COPPER

For the determination of Copper in Human Serum/Plasma (DiBr-PAESA Method)

Summary

Copper is a trace element widely distributed in the various organs of the body. The copper in serum mainly exists in the form of ceruloplasmin-copper and albumin-Cu^{2+}. Copper is an important component of copper-containing enzymes and plays an important role in cell, respiratory, nerve and endocrine functions. It can be used to assist in the diagnosis of Wilson disease, Menkes syndrome, bone disease, hepatobiliary system disease by measuring the concentration of copper in the blood.

PRINCIPLE

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.

$$CER - Cu^{2+} \xrightarrow{\text{Acidic Conditions}} CER + Cu^{2+}$$

$$Albumin - Cu^{2+} \xrightarrow{\text{Acidic Conditions}} Albumin + Cu^{2+}$$

$$Cu^{2+} + VC\ (reduced \longrightarrow Cu^{2+} = VC\ (Oxidized)$$

$$Cu^{2+} = 3,5\text{-DiBr-PAESA} \longrightarrow Complex\ (Blue)$$

Calculate the copper concentration by the absorbance at 600 nm.

Contents

R1 : Buffer Reagent
R2 : Enzyme Reagent
Calibrator : Copper Calibrator

Storage/Stability

Contents are stable at 2-8° C till the expiry mentioned on the labels and contaminations are prevented during their use. Do not use reagents over the expiration date.

Reagent Preparation

Reagents are ready to use. Protect from bright light.

Sample material

Fresh Serum, plasma (heparin anticoagulation). Samples are stable for 7 days when stored at 2-8° C, and for 1 month when stored at -20°C. Only freeze once..

Normal reference values

Female : 12.6 – 24.4 µmol/l
Male : 10.0 – 24.0 µmol/l
It is recommended that each laboratory establish its own normal range representing its patient population.

Procedure

Wavelength/filter : 630 nm
Temperature : 37°C.
Light path : 10 mm

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

Addition Sequence	Sample/Calibrator
Buffer Reagent R1	150 µl
Sample	10 µl
Mix and incubate for 5 min at 37°C & add Reagent 2	
Enzyme Reagent 2	50 µl
Mix well and read the absorbance (A1) immediately and after 5 min read (A2) of the sample addition.	

Calculations

$$\text{Copper (µmol/L)} = \frac{\Delta A\ \text{Sample}}{\Delta A\ \text{Cal}} \times \text{Conc. Of Cal (µmol/L)}$$

Quality Control

For internal quality control any normal and abnormal controls should be assayed with each batch of samples. Each laboratory should establish its own Quality Control scheme and corrective actions if controls do not meet the acceptable tolerances.

Performance Characteristics and Measuring Range

The test has been developed to determine Copper activities within a measuring range from 2.5 – 79 µmol/l. If such value is exceeded, the sample should be diluted 1+4 with NacL solution (9 g/l) and result multiplied by 5.

Interferences

No interference was observed by, Bilirubin up to 20 mg/dl, Uric Acid up to 25 mg/dl, Ascorbic Acid 20 mg/dl.

Sensitivity/Limit of Detection

The lower limit of detection is 2.5 µmol/L.

Linearity

The higher limit of detection is 79 µmol/L.

Precision

Intra-Assay n=20	Mean (µmol/L)	SD (µmol/L)	CV(%)
Sample 1	15.950	0.30	1.93
Sample 2	26.245	0.29	1.12

Inter-Assay n=20	Mean (µmol/L)	SD (µmol/L)	CV(%)
Sample 1	15.8	0.79	5.0
Sample 2	26.8	1.07	4.0

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References

1. Abc, S. Yamashita, A. Noma, Sensitive, direct metric assay for copper in serum, Clin Chem, 35,552-554 (1989).
2. Miwa Nishi: Japanese Clinic, 1995, 53 (1995 Supplement): 815 Kanai Izumi, He: Summary of Clinical Laboratory Methid, 1998, Revised Edition, 31st Edition, Kanehara Publishing, Tokyo: P.1433.
3. Flowing

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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