Human Caeruloplasmin Kit for use on SPAPLUS®

For *in vitro* diagnostic use

Product Code: NK045.S

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FDA (USA) Information
Analyte Name: Ceruloplasmin
Complexity Cat.: Moderate

CE

1 INTENDED USE

The Human Caeruloplasmin Kit for use on SPAPLUS is intended for the quantitative *in vitro* measurement of human caeruloplasmin in serum using the SPAPLUS turbidimetric analyser. The measurement of caeruloplasmin levels in serum is an aid in the diagnosis of copper metabolism disorders. This test should be used in conjunction with other laboratory and clinical findings.

2 SUMMARY AND EXPLANATION

Caeruloplasmin is synthesised in the liver and has a major role in copper metabolism, carrying approximately 95% of the total copper in serum. Decreased levels of caeruloplasmin can be caused by hereditary disorders of copper metabolism, for example; inability to transport oxidised copper (Cu^{2+}) from the gastrointestinal epithelium into the circulation (as in Menkes disease), or the inability to insert Cu^{2+} into the developing caeruloplasmin molecule (as in Wilson's disease). Dietary copper insufficiency, including malabsorption, also reduces serum caeruloplasmin concentrations. Serum caeruloplasmin concentrations can increase as a result of acute-phase reactions, pregnancy or use of oral contraceptives (ref. 1).

3 PRINCIPLE

The determination of soluble antigen concentration by turbidimetric methods involves the reaction with specific antiserum to form insoluble complexes. When light is passed through the suspension formed a portion of the light is transmitted and focused onto a photodiode by an optical lens system. The amount of transmitted light is indirectly proportional to the specific protein concentration in the test sample. Concentrations are automatically calculated by reference to a calibration curve stored within the instrument.

4 REAGENTS

4.1 **Human Caeruloplasmin Antiserum:** This antiserum is monospecific for caeruloplasmin and is supplied in stabilised liquid form. It contains 0,099% sodium azide, 0.1% EACA, 0.1% EDTA and 0.01% benzamidine as preservatives.
4.2 **Calibrators**: These consist of pooled human serum and are supplied lyophilised.
4.3 **Controls:** These consist of pooled normal human serum and are supplied in lyophilised form. They contain 0.099% sodium azide, 0.1% EACA and 0.01% benzamidine as preservatives.
4.4 **Reaction Buffer:** Containing 0.099% sodium azide as a preservative.

5 CAUTION

All donors of human serum supplied in this kit have been serum tested and found negative for hepatitis B surface antigen (HBsAg) and antibodies to human immunodeficiency virus (HIV1 and HIV2) and hepatitis C virus. The assays used were either cleared by the FDA (USA) or cleared for *in vitro* diagnostic use in the EU (Directive 98/79/EC, Annex II); however, these tests cannot guarant**e**e the absence of infective agents. Proper handling and disposal methods should be established as for all potentially infective material, including (but not limited to) users wearing suitable protective equipment and clothing at all times. Only personnel fully trained in such methods should be permitted to perform these procedures.

WARNING: This product contains sodium azide and must be handled with caution; suitable gloves and other protective clothing should be worn at all times when handling this product. Do not ingest or allow contact with the skin (particularly broken skin or open wounds) or mucous membranes. If contact does occur wash with a large volume of water and seek urgent medical advice. Explosive metal azides may be formed on prolonged contact of sodium azide with lead and copper plumbing; on disposal of reagent, flush with a large volume of water to prevent azide build up.

This product should only be used by suitably trained personnel for the purposes stated in the Intended Use. Strict adherence to these instructions is essential at all times. Results are likely to be invalid if parameters other than those stated in these instructions are used.

Reagents from different batch numbers of kits are **NOT** interchangeable. If large numbers of tests are performed care should be taken to ensure that all the reagents are from the same batch.

6 STORAGE AND STABILITY

The unopened kit should be stored at 2-8°C and can be used until the expiry date shown on the kit box label. DO NOT FREEZE. The human caeruloplasmin antiserum and reaction buffer may be stored for up to 2 months after opening providing that they are capped to avoid evaporation and kept at 2-8°C in a refrigerator. The human caeruloplasmin antiserum and reaction buffer may be stored, uncapped, on the SPAPLUS analyser for up to 30 days, provided that the main power switch (located at the rear of the left hand panel) is left switched on. The caeruloplasmin calibrator set and controls are stable until the expiration date on the vial label when stored at 2-8°C. Once reconstituted, the solution must be stored at 2-8°C and used within 7 days for the calibrator set and 30 days for the controls. Discard any unused solution after this time.

