. Time	10 min.
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

Symbols

IVD	In Vitro Diagnostics		Caution
LOT	Batch No.		Product Expiry Date
CONT	Content		Manufactured By
	Read Instructions		Date of Manufacture
	Storage Temperature		Keep Dry
REF	Catalogue No.		Fragile
			Keep away from sun light

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