HAPT2

cobas®

Tina-quant Haptoglobin ver.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05992001190	05992001500	Tina-quant Haptoglobin ver.2 150 tests	System-ID 03 9009 5	**cobas c** 701/702

Materials required (but not provided):

11355279216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 656	
10557897122	Precinorm Protein (3 x 1 mL)	Code 302	
11333127122	Precipath Protein (3 x 1 mL)	Code 303	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05172152190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3	

English

System information

HAPT2: ACN 8228

Intended use

In vitro test for the quantitative determination of haptoglobin in human serum and plasma on **cobas c** systems.

Summary

Haptoglobin is produced primarily in the liver and is important for binding free hemoglobin produced from lysed red blood cells in vivo, thereby preventing its toxic effects. In the presence of large amounts of free hemoglobin, haptoglobin levels decrease, so reduced haptoglobin levels are a marker of hemolysis or decreased liver function. Increased haptoglobin levels are observed under inflammatory conditions. Therefore, haptoglobin measurements, performed with this assay in human serum and plasma can be used as an aid in diagnosis and monitoring of diseases characterized by increased red blood cell destruction (e.g. hemolytical anemia), and in evaluating conditions associated with altered haptoglobin blood levels such as acute inflammation and liver dysfunction.

Haptoglobin is a transport and acute phase protein which is synthesized in hepatocytes. It is a glycoprotein which consists of two light α-chains and two heavy β-chains.[1] The genetic polymorphism of the α-chains leads to three phenotypes Hp 1-1, Hp 2-1 and Hp 2-2 differing in molecular weight.[2]

Haptoglobin binds hemoglobin in a strong haptoglobin-hemoglobin complex (Hp-Hb), the hemoglobin resulting from pathologically elevated hemolysis. These complexes are deposited and degraded in the hepatocytes, the deposition process having a half-life of less than 10 minutes. Hemoglobin is then enzymatically metabolized to prevent the occurrence of hemoglobinuria with excess renal loss of iron.[3,4]

Since haptoglobin is degraded after complexing with hemoglobin, serum haptoglobin levels are significantly depleted following either intravascular or extravascular hemolysis.[1] Differential levels of serum haptoglobin depletion accompany different hemolytic disease states and may be useful in the assessment of the severity and stage of hemolysis.[3,5] There is no 'gold standard' test to confirm a diagnosis of hemolysis, therefore a combination of clinical factors and parallel assessment with other parameters should form the complete diagnostic workup.[3] Decreased haptoglobin levels can also occur in the absence of hemolysis, including in cirrhotic liver disease, splenomegaly, ineffective hematopoiesis, and malnutrition.[5]

Serum haptoglobin can rise in response to stress, infection, acute inflammation, or tissue necrosis.[2] As an acute phase reactant, haptoglobin is produced in increased quantities in response to inflammatory cytokines such as IL-1 and IL-6.[3,6]

Increased serum haptoglobin levels have also been associated with increased mortality risk in a number of cancer types including breast, colorectal and lung cancer.[7,8,9,10]

Various methods including nephelometry, radial immunodiffusion (RID) and turbidimetric methods are available for the determination of haptoglobin.[1] The haptoglobin assay from Roche is based on the principle of immunological agglutination.

Test principle

Immunoturbidimetric assay.
Human haptoglobin forms a precipitate with a specific antiserum which is determined turbidimetrically.

Reagents - working solutions

R1 Phosphate buffer: 12.7 mmol/L, pH 7.2; NaCl: 130 mmol/L; PEG: 40 g/L; preservative

R3 Anti-human haptoglobin antibody (rabbit): > 1.1 g/L; NaCl: 100 mmol/L; preservative

R1 is in position B and R3 is in position C.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

Reagent handling

Ready for use

Storage and stability

Shelf life at 2-8 °C:	See expiration date on **cobas c** pack label.
On-board in use and refrigerated on the analyzer:	12 weeks
On-board on the Reagent Manager:	24 hours

Specimen collection and preparation

For specimen collection and preparation only use suitable tubes or collection containers.

Only the specimens listed below were tested and found acceptable.
Serum.
Plasma: Li-heparin and K_2-EDTA plasma

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates before performing the assay.

See the limitations and interferences section for details about possible sample interferences.

Stability:[11] 3 months at 15-25 °C

8 months at 2-8 °C

Materials provided

See "Reagents – working solutions" section for reagents.

Materials required (but not provided)

- See "Order information" section
- General laboratory equipment

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

The performance of applications not validated by Roche is not warranted and must be defined by the user.

