0108056668500V7.0

A1CX3

Tina-quant Hemoglobin A1cDx Gen.3

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056668190	08056668500	Tina-quant Hemoglobin A1cDx Gen.3 (500 tests)	System-ID 2066 001	**cobas c** 303, **cobas c** 503
08445699190	08056668500	Tina-quant Hemoglobin A1cDx Gen.3 (200 tests)	System-ID 2066 002	

Materials required (but not provided):

04528417190	Calibrator f.a.s. HbA1c (3 × 2 mL)	Code 20674	
05479207190	PreciControl HbA1c norm (4 × 1 mL)	Codes 20002-20003	
05912504190	PreciControl HbA1c path (4 × 1 mL)	Codes 20012-20013	
08463107190	A1CD (Hemolyzing Reagent) (50 mL)	System-ID 2069 001	
08463093190	SCCS (Special Cell Cleaning Solution) (50 mL)	System-ID 2905 001	
11488457122	Hemolyzing Reagent for Tina-quant HbA1c (1000 mL)	For Hemolysate Application only	

English

System information

Whole Blood Application - Standardized according to IFCC transferable to DCCT/NGSP

HBW3:	ACN 20660	Hemoglobin (Hb)
A1W3:	ACN 20661	Hemoglobin A1c (HbA1c)
RWD3:	ACN 20662	Ratio % HbA1c (acc. to DCCT/NGSP)
RIW3:	ACN 20667	Ratio mmol/mol HbA1c (acc. to IFCC)
A1CD:	ACN 20690	Hemolyzing reagent

Hemolysate Application - Standardized according to IFCC transferable to DCCT/NGSP

HBH3:	ACN 20663	Hemoglobin (Hb)
A1H3:	ACN 20664	Hemoglobin A1c (HbA1c)
RHD3:	ACN 20665	Ratio % HbA1c (acc. to DCCT/NGSP)
RIH3:	ACN 20666	Ratio mmol/mol HbA1c (acc. to IFCC)
A1CD:	ACN 20690	Hemolyzing reagent

Intended use

In vitro test for the quantitative determination of mmol/mol hemoglobin A1c (IFCC) and % hemoglobin A1c (DCCT/NGSP) in whole blood or hemolysate on **cobas c** systems. HbA1c determinations are useful for monitoring of long-term blood glucose control in individuals with diabetes mellitus. Moreover, this test is to be used as an aid in diagnosis of diabetes and identifying patients who may be at risk for developing diabetes.

Summary

Hemoglobin A1c measurements performed with this assay in whole blood or hemolysate, are useful for monitoring of long-term blood glucose control in individuals with diabetes mellitus. Moreover, this test is to be used as an aid in diagnosis of diabetes and identifying patients who may be at risk for developing diabetes.

Hemoglobin (Hb) is the red-pigmented protein located in the erythrocytes, whose primary function is the transport of oxygen and carbon dioxide in blood. Hb is a globular protein composed of four globin subunits, each containing a heme moiety able to bind one oxygen molecule. Therefore, each Hb molecule can bind up to four oxygen molecules.[1] Hb consists of a variety of subfractions and derivatives, including glycated hemoglobins, formed by the attachment of various sugars to the Hb molecule. The set of glycated hemoglobins includes HbA1 and other non-enzymatically formed hemoglobin-glucose adducts; HbA1 is made up of HbA1a, HbA1b, and HbA1c. HbA1c is the major fraction of glycohemoglobin. It is formed in 2 steps by the non-enzymatic reaction of glucose with the N-terminal amino group of the β-chain of normal adult Hb (HbA). The first step is reversible and yields labile HbA1c. This is rearranged to form stable HbA1c in a second reaction step.[2]

In the erythrocytes, the relative amount of HbA converted to stable HbA1c increases with the average concentration of glucose in the blood. The conversion to stable HbA1c is limited by the erythrocyte's life span of approximately 100 to 120 days. As a result, HbA1c reflects the average blood glucose level during the preceding 2 to 3 months. HbA1c is thus suitable to monitor long-term blood glucose control in individuals with diabetes mellitus.[3,4] Glucose levels closer to the time of the assay have a greater influence on the HbA1c level, since the plasma glucose in the preceding month determines 50 % of the HbA1c concentration, whereas days 60 to 120 determines only 25 %. HbA1c is relatively unaffected by recent acute fluctuations in glucose levels.[2]

The approximate relationship between HbA1c and mean blood glucose values was analyzed in several studies.[5,6,7] The following correlations have been described:

According to IFCC standardization[8]

- Estimated average glucose [mmol/L] = 0.146 x HbA1c (mmol/mol) + 0.834

or

- Estimated average glucose [mg/dL] = 2.64 x HbA1c (mmol/mol) + 15.03

According to DCCT/NGSP standardization[9]

- Estimated average glucose [mmol/L] = 1.59 x HbA1c (%) - 2.59

or

- Estimated average glucose [mg/dL] = 28.7 x HbA1c (%) - 46.7

With these reference systems, HbA1c results are reported globally in IFCC units (mmol/mol) and derived NGSP units (percent of total hemoglobin).