7 SPECIMEN COLLECTION AND PREPARATION

Use fresh or deep frozen serum samples. Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to 3 days prior to assay, or for prolonged storage kept at -20°C or below for up to 4 weeks provided they are frozen within 24 hours after collection (ref. 2). Repeated freezing and thawing should be avoided. Microbially contaminated, haemolysed and lipaemic serum and samples containing particulate matter should not be used.

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 50 Tests *Human Caeruloplasmin Antiserum SPAPLUS*
8.1.2 2 x *Human Caeruloplasmin SPAPLUS Calibrator sets 1-6* (12 vials, supplied lyophilised)
8.1.3 2 x *Human Caeruloplasmin SPAPLUS High Control (*supplied lyophilised)
8.1.4 2 x *Human Caeruloplasmin SPAPLUS Low Control (*supplied lyophilised)
8.1.5 1 x 50 Tests *Caeruloplasmin Reaction Buffer SPAPLUS*

8.2 Materials required but not provided

8.2.1 Equipment for collection and preparation of test samples e.g. sample tubes, centrifuge etc.
8.2.2 A fully operational and equipped SPAPLUS analyser.
8.2.3 Current analyser operating instructions: SPAPLUS Reference Guide, Insert Code FIN012.
8.2.4 SPAPLUS Sample Diluent 2 (100: Dil 2) Product Code: SN114.S
8.2.5 Distilled water

8.3 Calibrator Set, Controls and Reagent preparation

8.3.1 The calibrator set and controls are supplied in lyophilised form. Each vial must be reconstituted in the volume of distilled water stated on the Quality Control Certificate (SIN242.QC) and vial label. Remove the cap and gently tap down all lyophilised material to the bottom of the vial. Add the required volume of distilled water and leave to stand for 20 minutes. Invert the vial and allow the fluid to cover the stopper for 20-30 seconds with gentle shaking. Return the vial to the upright position and leave to stand for 10 minutes, gently shake before use.
8.3.2 Before loading the reagent, gently mix by inversion ensuring no foam or bubbles are generated or remain on the surface as these may interfere with reagent aspiration.

8.4 Test procedure

The user should be familiar with the operation of the SPAPLUS analyser before attempting to carry out the test procedures. The analyser should be prepared for use according to the manufacturer's instructions and the assay protocol entered as described below.

For full details of analyser operation refer to the SPAPLUS Reference Guide (FIN012) supplied with the analyser.

8.4.1 Test parameters

Assay parameters are entered into item number 39.

Item Name 39 Caer
DATA INFORMATION
Units g/L
Decimals 3

ANALYSIS
Type End ▼

Main W.Length 1 340 ▼
Sub W.Length ▼
Method

CORR.
SLOPE INTER
Y = 1 X + 0

CALIBRATION
Type Spline 1 ▼ Auto Fill
Standard
1 # 4 #
2 # 5 #
3 # 6 #

NORMAL RANGE

	MALE LOW	MALE HIGH	FEMALE LOW	FEMALE HIGH
Serum	[ ]	[ ]	[ ]	[ ]
Urine	[ ]	[ ]	[ ]	[ ]
Plasma	[ ]	[ ]	[ ]	[ ]
CSF	[ ]	[ ]	[ ]	[ ]
Dialysis	[ ]	[ ]	[ ]	[ ]
Other	[ ]	[ ]	[ ]	[ ]

Page : 1 Print Hard Copy Next Page Save Return

Item Name 39 Caer

ASPIRATION
KIND ○ Single ● Double
VOLUME
SAMPLE 30
REAGENT1 VOL 200 µL
REAGENT2 VOL 20

Third mix ● **OFF** ○ **ON**
R1 Blank ● **Water – Blank**

MONITOR
0 LEVEL SPAN 1
SPAN 3.0

DATA PROCESS
READ **ABSORBANCE LIMIT**
START END
MAIN 53 54 LOW -3.0
SUB 30 31 HIGH #

FACTOR **Reaction Check**
Blank correction * ○ ON ● OFF
ENDPOINT LIMIT 2.0 CHECK POINT
LINEAR CHECK (%) 0 LOW -3
HIGH 3