Application for serum and plasma

cobas c 701/702 **test definition**

Assay type	2 Point End		
Reaction time / Assay points	10 / 18-38		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	110 µL	–	
R3	50 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	5.5 µL	9 µL	180 µL
Decreased	5.5 µL	4 µL	164 µL
Increased	5.5 µL	15 µL	150 µL

Calibration

Calibrators	S1: H_2O S2-S6: C.f.a.s. Proteins
Calibration mode	RCM2
Calibration frequency	Full calibration - after reagent lot change - as required following quality control procedures

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Traceability: This method has been standardized against the certified reference material in human serum of the IRMM (Institute for Reference Materials and Measurements) ERM-DA470k/IFCC.

Quality control

For quality control, use control materials as listed in the "Order information" section.

In addition, other suitable control material can be used.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

cobas c systems automatically calculate the analyte concentration of each sample.

Conversion factors: g/L x 10.0 = µmol/L

g/L x 100 = mg/dL

Limitations - interference

Citerion: Recovery within ≤ ± 0.03 g/L of initial values of samples ≤ 0.3 g/L and ≤ ± 10 % for samples > 0.3 g/L.

Icterus:[12] No significant interference up to an I index of 60 for conjugated bilirubin and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL).

Hemolysis:[12] No significant interference up to an H index of 10 (approximate hemoglobin concentration: 6 µmol/L or 10 mg/dL).

The Glick model which is normally used for assessment of hemoglobin interference is not suitable in the case of haptoglobin. Binding of free hemoglobin is the physiological function of haptoglobin. In the Glick study, hemolysate is added to the sample resulting in the formation of the haptoglobin-hemoglobin complex. This complex is present in the reagent tube and causes a 10-15 % decrease in haptoglobin values. However, the effect is of no relevance for the results in native samples because in vivo the haptoglobin-hemoglobin complex is rapidly eliminated from the circulation and is practically not present in the blood.

Lipemia (Intralipid):[12] No significant interference up to an L index of 600. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors up to 250 IU/mL do not interfere.

High dose hook-effect: No false result occurs up to a haptoglobin concentration of 12 g/L (120 µmol/L, 1200 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[13,14]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[15]

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

ACTION REQUIRED

Special Wash Programming: The use of special wash steps is mandatory when certain test combinations are run together on **cobas c** systems. All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SmpCln1+2/SCCS Method Sheet and for further instructions refer to the operator's manual.

Where required, special wash/carry-over evasion programming must be implemented prior to reporting results with this test.

Limits and ranges

Measuring range

0.1-5.7 g/L (1.0-57 µmol/L, 10-570 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:2 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 2.

Lower limits of measurement

Lower detection limit of the test

0.1 g/L (1.0 µmol/L, 10 mg/dL)

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying 3 standard deviations above that of the lowest standard (standard 1 + 3 SD, repeatability, n = 21).

Values below the lower detection limit (< 0.1 g/L) will not be flagged by the instrument.

Expected values[16]

0.3-2.0 g/L (3.0-20.0 µmol/L, 30-200 mg/dL)

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using human samples and controls in an internal protocol with repeatability (n = 21) and intermediate precision (3 aliquots per run, 1 run per day, 21 days). The following results were obtained on the **cobas c** 701 analyzer:

Repeatability	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	1.15 (11.5, 115)	0.02 (0.2, 2)	1.7
Precipath Protein	1.98 (19.8, 198)	0.03 (0.3, 3)	1.5
Human serum A	2.25 (22.5, 225)	0.04 (0.4, 4)	1.6
Human serum B	5.04 (50.4, 504)	0.06 (0.6, 6)	1.1
Human serum C	0.177 (1.77, 17.7)	0.005 (0.05, 0.5)	2.7
Intermediate precision	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	1.04 (10.4, 104)	0.01 (0.1, 1)	1.2
Precipath Protein	1.73 (17.3, 173)	0.02 (0.2, 2)	1.1
Human serum 3	1.05 (10.5, 105)	0.01 (0.1, 1)	1.2
Human serum 4	1.57 (15.7, 157)	0.02 (0.2, 2)	1.2

Results for intermediate precision were obtained on the **cobas c** 501 analyzer.

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 701 analyzer(s).

Method comparison

Haptoglobin values for human serum and plasma samples obtained on a **cobas c** 701 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 128

Passing/Bablok[17]	Linear regression
y = 0.964x + 0.026 g/L	y = 0.950x + 0.052 g/L
τ = 0.983	r = 0.999

The sample concentrations were between 0.110 and 5.43 g/L (1.10 and 54.3 µmol/L, 11.0 and 543 mg/dL).

References

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A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard:

CONTENT	Contents of kit
→	Volume for reconstitution
GTIN	Global Trade Item Number
Rx only	For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

CE 0123

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