Fasting plasma glucose, two-hour plasma glucose during a 75 g oral glucose tolerance test (OGTT), or HbA1c may be used for diagnostic testing of diabetes mellitus. HbA1c testing every 2 to 6 months is recommended for monitoring of long-term glycemic control. In certain clinical situations, such as gestational diabetes, or after a major change in therapy, it may be useful to measure HbA1c more frequently than usual (e.g., monthly). Presence of impaired fasting glucose and/or impaired glucose tolerance and/or HbA1c levels slightly above normal reference ranges, define an increased risk for diabetes and cardiovascular disease (CVD). The risk of diabetic complications, such as diabetic nephropathy and retinopathy, increases with poor metabolic control. In accordance with its function as an indicator for the mean blood glucose level, HbA1c predicts the development of diabetic complications in diabetes patients.[10,11,12,13,14,15,16,17,18,19]

Test principle[20,21,22]

This method uses TTAB* as the detergent in the hemolyzing reagent to eliminate interference from leukocytes (TTAB does not lyse leukocytes). Sample pretreatment to remove labile HbA1c is not necessary.

All hemoglobin variants which are glycated at the β-chain N-terminus and which have antibody-recognizable regions identical to that of HbA1c are determined by this assay. Consequently, the metabolic state of patients having uremia or the most frequent hemoglobinopathies (HbAS, HbAC, HbAE, HbAD) can be determined using this assay.[23,24,25]

*Tetradecyltrimethylammonium bromide

Hemoglobin A1c

The HbA1c determination is based on the turbidimetric inhibition immunoassay (TINIA) for hemolyzed whole blood.

- Sample and addition of R1 (buffer/antibody):

  Glycohemoglobin (HbA1c) in the sample reacts with anti-HbA1c antibody to form soluble antigen-antibody complexes. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, formation of insoluble complexes does not take place.

- Addition of R3 (buffer/polyhapten) and start of reaction:

  The polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be determined turbidimetrically.

Hemoglobin

Liberated hemoglobin in the hemolyzed sample is converted to a derivative having a characteristic absorption spectrum which is measured bichromatically during the preincubation phase (sample + R1) of the above immunological reaction. A separate Hb reagent is consequently not necessary.

The final result is expressed as mmol/mol HbA1c or % HbA1c and is calculated from the HbA1c/Hb ratio as follows:

Protocol 1 (mmol/mol HbA1c acc. to IFCC):

HbA1c (mmol/mol) = (HbA1c/Hb) × 1000

Protocol 2 (% HbA1c acc. to DCCT/NGSP):

HbA1c (%) = (HbA1c/Hb) × 91.5 + 2.15

Reagents - working solutions

R1 Antibody Reagent

MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c antibody (ovine serum): ≥ 0.5 mg/mL; detergents; stabilizers; preservative

R3 Polyhapten Reagent

MES buffer: 0.025 mol/L; TRIS buffer: 0.015 mol/L, pH 6.2; HbA1c polyhapten: ≥ 8 µg/mL; detergents; stabilizers; 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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing mist or vapours.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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:	4 weeks

The reagents cannot be frozen. If freezing of a cassette is suspected a control measurement with this cassette is recommended.

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. Anticoagulated venous or capillary blood or hemolysate.

The only acceptable anticoagulants are Li-heparin, K_2-EDTA, K_3-EDTA, Fluoride/Na_2-EDTA, Na-Heparin and Fluoride/potassium oxalate.

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.

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

Stability:	3 days at 15-25 °C
	7 days at 2-8 °C
	6 months at (–15)-(–25) °C

Freeze only once. Mix specimen thoroughly after thawing.

Hemolysate preparation for Hemolysate Application

Manual hemolysate preparation:

1. Allow blood specimen and Hemolyzing Reagent for Tina-quant HbA1c (Cat. No. 11488457122) to equilibrate at room temperature before use.
2. Moderately mix the sample immediately prior to pipetting, to ensure homogeneous mixture of erythrocytes. Take care to avoid the formation of foam.
3. Dilute the sample with Hemolyzing Reagent for Tina-quant HbA1c in the ratio 1:101 (1+100) using one of the following pipetting schemes.

   Pipette into tubes:

   Hemolyzing Reagent for Tina-quant HbA1c: **500 µL**
   Specimen (patient or control): **5 µL**
   or
   Hemolyzing Reagent for Tina-quant HbA1c: **1000 µL**
   Specimen (patient or control): **10 µL**
   or
   Hemolyzing Reagent for Tina-quant HbA1c: **2000 µL**
   Specimen (patient or control): **20 µL**
4. Mix using a vibration mixer or by gentle swirling.
5. The hemolysate can be used after the solution has changed color from red to brownish-green (approximately 1-2 min).

Stability of the hemolysate:	4 hours at 15-25 °C
	24 hours at 2-8 °C
	6 months at (–15)-(–25) °C

Freeze only once. Mix specimen thoroughly after thawing.

Materials provided

See “Reagents – working solutions” section for reagents.

Materials required (but not provided)

See “Order information” section

General laboratory equipment

A1CX3

cobas®

Tina-quant Hemoglobin A1cDx Gen.3

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.

