HAPT2

cobas®

Tina-quant Haptoglobin ver.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08106045190	08106045500	Tina-quant Haptoglobin ver.2 (200 tests)	System-ID 2064 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

11355279216	Calibrator f.a.s. Proteins (5 x 1 mL)	Code 20656	
10557897122	Precinorm Protein (3 x 1 mL)	Code 20302	
11333127122	Precipath Protein (3 x 1 mL)	Code 20303	
05117003190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 20392	
08063494190	Diluent NaCl 9 % (123 mL)	System-ID 2906 001	

English

System information

HAPT2: ACN 20640

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 2 light α-chains and 2 heavy β-chains.[1] The genetic polymorphism of the α-chains leads to 3 phenotypes Hp 1-1, Hp 2-1 and Hp 2-2 differing in molecular weight.

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.[2,3]

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.[2,4] 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.[2] Decreased haptoglobin levels can also occur in the absence of hemolysis, including in cirrhotic liver disease, splenomegaly, ineffective hematopoiesis, and malnutrition.[4]

Serum haptoglobin can rise in response to stress, infection, acute inflammation, or tissue necrosis.[5] As an acute phase reactant, haptoglobin is produced in increased quantities in response to inflammatory cytokines such as IL-1 and IL-6.[2,6]

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:	26 weeks

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.

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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

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	78 µL	–	
R3	36 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3.9 µL	5 µL	100 µL
Decreased	3.9 µL	2 µL	82 µL
Increased	3.9 µL	5 µL	100 µL

For further information about the assay test definitions refer to the application parameters setting screen of the corresponding analyzer and assay.

Calibration

Calibrators	S1: H_2O
	S2-S6: C.f.a.s. Proteins
Calibration mode	Non-linear
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. It is recommended to perform quality control always after lot calibration and subsequently at least every 26 weeks. 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 in the unit g/L (µmol/L, mg/dL).

Conversion factors:	g/L × 10.0 = µmol/L
	g/L × 100 = mg/dL

Limitations - interference

Criterion: 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 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 200. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 250 IU/mL.

High-dose hook effect: No false result occurs up to a haptoglobin concentration of 12 g/L.

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. The latest version of the carry-over evasion list can be found with the NaOHD/SMS/SCCS Method Sheet. For further instructions, refer to the operator's manual.

Limits and ranges

Measuring range

0.1-5.7 g/L (1.0-57 µmol/L)

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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.1 g/L (1.0 µmol/L)
Limit of Detection	= 0.1 g/L (1.0 µmol/L)
Limit of Quantitation	= 0.1 g/L (1.0 µmol/L)

The Limit of Blank, Limit of Detection and Limit of Quantitation were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A2 requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples.

The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

The Limit of Quantitation is the lowest analyte concentration that can be reproducibly measured with a total error of 20 %. It has been determined using low concentration haptoglobin samples.

Expected values[16]

0.3-2.0 g/L (3.0-20.0 µmol/L*)

*calculated by unit conversion factor

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. These data represent the performance of the analytical procedure itself.

Results obtained in individual laboratories may differ due to heterogenous sample materials, aging of analyzer components and mixture of reagents running on the analyzer.

Precision

Precision was determined using human samples and controls in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP05-A3 requirements with repeatability (n = 84) and intermediate precision (2 aliquots per run, 2 runs per day, 21 days). Results for repeatability and intermediate precision were obtained on the **cobas c** 503 analyzer.

Repeatability	*Mean* *g/L*	*SD* *g/L*	*CV* *%*
PCCC1[a)]	0.780	0.00469	0.6
PCCC2[b)]	1.38	0.0114	0.8
Human serum 1	0.250	0.00354	1.4
Human serum 2	1.13	0.00880	0.8
Human serum 3	1.90	0.0178	0.9
Human serum 4	2.81	0.0268	1.0
Human serum 5	4.99	0.0487	1.0

Intermediate precision	*Mean* *g/L*	*SD* *g/L*	*CV* *%*
PCCC1[a)]	0.796	0.0146	1.8
PCCC2[b)]	1.38	0.0205	1.5
Human serum 1	0.250	0.0151	6.1
Human serum 2	1.15	0.0172	1.5
Human serum 3	1.90	0.0248	1.3
Human serum 4	2.81	0.0337	1.2
Human serum 5	5.08	0.0540	1.1

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

The data obtained on **cobas c** 503 analyzer(s) are representative for **cobas c** 303 analyzer(s) and **cobas c** 703 analyzer(s).

Method comparison

Haptoglobin values for human serum and plasma samples obtained on a **cobas c** 503 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 99

Passing/Bablok[17]	Linear regression
y = 1.012x + 0.007 g/L	y = 0.971x + 0.0462 g/L
τ = 0.989	r = 0.999

The sample concentrations were between 0.110 and 5.62 g/L.

Haptoglobin values for human serum and plasma samples obtained on a **cobas c** 303 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 501 analyzer (x).

Sample size (n) = 70

Passing/Bablok[17]	Linear regression
y = 1.014x + 0.0161 g/L	y = 0.969x + 0.0586 g/L
τ = 0.977	r = 0.999

The sample concentrations were between 0.150 and 5.60 g/L.

Haptoglobin values for human serum and plasma samples obtained on a **cobas c** 703 analyzer (y) were compared with those determined using the corresponding reagent on a **cobas c** 503 analyzer (x).

Sample size (n) = 72

Passing/Bablok[17]	Linear regression
y = 0.983x - 0.00750 g/L	y = 0.962x + 0.0126 g/L
τ = 0.987	r = 1.000

The sample concentrations were between 0.123 and 5.60 g/L.

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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