DILUTION
Diluent ○ 99: Dil 1 ●100: Dil 2
Pre Dilution Rate 10 ▼
Auto Rerun Dilution Rate High 20 ▼
Auto Rerun Dilution Rate Low ▼

PROZONE CHECK 0
START END LIMIT (%) Min dOD
FIRST [ ] [ ]
SECOND [ ] [ ] [ ] ○ Low ● High
THIRD [ ] [ ] [ ] ○ Low ● High

Page : 2 Print Hard Copy Prev Page Next Page Save Return

*automatically calculated

Item Name 39 Caer

Auto Rerun SW
● On ○ Off
Auto Rerun Range (Result)
○ On ● Off ● On ○ Off

	Lower	Higher
Serum	#	#
Urine	#	#
Plasma	#	#
CSF	#	#
Dialysis	#	#
Other	#	#

Bottle Size (ml)

24 Items		36 Items	
Reagent1	60	Reagent1	0
Reagent2 R1	10.5	Reagent2 R1	0
Reagent2 R2	1.5	Reagent2 R2	0

Auto Rerun Condition (Absorbance)

Absorbance Range
Lower ○ On ● Off
Higher ● On ○ Off

Prozone Range ○ On ● Off

Page : 3 Print Prev Page Save Return

Item Name 39 Caer

Out-of-Range Table

	NEAT		Pre Dilution (*10)		Auto-rerun Dilution (*20)	
	ABOVE Cal#1	BELOW Cal#2-Cal#6	ABOVE Cal#1	BELOW Cal#2-Cal#6	ABOVE Cal#1	BELOW Cal#2-Cal#6
Serum	#	#	#	#	#	#
Urine	#	#	#	#	#	#
Plasma	#	#	#	#	#	#
CSF	#	#	#	#	#	#
Dialysis	#	#	#	#	#	#
Other	#	#	#	#	#	#

Page : 4 Prev Page Save Return

The calibrator (Standard #), high absorbance limit (High #), Auto Rerun Range (Result) and Out-of-Range Table values are found in the Quality Control Certificate (SIN242.QC). Calibrator values on Page 1 should be entered in ascending order, i.e. the lowest value first, and the Auto Fill button pressed after typing the value for calibrator 6. The high absorbance value (High #) should be entered on Page 2.
IMPORTANT: Calibrator values on page 1 must be updated before the Auto Rerun Range (Result) (Page 3) and Out of Range (page 4) tables (sample type #). These tables must be checked and manually updated using the values supplied in the Quality Control certificate (SIN242.QC).

* The Blank correction factor is automatically calculated by the instrument.

8.5 Measuring range

The approximate measuring range of the caeruloplasmin assay is shown in the table below.

SPAPLUS Analyser Dilution	Approximate range (g/L)
1/10	**0.03-0.82**
1/20	0.06-1.64

9 QUALITY CONTROL

9.1 At least two levels of appropriate control material should be tested a minimum of once a day. In addition, controls should be tested after calibration, with each new lot of reagent and after specific maintenance or troubleshooting steps described in the SPAPLUS Operation Manual.

9.2 Quality control testing should be performed in accordance with regular requirements and each laboratory's standard procedure. Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to the local technical support organisation.

9.3 The concentrations of the controls provided are stated on the accompanying QC certificate (SIN242.QC). Sample results obtained should only be accepted if the control results are within ±15% of the concentration(s) stated.

10 LIMITATIONS

10.1 Turbidimetric assays are not suitable for measurement of highly lipaemic or haemolysed samples or samples containing high levels of circulating immune complexes (CICs) due to the unpredictable degree of non-specific scatter these sample types may generate. Unexpected results should be confirmed using an alternative assay method.

10.2 This assay has not been validated using paediatric samples.

10.3 Should a control measurement be out of range when assayed with a stored curve the assay must be recalibrated. If on recalibration the control values measured with the new curve are still out of range, the instrument and the assay parameters should be checked before repeating the assay. If problems persist, refer to supplier.

10.4 Diagnosis cannot be made and treatment must not be given on the basis of caeruloplasmin measurements alone. Clinical history and other laboratory findings must be taken into account.