Whole Blood application for Hb (HBW3) and HbA1c (A1W3)

Test definition Hb (HBW3)

Reporting time	10 min
Wavelength (sub/main)	660/376 nm

Reagent pipetting		Diluent (H_2O)
R1	76 µL	-
R3	15 µL	

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	3.2 µL	1.3 µL	130 µL
Decreased	3.2 µL	1.3 µL	130 µL
Increased	3.2 µL	1.3 µL	130 µL

Test definition HbA1c (A1W3)

Reporting time	10 min
Wavelength (sub/main)	660/340 nm

Reagent pipetting		Diluent (H_2O)
R1	76 µL	-
R3	15 µL	

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	3.2 µL	1.3 µL	130 µL
Decreased	3.2 µL	1.3 µL	130 µL
Increased	3.2 µL	1.3 µL	130 µL

Ratio definition for mmol/mol HbA1c and % HbA1c calculation

Protocol 1 (mmol/mol HbA1c acc. to IFCC):

Abbreviated ratio name	**RWI3 (20667)**
Equation	(A1W3/HBW3) × 1000
Unit	mmol/mol

Protocol 2 (% HbA1c acc. to DCCT/NGSP):

Abbreviated ratio name	**RWD3 (20662)**
Equation	(A1W3/HBW3) × 91.5 + 2.15
Unit	%

The protocols are already implemented in the application (ACNs 20667 and 20662). It is recommended to report % HbA1c values (DCCT/NGSP) to one decimal place and mmol/mol HbA1c values (IFCC) without decimal places.

Hemolysate application for Hb (HBH3) and HbA1c (A1H3)

Test definition Hb (HBH3)

Reporting time	10 min
Wavelength (sub/main)	660/376 nm

Reagent pipetting		Diluent (H_2O)
R1	76 µL	-
R3	15 µL	

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	3.2 µL	-	-
Decreased	3.2 µL	-	-
Increased	3.2 µL	-	-

Test definition HbA1c (A1H3)

Reporting time	10 min
Wavelength (sub/main)	660/340 nm

Reagent pipetting		Diluent (H_2O)
R1	76 µL	-
R3	15 µL	

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (Hemolyzing reagent)*
Normal	3.2 µL	-	-
Decreased	3.2 µL	-	-
Increased	3.2 µL	-	-

Ratio definition for HbA1c (mmol/mol (IFCC) or % (DCCT/NGSP)) calculation

Protocol 1 (mmol/mol HbA1c acc. to IFCC):

Abbreviated ratio name	**RHI3 (20666)**
Equation	(A1H3/HBH3) × 1000
Unit	mmol/mol

Protocol 2 (% HbA1c acc. to DCCT/NGSP):

Abbreviated ratio name	**RHD3 (20665)**
Equation	(A1H3/HBH3) × 91.5 + 2.15
Unit	%

The protocols are already implemented in the application (ACNs 20666 and 20665). It is recommended to report % HbA1c values (DCCT/NGSP) to one decimal place and mmol/mol HbA1c values (IFCC) without decimal places.

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

Calibration for Whole Blood and Hemolysate Application

Hb

Calibrators	S1-S2: C.f.a.s. HbA1c
Calibration mode	Linear

HbA1c

Calibrators	S1-S6: C.f.a.s. HbA1c
Calibration mode	Non-linear
Calibration frequency	Hb: 2-point calibration is recommended HbA1c: full calibration is recommended ▪ every 29 days during shelf life ▪ after reagent lot change ▪ as required following quality control procedures

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

Always calibrate both assays (Hb and HbA1c) in parallel.

Traceability: This method has been standardized against the approved IFCC reference method for the measurement of HbA1c in human blood[26,27] and can be transferred to results traceable to DCCT/NGSP by calculation.

Note for Whole Blood and Hemolysate Application

For these applications C.f.a.s. HbA1c calibrator values are reagent lot matched. For each application and each combination of C.f.a.s. HbA1c calibrator lot and Tina-quant Hemoglobin A1cDx Gen.3 reagent lot the exact calibrator values are given in the respective electronically available value sheet. The lot-specific calibrator values are automatically linked to the correct reagent lot via the software of the analyzer.

The **cobas c** pack A1CD (Hemolyzing Reagent, 50 mL), Cat. No. 08463107190, needs to be available on the analyzer otherwise the calibration cannot be performed.

Quality control for Whole Blood and Hemolysate Application

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 4 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 for Whole Blood and Hemolysate Application

Hb, HbA1c

cobas c systems automatically calculate the analyte concentration of each sample in the unit mmol/L (g/dL).

Conversion factor: mmol/L × 1.61 = g/dL

HbA1c ratio calculation:

For calculation of the mmol/mol HbA1c value (IFCC) and the percent HbA1c value (DCCT/NGSP), refer to the **Test principle** and **Ratio definition for mmol/mol HbA1c and % HbA1c calculation** sections in this method sheet.

Limitations - interference[23,24,28,29,30,31,32,33,34,35]

