



Intended Use

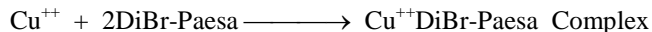
For **In Vitro Diagnostic** use in the automated, quantitative determination of Copper.

Clinical Significance (1,2)

Copper is one of the essential trace elements, stored mainly in the liver and released from the liver to maintain blood concentrations essential to normal physiological function. The most significant application of copper determination is in the diagnosis of hepatolenticular degeneration (Wilson's disease). The concentration of copper is generally decreased because of low levels of the ferroxidase enzyme ceruloplasmin. Increased serum copper levels (Hypercupremia) are found in a number of acute and chronic diseases, such as malignancies, leukemia and others.

Method Principle (3)

Under acid conditions protein unraveling agents free the copper ions. The dissociated copper ions then engage in a subsequent reaction with 4-(3,5-dibromo-2-pyridylazo)-N-ethyl-N-sulfopropylaniline (DiBr-PAESA) to produce a colored chelate complex with maximum absorption at 580nm. The intensity of the color thus produced is directly proportional to the Copper ions in the serum sample. The reaction scheme below depicts the steps that occur in this assay method.



REAGENTS

Copper Color Reagent

Each liter contains:

Buffer

DiBr-PAESA

0.025 mmol

Stabilizer and nonreactive ingredients

Precautions

Handle these reagents using good laboratory practice. **DO NOT PIPETTE REAGENT BY MOUTH.** Avoid contact with skin and eyes. If contact occurs, wash affected area with plenty of cold water. Clean spills immediately.

Reagent Storage And Stability

Store the Copper Reagent at 2-8°C. When stored as directed the reagent is stable until expiration date stated on the label.

Working Reagent Preparation

The Copper reagent is packaged ready for use. No preparation is required. Once opened the Working Copper Reagent is stable until the expiration date indicated on the label when stored capped at 2-8°C.

Reagent Indications of Deterioration

- Turbidity
- Quality control values out of assigned ranges

If these reagent characteristics are observed call Catachem's technical representative.

Specimen Collection And Stability

Clear unhemolyzed sera are the specimens of choice. Serum should be separated immediately from the clot and analyzed promptly or stored at 2-8°C. Copper in serum is stable 7 days at room temperature, 10 days refrigerated at 2-8°C and for many months frozen at -20°C. (2)

Procedure

These instructions are outlined for performing the Catachem Copper assay using an automated procedure.

Materials Provided

Catachem Copper Reagent

Materials Required But Not Provided

- Automated analyzer
- Catachem Copper Calibrator material with assigned Copper values
- Catachem Quality control material with assigned Copper values

Calibration

Catachem Copper Calibrator, which contains a known Copper value, is recommended.

Calibration Schedule

Calibrate the Catachem Copper method each time the procedure is performed.

Quality Control

To monitor the quality performance of the procedure used, Catachem Copper Control Level I and Copper Control Level II with assigned Copper values should be included in the assay procedure as follows:

- Each time the Copper procedure is performed
- Replacement of reagent lot numbers
- Analyzer service/ maintenance

Directions For Use

The Catachem Copper method requires a single Working Reagent that comes ready for use.

Procedure

Program the CataChemwell-T analyzer for Catachem's Copper method as follows:

COPPER CATACHEMWELL-T APPLICATION SPECIFIC TEST PARAMETERS			
Assay Name	Copper	Units	µg/dL
Type	Chemistry	No. of Decimals	1
Temp.	37°C	Normal Range	70-155
Mode	One by One	Linearity	0-500
Assay Steps:		Reaction Type	+
Reagent 1 Vol µl	250	Standards:	
Incubation Sec.	120	#1	*
Read:		#2	**
Primary WL	580 nm	#3	
Secondary WL	630 nm	#4	
Sample Vol. µl	15		
Incubation Sec.	180	Blank Used	No
Read:			
Primary WL	580 nm	Controls:	
Secondary WL	630 nm	#1	***
		#2	***
Clean Probe		#3	
Rinse Probe		#4	
* Use Deionized Water or Saline ** Enter value from Catachem Calibrator Package insert ***Enter value from Catachem Copper Control I and II Package Insert.			

Procedure Limitations

Samples with copper values greater than 500 µg/dL should be diluted 1:2 with distilled or deionized water and reassayed. Multiply results obtained by 2 to adjust for the sample dilution.

Normal Values

Adult Men 70-140 µg/dL
Adult Women 80-155 µg/dL

The values given here are only to be used as a guideline. It is recommended that each laboratory establish the normal range for the species under test and for the area in which it is located.

Interfering Substances

Certain substances and certain drugs are also known to influence the Copper values. A summary of the influence of drugs on clinical laboratory procedures may be found by consulting D.S. Young et al (5).

Method Performance Characteristics

Sensitivity: TBD

Linearity: In this procedure there is no significant nonlinearity over the range of 5-500 µg/dL; (78.5 umol/L)

Precision: Precision data was obtained using three levels of protein based controls and following the NCCLS EP5-T2 procedure (3). The following results were observed.

Precision

Copper	Within-Run Precision		Total Precision	
Mean	SD	CV	SD	CV
µg/dL	µg/dL	%	µg/dL	%
50.58	2.97	5.88	3.43	6.78
97.60	2.34	2.40	3.25	3.33
165.44	2.50	1.52	2.96	1.79

ACCURACY

Correlation studies were carried out between this automated Copper method (Y) and a reference Atomic Absorption method (X). Serum samples were assayed and the results compared by the least squares regression. The following statistics were observed:

N = 30
Range = 37.5 – 184.9 µg/dL
Mean Y = 105.0
Mean X = 104.9
Y = 0.971x + 0.32
Sy.x = 0.995
r = 0.9847

Bibliography

1. Fundamentals of Clinical Chemistry. Edited by Norbert Tietz 2nd. Ed. Philadelphia: W.B. Saunders; 1976.
2. C.A. Burtis, E.R. Ashwood. Fundamentals of Clinical Chemistry. Edited by Norbert Tietz 5nd. Ed. 30:54 and 973.
3. Akira Abe, Sumiko Yamashita, Aldo Noma. Sensitive, Direct Colorimetric assay for copper in serum. Clin Chem. 35/4 552-554 (1989)
4. Evaluation of Precision Performance of Clinical Chemistry Devices. Second Edition. NCCLS Document EP5-T2. Vol. 12, No. 4
5. Young D.S., Pestamer L.C., Gibberman V. Effects of drugs on clinical laboratory tests. Clin. Chem. 21, No. 5, 1975.

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