HAPT2

cobas®

Tina-quant Haptoglobin ver.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
03005593 322	Tina-quant Haptoglobin ver.2, 100 tests	System-ID 07 9009 5	**cobas c** 311, **cobas c** 501/502

Materials required (but not provided):

11355279 216	Calibrator f.a.s. Proteins (5 × 1 mL)	Code 656	
11355279 160	Calibrator f.a.s. Proteins (5 × 1 mL, for USA)	Code 656	
10557897 122	Precinorm Protein 3 x 1 mL	Code 302	
10557897 160	Precinorm Protein (3 × 1 mL, for USA)	Code 302	
11333127 122	Precipath Protein (3 × 1 mL)	Code 303	
11333127 160	Precipath Protein (3 × 1 mL, for USA)	Code 303	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

HAPT2: ACN 228

For **cobas c** 502 analyzer:

HAPT2: ACN 8228

Intended use

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

Summary[1,2,3,4,5,6,7,8]

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. 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.

Haptoglobin binds hemoglobin in a strong haptoglobin-hemoglobin complex (Hp-Hb), the hemoglobin resulting from pathologically elevated hemolysis. These complexes are deposited in the hepatocytes, the deposition process having a half-life of less than 10 minutes. Hemoglobin is enzymatically metabolized and haptoglobin is liberated after approximately 3 days. Complex formation and the extremely rapid elimination from circulating blood prevent the occurrence of hemoglobinuria with excess renal loss of iron. A reduction in the level of free haptoglobin is indicative of intravascular hemolysis.

As a strong positive acute phase reactant, a hemolysis-mediated reduction or, to a certain extent, an elevation with accompanying acute inflammation can be compensated for. Indications for haptoglobin assays have been published and include the assessment of the severity and stage of intravascular hemolysis, evaluation of acute inflammatory processes.

Various methods including nephelometry, radial immunodiffusion (RID) and turbidimetric methods are available for the determination of haptoglobin. 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

R2 Anti-human haptoglobin antibody (rabbit): > 1.1 g/L; NaCl: 100 mmol/L; preservative

R1 is in position B and R2 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.

For USA: Caution: Federal law restricts this device to sale by or on the order of a physician.

Reagent handling

Ready for use

Storage and stability

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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

Diluent NaCl 9 %

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

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.

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Centrifuge samples containing precipitates before performing the assay.

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

Stability:[9] 3 months at 15-25 °C

8 months at 2-8 °C

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the method sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

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 311 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 6-24		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	110 µL	–	
R2	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	9 µL	180 µL

cobas c 501 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-48		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	110 µL	–	
R2	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	9 µL	180 µL

cobas c 502 **test definition**

Assay type	2-Point End		
Reaction time / Assay points	10 / 10-48		
Wavelength (sub/main)	700/340 nm		
Reaction direction	Increase		
Units	g/L (µmol/L, mg/dL)		
Reagent pipetting		Diluent (H_2O)	
R1	110 µL	–	
R2	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	18 µL	180 µL

Calibration

Calibrators	S1: H_2O	
	S2-S6: C.f.a.s. Proteins	
	Multiply the lot-specific C.f.a.s. Proteins calibrator value by the factors below to determine the standard concentrations for the 6-point calibration curve:	
	S2: 0.0955	S5: 1.45
	S3: 0.382	S6: 2.28
	S4: 0.840	
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	mg/dL x 0.100 = µmol/L
	g/L x 100 = mg/dL	mg/dL x 0.01 = g/L

Limitations - interference

Criterion: Recovery within ± 10 % of the initial value at a haptoglobin concentration of 0.3 g/L (3.0 µmol/L, 30 mg/dL).

Icterus:[10] 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:[10] 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):[10] 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 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.[11,12]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[13]

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. The latest version of the carry-over evasion list can be found with the NaOHD-SMS-SmpCln1+2-SCCS Method Sheets. For further instructions refer to the operator's manual. **cobas c** 502 analyzer: All special wash programming necessary for avoiding carry-over is available via the **cobas** link, manual input is required in certain cases.

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).

Expected values[14]

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:

Repeatability	*Mean*	*SD*	*CV*
	g/L (µmol/L, mg/dL)	*g/L (µmol/L, mg/dL)*	*%*
Precinorm Protein	1.05 (10.5, 105)	0.01 (0.1, 1)	0.7
Precipath Protein	1.75 (17.5, 175)	0.01 (0.1, 1)	0.7
Human serum 1	1.03 (10.3, 103)	0.00 (0.0, 0)	0.4
Human serum 2	1.40 (14.0, 140)	0.02 (0.2, 2)	1.3

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

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

Method comparison

Haptoglobin values for human serum and plasma samples obtained on a **cobas c** 501 analyzer (y) were compared with those determined using the corresponding reagent on a Roche/Hitachi 917 analyzer (x).

Sample size (n) = 304

Passing/Bablok[15]	Linear regression
y = 0.996x + 0.014 g/L	y = 0.998x + 0.011 g/L
τ = 0.974	r = 0.999

The sample concentrations were between 0.030 and 5.32 g/L

(0.300 and 53.2 µmol/L, 3.00 and 532 mg/dL).

The data obtained on **cobas c** 501 analyzer(s) are representative for **cobas c** 311 analyzer(s).

References

1 Lievens M, Bienvenu J, Buitrago JMG, et al. Evaluation of four new Tina-quant assays for determination of α1-acid glycoprotein, α1-antitrypsin, haptoglobin and prealbumin. Clin Lab 1996;42:515-520.

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12 Sonntag O, Scholer A. Drug interference in clinical chemistry: recommendation of drugs and their concentrations to be used in drug interference studies. Ann Clin Biochem 2001;38:376-385.

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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 (for USA: see dialog.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

FOR US CUSTOMERS ONLY: LIMITED WARRANTY

Roche Diagnostics warrants that this product will meet the specifications stated in the labeling when used in accordance with such labeling and will be free from defects in material and workmanship until the expiration date printed on the label. THIS LIMITED WARRANTY IS IN LIEU OF ANY OTHER WARRANTY, EXPRESS OR IMPLIED, INCLUDING ANY IMPLIED WARRANTY OF MERCHANTABILITY OR FITNESS FOR PARTICULAR PURPOSE. IN NO EVENT SHALL ROCHE DIAGNOSTICS BE LIABLE FOR INCIDENTAL, INDIRECT, SPECIAL OR CONSEQUENTIAL DAMAGES.

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