1. For diagnostic purposes, mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) should be used in conjunction with information from other diagnostic procedures and clinical evaluations.
2. The test is designed only for accurate and precise measurement of mmol/mol HbA1c (IFCC) and % HbA1c (DCCT/NGSP). The individual results for total Hb and HbA1c concentration should not be reported.
3. As a matter of principle, care must be taken when interpreting any HbA1c result from patients with Hb variants. Abnormal hemoglobins might affect the half life of the red cells or the in vivo glycation rates. In these cases even analytically correct results do not reflect the same level of glycemic control that would be expected in patients with normal hemoglobin.[33] Whenever it is suspected that the presence of an Hb variant (e.g. HbSS, HbCC or HbSC) affects the correlation between the HbA1c value and glycemic control, HbA1c must not be used for the diagnosis of diabetes mellitus.
4. Any cause of shortened erythrocyte survival or decrease in mean erythrocyte age will reduce exposure of erythrocytes to glucose with a consequent decrease in mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP), even though the time-averaged blood glucose level may be elevated. Causes of shortened erythrocyte lifetime might be hemolytic anemia or other hemolytic diseases, homozygous sickle cell trait, pregnancy, recent significant or chronic blood loss, etc. Similarly, recent blood transfusions can alter the mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP). Caution should be used when interpreting the HbA1c results from patients with these conditions. HbA1c must not be used for the diagnosis of diabetes mellitus in the presence of such conditions.
5. Glycated HbF is not detected by the assay as it does not contain the glycated β-chain that characterizes HbA1c. However, HbF is measured in the total Hb assay and as a consequence, specimens containing high amounts of HbF (> 7 %) may result in lower than expected mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP).[24,35]
6. mmol/mol HbA1c values (IFCC) and % HbA1c values (DCCT/NGSP) are not suitable for the diagnosis of gestational diabetes.[36]
7. In very rare cases of rapidly evolving type 1 diabetes the increase of the HbA1c values might be delayed compared to the acute increase in glucose concentrations. In these conditions diabetes mellitus must be diagnosed based on plasma glucose concentrations and/or the typical clinical symptoms.[36]

Criterion: Recovery within ± 7 % of initial value.

Icterus:[32] 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).

Lipemia (Intralipid):[32] No significant interference up to an Intralipid concentration of 600 mg/dL. There is poor correlation between triglycerides concentration and turbidity.

Glycemia: No significant interference from glucose up to a concentration of 55.5 mmol/L (1000 mg/dL). A fasting sample is not required.

Rheumatoid factors: No significant interference from rheumatoid factors up to a concentration of 750 IU/mL.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[37,38]

Other: No cross reactions with HbA0, HbA1a, HbA1b, acetylated hemoglobin, carbamylated hemoglobin, glycated albumin and labile HbA1c were found for the anti-HbA1c antibodies used in this kit.

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

A special wash with the Special Cell Cleaning Solution is performed automatically after the fifth usage of each cuvette. For this purpose the **cobas c** pack SCCS (Special Cell Cleaning Solution, 50 mL), Cat. No. 08463093190 needs to be available on the analyzer otherwise the washing cannot be performed.

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

Hemoglobin: 2.48-24.8 mmol/L

HbA1c: 0.186-1.61 mmol/L

This corresponds to a measuring range of 23-196 mmol/mol HbA1c (IFCC) and 4.2-20.1 % HbA1c (DCCT/NGSP) at a typical hemoglobin concentration of 8.2 mmol/L.

In rare cases of ">Test" flags which might occur with the use of the whole blood application, remix the whole blood sample and repeat the analysis with the same settings.

It is recommended to switch the auto rerun function off.

Lower limits of measurement

Limit of Blank and Limit of Detection

Hemoglobin:	
Limit of Blank	= 0.31 mmol/L
Limit of Detection	= 0.62 mmol/L
HbA1c:	
Limit of Blank	= 0.12 mmol/L
Limit of Detection	= 0.18 mmol/L

The Limit of Blank and Limit of Detection 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 sample concentration which leads with a probability of 95 % to a measurement result above the Limit of Blank.

Expected values

Protocol 1 (mmol/mol HbA1c acc. to IFCC): 29-42 mmol/mol HbA1c[39]

Protocol 2 (% HbA1c acc. to DCCT/NGSP): 4.8-5.9 % HbA1c[39]

This reference range was obtained by measuring 482 well-characterized healthy individuals without diabetes mellitus. HbA1c levels higher than the upper end of this reference range are an indication of hyperglycemia during the preceding 2 to 3 months or longer. According to the recommendations of the American Diabetes Association values above 48 mmol/mol HbA1c (IFCC) or 6.5 % HbA1c (DCCT/NGSP) are suitable for the diagnosis of diabetes mellitus.[36,40] Patients with HbA1c values in the range of 39-46 mmol/mol HbA1c (IFCC) or 5.7-6.4 % HbA1c (DCCT/NGSP) may be at risk of developing diabetes.[36,40]

HbA1c levels may reach 195 mmol/mol (IFCC) or 20 % (DCCT/NGSP) or higher in poorly controlled diabetes. Therapeutic action is suggested at levels above 64 mmol/mol HbA1c (IFCC) or 8 % HbA1c (DCCT/NGSP). Diabetes patients with HbA1c levels below 53 mmol/mol (IFCC) or 7 % (DCCT/NGSP) meet the goal of the American Diabetes Association.[31,30]

HbA1c levels below the established reference range may indicate recent episodes of hypoglycemia, the presence of Hb variants, or shortened lifetime of erythrocytes.

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 (data based on DCCT/NGSP values):

Whole Blood Application:

Repeatability	*Mean % HbA1c*	*SD % HbA1c*	*CV %*
PreciControl HbA1c norm	5.54	0.04	0.7
PreciControl HbA1c path	11.3	0.06	0.5
Human sample 1	4.90	0.04	0.9
Human sample 2	6.53	0.03	0.4
Human sample 3	7.29	0.03	0.5
Human sample 4	8.33	0.05	0.5
Human sample 5	12.5	0.06	0.5

Intermediate precision	*Mean % HbA1c*	*SD % HbA1c*	*CV %*
PreciControl HbA1c norm	5.54	0.06	1.1
PreciControl HbA1c path	11.3	0.09	0.8
Human sample 1	4.89	0.06	1.3
Human sample 2	6.67	0.05	0.7
Human sample 3	7.46	0.05	0.7
Human sample 4	8.33	0.08	0.9
Human sample 5	12.8	0.09	0.7