10.5 Variation in reagent temperature may affect results. Ensure that reagents are transferred directly from the refrigerator to the refrigerated reagent compartment of the analyser – do not allow to warm to room temperature.

11 EXPECTED VALUES

The ranges provided have been obtained from a limited number of samples and are intended for guidance purposes only. Wherever possible it is strongly recommended that local ranges are generated.

Adult serum range

The reference range for this kit was transferred from a published literature reference (ref. 3). It is recommended that each laboratory establishes its own reference range.

	Range (g/L)
Caeruloplasmin	0.2 – 0.60

12 PERFORMANCE CHARACTERISTICS

12.1 Precision

A study was performed following CLSI *Evaluation of Precision Performance of Clinical Quantitative Measurement Methods; Approved Guideline* (CLSI Document EP5-A2)**.** The study was performed over 21 working days, with two runs per day. One user assessed four different samples using three different reagent lots on three analysers.

Caeruloplasmin Precision summary									
	Mean (g/L)	Within run		Between run		Between day		Total	
		SD	CV %	SD	CV %	SD	CV %	SD	CV %
Serum 1	0.809	0.013	1.6	0.012	1.5	0.042	5.2	0.045	5.6
Serum 2	0.214	0.002	1.1	0.004	1.6	0.019	8.9	0.020	9.1
Serum 3	0.047	0.001	2.1	0.002	4.3	0.005	10.6	0.006	11.6
Serum 4*	0.750	0.009	1.1	0.025	3.4	0.055	7.4	0.061	8.2

*performed on the 1/20 sample dilution

12.2 Comparison

A correlation study was performed on 102 samples (using a variety of normal and clinical serum samples) using this kit on a SPAPLUS and an alternative commercially available caeruloplasmin assay. The study demonstrated excellent agreement with the following Passing and Bablok regression plot:

y = 1.08x - 0.02 (g/L) (y = SPAPLUS caeruloplasmin; x = alternative assay)

R^2 = 0.93 (calculated by linear regression)

12.3 Limit of Quantitation

Based on *CLSI document EP17-A - Protocols for Determination of Limits of Detection and Limits of Quantitation; Approved Guideline* the limit of quantitation for this assay is defined as the lowest point of the calibration curve i.e. 0.03g/L based upon measuring neat sample.

12.4 Linearity

A linearity study was performed following CLSI (formerly NCCLS) *Evaluation of the Linearity of Quantitative Measurement Procedures* document EP6-A. One user assessed the linearity of a pool of high samples using one lot of reagent on one analyser. This gave a regression plot of y = 1.0674x – 0.0094 (y = measured Caeruloplasmin concentration, x = theoretical concentration) over the range of 0.024 – 1.066 g/L using the 1/10 sample dilution.

12.5 Interference

Interference by 1550 formazine turbidity units (FTU) of chyle, 200mg/L bilirubin, 4.9g/L haemoglobin has been determined to be below d_{max}, defined as the maximum level of interference considered acceptable.

	Bilirubin	Hb	Chyle
Interference at higher sample dilution (d_{max} 0.045)			
d_{obs} (g/L)	0.000	-0.002	-0.002
d_{obs}, 95% CI (g/L)	-0.022 – 0.022	-0.024 – 0.020	-0.024 – 0.020
Interference at lower sample dilution (d_{max} 0.019)			
d_{obs} (g/L)	-0.002	-0.001	-0.005
d_{obs}, 95% CI (g/L)	-0.019 – 0.015	-0.018 – 0.016	-0.022 – 0.012

Rheumatoid Factor interference has not been evaluated.

12.6 Antigen excess

No antigen excess was observed to a level of two times the top point of the assay, approximately 2.0 g/L (at the initial sample dilution of 1/10).

13 BIBLIOGRAPHY

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2. Tietz Clinical guide to laboratory tests, 4th edn. (2006). Ed. AHB Wu. Publ. WB Saunders Company, Philadelphia, US, 230.
3. Dati, F *et al.* (1996). Consensus of a Group of Professional Societies and Diagnostic Companies on Guidelines for Interim Reference Ranges for 14 Proteins in Serum based on the Standardization against IFCC/BCR/CAP Reference Material (CRM 470). International Federations of Clinical Chemistry. Community Bureau of Reference of the Commission of the European Communities. College of American Pathologists. Eur J Clin Chem Clin Biochmem; 34:517-20.