Hemolysate Application:

Repeatability	*Mean % HbA1c*	*SD % HbA1c*	*CV %*
PreciControl HbA1c norm	5.57	0.03	0.5
PreciControl HbA1c path	11.1	0.07	0.6
Human sample 1	4.97	0.03	0.5
Human sample 2	6.57	0.03	0.5
Human sample 3	7.26	0.04	0.5
Human sample 4	8.24	0.04	0.5
Human sample 5	12.4	0.06	0.5

Intermediate precision	*Mean % HbA1c*	*SD % HbA1c*	*CV %*
PreciControl HbA1c norm	5.57	0.10	1.8
PreciControl HbA1c path	11.1	0.14	1.3
Human sample 1	4.98	0.11	2.2
Human sample 2	6.68	0.09	1.3
Human sample 3	7.39	0.09	1.2
Human sample 4	8.41	0.09	1.1
Human sample 5	12.8	0.17	1.3

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

Method comparison

Evaluation of method comparison data is according to former NGSP certification criteria. The mean difference between the two methods and the 95 % confidence intervals of the differences in the range from 4-10 % (DCCT/NGSP) are given. 95 % of the differences between the values obtained for individual samples with both methods fall within the range defined by the lower and upper 95 % confidence intervals of the differences.

Whole Blood Application:

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 503 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application on a **cobas c** 501 analyzer (x).

Sample size (n) = 151

Mean difference: -0.050 % HbA1c

Lower 95 % confidence interval of differences: -0.274 % HbA1c

Upper 95 % confidence interval of differences: 0.174 % HbA1c

The sample concentrations were between 4.55 % and 9.97 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 503 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application (y) were compared to those determined using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application on a **cobas c** 513 analyzer (x).

Sample size (n) = 159

Mean difference: 0.052 % HbA1c

Lower 95 % confidence interval of differences: -0.190 % HbA1c

Upper 95 % confidence interval of differences: 0.294 % HbA1c

The sample concentrations were between 4.77 % and 9.97 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 303 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.3 reagent with the whole blood application on a **cobas c** 501 analyzer (x).

Sample size (n) = 145

Mean difference: -0.023 % HbA1c

Lower 95 % confidence interval of differences: -0.371 % HbA1c

Upper 95 % confidence interval of differences: 0.324 % HbA1c

The sample concentrations were between 4.83 % and 9.93 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 303 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application (y) were compared to those determined using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the whole blood application on a **cobas c** 503 analyzer (x).

Sample size (n) = 147

Mean difference: 0.012 % HbA1c

Lower 95 % confidence interval of differences: -0.162 % HbA1c

Upper 95 % confidence interval of differences: 0.185 % HbA1c

The sample concentrations were between 4.67 % and 9.97 % HbA1c (DCCT/NGSP values).

Hemolysate Application:

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 503 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.3 reagent with the hemolysate application on a **cobas c** 501 analyzer (x).

Sample size (n) = 157

Mean difference: 0.037 % HbA1c

Lower 95 % confidence interval of differences: -0.311 % HbA1c

Upper 95 % confidence interval of differences: 0.385 % HbA1c

The sample concentrations were between 4.38 % and 9.94 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 503 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application (y) were compared to those determined using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application on a **cobas c** 513 analyzer (x).

Sample size (n) = 160

Mean difference: 0.083 % HbA1c

Lower 95 % confidence interval of differences: -0.038 % HbA1c

Upper 95 % confidence interval of differences: 0.203 % HbA1c

The sample concentrations were between 4.72 % and 9.98 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 303 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application (y) were compared to those determined using the Tina-quant Hemoglobin A1c Gen.3 reagent with the hemolysate application on a **cobas c** 501 analyzer (x).

Sample size (n) = 148

Mean difference: 0.161 % HbA1c

Lower 95 % confidence interval of differences: -0.116 % HbA1c

Upper 95 % confidence interval of differences: 0.438 % HbA1c

The sample concentrations were between 4.45 % and 9.87 % HbA1c (DCCT/NGSP values).

% HbA1c (DCCT/NGSP) values for human blood samples obtained on a **cobas c** 303 analyzer using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application (y) were compared to those determined using the Tina-quant Hemoglobin A1cDx Gen.3 reagent with the hemolysate application on a **cobas c** 503 analyzer (x).

Sample size (n) = 148

Mean difference: 0.178 % HbA1c

Lower 95 % confidence interval of differences: -0.038 % HbA1c

Upper 95 % confidence interval of differences: 0.393 % HbA1c

The sample concentrations were between 4.77 % and 9.72 % HbA1c (DCCT/NGSP values).

Analytical specificity

Hb derivatives	Labile HbA1c (pre-HbA1c), acetylated Hb, and carbamylated Hb do not affect the assay results.
Hb variants	Specimens containing high amounts of HbF (> 7 %) may yield lower than expected HbA1c results.

Please note

According to the consensus statement of the American Diabetes Association (ADA), the European Association for the Study of Diabetes (EASD), the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) and International Diabetes Federation (IDF) HbA1c results should be reported in parallel, both in mmol/mol (IFCC) and % (DCCT/NGSP) values.[41] In addition an HbA1c derived estimated average glucose concentration can be reported which can be calculated according to the equations given in the Summary section of this method sheet. Former % HbA1c (IFCC) values must not be used due to the risk of mix up / misinterpretation with the % HbA1c (DCCT/NGSP) values.

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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.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

ACP2

cobas®

Acid Phosphatase Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08101442190	08101442500	Acid Phosphatase Gen.2 4 × 140 tests ACP2/140 tests NPP2 or 4 × 280 tests ACP2-T	System-ID 2005 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 × 3 mL)	Code 20401	
05117003190	PreciControl ClinChem Multi 1 (20 × 5 mL)	Code 20391	
05947626190	PreciControl ClinChem Multi 1 (4 × 5 mL)	Code 20391	
05117216190	PreciControl ClinChem Multi 2 (20 × 5 mL)	Code 20392	
05947774190	PreciControl ClinChem Multi 2 (4 × 5 mL)	Code 20392	

English

System information

ACP2: ACN 20050 (Total acid phosphatase)

NPP2: ACN 20051 (Non-prostatic acid phosphatase)

ACP2-T: ACN 20052 (Total acid phosphatase only)

Intended use

In vitro test for the quantitative determination of acid phosphatase in human serum on **cobas c** systems.

Summary

Measurement of the activity of acid phosphatase (ACP) in serum with this assay, is used to aid in the diagnosis and management of prostate cancer.

Acid phosphatases (ACPs) are a group of enzymes with optimal activity at a pH below 7.0 and can be differentiated according to their immunological properties, tissue distribution and subcellular localisation. To date, at least 5 different ACPs have been reported in human tissues. Lysosomal acid phosphatase is stored in the lysosomes of all body cells, while the highest concentrations of extralysosomal ACP activity occur in the prostate, bone (osteoclasts), spleen, platelets and erythrocytes. ACP activity in blood serum is usually distinguished into tartrate-resistant and tartrate-refractory.[1,2,3] A specific form of ACP sensitive to tartrate inhibition is the secretory prostatic acid phosphatase (PAP), which is normally secreted by prostate tissue. In prostate cancer, circulating levels of PAP are increased.[3,4] PAP has therefore extensively been used as a serum marker for prostate cancer until the introduction of the current gold standard prostate-specific antigen (PSA).[5] Serum PAP levels are particularly increased in individuals with metastatic prostate cancer and correlate with tumor stage. It has been suggested that PAP has clinical application in patient management, in predicting disease recurrence or monitoring the effects of treatment.[4,6] However, PSA is indicated as the preferred test for screening, monitoring and predicting prostate cancer outcomes. Presence or absence of malignant disease can only be confirmed with a prostate biopsy. A multi-parametric magnetic resonance imaging (mpMRI) is recommended before prostate biopsy to facilitate the targeting of suspected lesions.[7,8,9,10]

Activity of total acid phosphatase increases in pathologic conditions of increased osteolysis and bone remodeling, in case of bone metastasis and other types of malignancies, in Gaucher's and Niemann-Pick diseases. Prostatic and total acid phosphatase levels increase after prostate surgery, biopsy, manipulation or catheterization, in the presence of benign prostate hypertrophy, prostatitis and prostate infarction.[1,2,11,12,13] Increased PAP levels should not be considered an absolut test for malignancy and PAP results should always be interpreted in combination with the patient's medical history and further diagnostic evaluations.

With this assay, PAP is detected with an indirect method by subtraction between ACP and non-prostatic acid phosphatase (NPP). The assay used here is a modification of the method described by Hillmann. Addition of 1,5-pentanediol increases the activity of prostatic acid phosphatase.[14]

Test principle[14]

Colorimetric test
The 1-naphthol released during the enzymatic hydrolysis of 1-naphthyl phosphate is converted to an azo dye by coupling with diazotized fast red TR*. The tartrate is used as a specific inhibitor for prostatic acid phosphatase.

* Fast red TR = 2-amino-5-chlorotoluene

$$\text{1-naphthyl phosphate} + H_2O \xrightarrow[\text{phosphatase}]{\text{acid}} \text{1-naphthol} + \text{phosphate}$$

$$\text{1-naphthol} + \text{fast red TR*} \longrightarrow \text{azo dye}$$

Reagents - working solutions

R1	Bottle R1: Citrate buffer: 150 mmol/L, pH 4.8; 1,5-pentanediol: 220 mmol/L; detergent: 3.3 mL/L Bottle R1a: 1-Naphthyl phosphate: 12.1 mmol/L; fast red TR salt: 1.2 mmol/L Bottle R1b: Sodium tartrate: 100 mmol/L (additionally for non-prostatic acid phosphatase determination)
CH_3COOH	Bottle 2: Acetic acid: 0.8 mol/L (sample stabilizer)

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H373	May cause damage to organs through prolonged or repeated exposure.

Prevention:

P260	Do not breathe mist or vapours.

Response:

P314	Get medical advice/attention if you feel unwell.

ACP2

Acid Phosphatase Gen.2

cobas®

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

Reagent handling

Total acid phosphatase

Connect 1 bottle **R1** to 1 bottle **R1a** using the enclosed adapter and dissolve the substrate/chromogen mixture completely in the buffer. Fill the mixture into **cobas c** pack position B.

For ACP2-T prepare the total acid phosphatase reagent in duplicate as described above and fill 1 bottle of the mixture into **cobas c** pack position B and the other into **cobas c** pack position C so that both **cobas c** pack positions contain the same mixture.

Non-prostatic acid phosphatase

Connect 1 bottle **R1** to 1 bottle **R1a** using the enclosed adapter and dissolve the substrate/chromogen mixture completely in the buffer. Add a reagent tablet from bottle **R1b** and dissolve by gently swirling. Fill the mixture into **cobas c** pack position C.

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:	5 days

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

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.

Separate the serum from the clot or cells promptly.

Perform determinations on the samples immediately. Samples which cannot be examined immediately should be stabilized as follows: Add 1 drop (30 µL) of solution from bottle **2** to 1.0 mL of serum and mix.

Centrifuge samples containing precipitates before performing the assay.

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

Stability:[15]	8 days at 15-25 °C
	8 days at 2-8 °C
	4 months at -20 °C (±5 °C)

Freeze only once.

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

Test definition

Total acid phosphatase and non-prostatic acid phosphatase

Reporting time	10 min		
Wavelength (sub/main)	700/415 nm		
Reagent pipetting		Diluent (H_2O)	
R1	77 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	6.4 µL	–	–
Decreased	2.1 µL	–	–
Increased	6.4 µL	–	–

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

Calibration

Total acid phosphatase:

Calibrators	S1: H_2O
	S2: C.f.a.s.

Non-prostatic acid phosphatase:

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	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 Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

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 5 days. 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 activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

A) Total acid phosphatase: See instrument printout.

B) Prostatic acid phosphatase:
The prostatic acid phosphatase can be determined manually by calculating the difference between the total acid phosphatase (ACP2) and the non-prostatic acid phosphatase (NPP2).

$$\text{Activity}_{\text{Prostatic acid phosphatase}} = \text{Activity}_{\text{Total acid phosphatase}} - \text{Activity}_{\text{Non-prostatic acid phosphatase}}$$

ACP2

Acid Phosphatase Gen.2

cobas®

Limitations - interference

Criterion: Recovery within ±10 % of initial value at a total acid phosphatase activity of 7 U/L or at a non-prostatic acid phosphatase activity of 4 U/L.

Icterus:[16] No significant interference up to an I index of 1 for conjugated and unconjugated bilirubin (approximate conjugated and unconjugated bilirubin concentration: 17 µmol/L or 1 mg/dL).

Hemolysis:[16] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):[16] No significant interference up to an L index of 200. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels. Exception: Methyldopa, cefoxitine and doxycycline cause artificially high non-prostatic acid phosphatase results.[17,18]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[19]

The addition of stabilizer to the sample interferes with the determination of other parameters.

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

Total acid phosphatase and non-prostatic acid phosphatase

0.5-200 U/L (0.01-3.34 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.5 U/L (0.01 µkat/L)
Limit of Detection	= 0.5 U/L (0.01 µkat/L)
Limit of Quantitation	= 0.5 U/L (0.01 µkat/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 activity 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 activity samples.

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

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

Expected values

U/L

Total acid phosphatase (37 °C)[20]

Men	< 6.6 U/L	Women	< 6.5 U/L

Prostatic acid phosphatase (37 °C)[20]

Men	< 3.5 U/L

µkat/L*

Total acid phosphatase (37 °C)[20]

Men	< 0.110 µkat/L	Women	< 0.108 µkat/L

Prostatic acid phosphatase (37 °C)[20]

Men	< 0.058 µkat/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.

Total acid phosphatase

Repeatability	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	26.2	0.0866	0.3
PCCC2[b)]	48.4	0.182	0.4
Human serum 1	1.39	0.0301	2.2
Human serum 2	6.61	0.0495	0.7
Human serum 3	38.4	0.135	0.4
Human serum 4	101	0.477	0.5
Human serum 5	181	0.748	0.4

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	26.3	0.287	1.1
PCCC2[b)]	48.5	0.517	1.1
Human serum 1	1.39	0.0477	3.4
Human serum 2	6.73	0.0711	1.1
Human serum 3	38.4	0.186	0.5
Human serum 4	101	0.571	0.6
Human serum 5	182	1.03	0.6

Non-prostatic acid phosphatase

Repeatability	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	13.8	0.0964	0.7
PCCC2[b)]	29.0	0.174	0.6
Human serum 1	1.30	0.0533	4.1
Human serum 2	3.61	0.0455	1.3
Human serum 3	51.2	0.186	0.4
Human serum 4	99.4	0.393	0.4
Human serum 5	172	0.787	0.5

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*

ACP2

Acid Phosphatase Gen.2

PCCC1[a)]	14.0	0.190	1.4
PCCC2[b)]	29.0	0.359	1.2
Human serum 1	1.30	0.0672	5.2
Human serum 2	3.61	0.0796	2.2
Human serum 3	49.7	1.65	3.3
Human serum 4	97.2	3.24	3.3
Human serum 5	172	6.09	3.5

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

Acid phosphatase values for human serum 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).

Total acid phosphatase:

Sample size (n) = 62

Passing/Bablok[21]	Linear regression
y = 1.010x + 0.169 U/L	y = 1.007x + 0.362 U/L
τ = 0.966	r = 0.999

The sample activities were between 0.800 and 192 U/L.

Non-prostatic acid phosphatase:

Sample size (n) = 65

Passing/Bablok[21]	Linear regression
y = 1.007x + 0.0189 U/L	y = 1.018x - 0.0518 U/L
τ = 0.961	r = 1.000

The sample activities were between 0.700 and 196 U/L.

Acid phosphatase values for human serum 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).

Total acid phosphatase:

Sample size (n) = 76

Passing/Bablok[21]	Linear regression
y = 1.016x - 0.0375 U/L	y = 0.964x + 0.180 U/L
τ = 0.927	r = 0.999

The sample activities were between 0.600 and 199 U/L.

Non-prostatic acid phosphatase:

Sample size (n) = 65

Passing/Bablok[21]	Linear regression
y = 1.011x + 0.0644 U/L	y = 1.013x + 0.0408 U/L
τ = 0.948	r = 1.000

The sample activities were between 0.800 and 192 U/L.

Acid phosphatase values for human serum 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).

Total acid phosphatase:

Sample size (n) = 81

Passing/Bablok[21]	Linear regression
y = 0.995x + 0.120 U/L	y = 0.988x + 0.176 U/L
τ = 0.964	r = 1.000

The sample activities were between 0.856 and 195 U/L.

Non-prostatic acid phosphatase:

Sample size (n) = 75

Passing/Bablok[21]	Linear regression
y = 0.984x + 0.0846 U/L	y = 0.980x - 0.0572 U/L
τ = 0.790	r = 0.999

The sample activities were between 0.588 and 195 U/L.

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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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

ALB2

cobas®

Albumin Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056692190	Albumin Gen.2 (750 tests)	System-ID 2009 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
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
ALB2-G: ACN 20090

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

Summary[1,2]
Albumin is a carbohydrate-free protein, which constitutes 55-65 % of total plasma protein. It maintains plasma oncotic pressure, and is also involved in the transport and storage of a wide variety of ligands and is a source of endogenous amino acids. Albumin binds and solubilizes various compounds, e.g. bilirubin, calcium and long-chain fatty acids. Furthermore, albumin is capable of binding toxic heavy metal ions as well as numerous pharmaceuticals, which is the reason why lower albumin concentrations in blood have a significant effect on pharmacokinetics.

Hyperalbuminemia is of little diagnostic significance except in the case of dehydration. Hypoalbuminemia occurs during many illnesses and is caused by several factors: compromised synthesis due either to liver disease or as a consequence of reduced protein uptake; elevated catabolism due to tissue damage (severe burns) or inflammation; malabsorption of amino acids (Crohn's disease); proteinuria as a consequence of nephrotic syndrome; protein loss via the stool (neoplastic disease). In severe cases of hypoalbuminemia, the maximum albumin concentration of plasma is 2.5 g/dL (380 µmol/L). Due to the low osmotic pressure of the plasma, water permeates through blood capillaries into tissue (edema). The determination of albumin allows monitoring of a controlled patient dietary supplementation and serves also as an excellent test of liver function.

Test principle[3]
Colorimetric assay

At a pH value of 4.1, albumin displays a sufficiently cationic character to be able to bind with bromcresol green (BCG), an anionic dye, to form a blue-green complex.

$$\text{Albumin} + \text{BCG} \xrightarrow{\text{pH 4.1}} \text{albumin-BCG complex}$$

The color intensity of the blue-green color is directly proportional to the albumin concentration in the sample and is measured photometrically.

Reagents - working solutions

R1 Citrate buffer: 95 mmol/L, pH 4.1; preservatives, stabilizers

R3 Citrate buffer: 95 mmol/L, pH 4.1; bromcresol green: 0.66 mmol/L; preservatives, stabilizers

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 15-25 °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

Do not use fluoride 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:[4]	2.5 months at 20-25 °C
	5 months at 4-8 °C
	4 months at -20 °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)	505/570 nm	
Reagent pipetting		Diluent (H_2O)

ALB2

Albumin Gen.2

R1	80 µL	–	
R3	16 µL	24 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.6 µL	–	–
Decreased	1.6 µL	25 µL	50 µL
Increased	1.6 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: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - after 4 weeks on-board - 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 reference preparation of the IRMM (Institute for Reference Materials and Measurements) BCR470/CRM470 (RPPHS - Reference Preparation for Proteins in Human Serum).[5]

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, g/dL).

Conversion factors:	g/L x 15.2 = µmol/L
	g/L x 0.1 = g/dL

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at an albumin concentration of 35 g/L (532 µmol/L).

Icterus:[6] 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:[6] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[6] No significant interference up to an L index of 550. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[7,8]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[9]

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

Colorimetric methods used for the determination of Albumin may lead to falsely elevated test results in patients suffering from renal failure or insufficiency due to interference with other proteins. Immunoturbidimetric methods are less affected.

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

2-60 g/L (30.4-912 µmol/L)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 3.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 2 g/L (30.4 µmol/L)
Limit of Detection	= 2 g/L (30.4 µmol/L)
Limit of Quantitation	= 3 g/L (45.6 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 albumin samples.

Expected values

g/L

Reference range study[10]	
Adults	39.7-49.4 g/L
Consensus values[11]	
Adults	35-52 g/L
Reference intervals according to Tietz[12]	
Newborn	
0-4 days	28-44 g/L
Children	
4 days-14 years	38-54 g/L
14-18 years	32-45 g/L

µmol/L*

* calculated by unit conversion factor

Reference range study[10]	
Adults	603-751 µmol/L
Consensus values[11]	
Adults	532-790 µmol/L
Reference intervals according to Tietz[12]	
Newborn	
0-4 days	426-669 µmol/L

ALB2

Albumin Gen.2

Children	
4 days-14 years	578-821 µmol/L
14-18 years	486-684 µmol/L

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)]	33.9	0.270	0.8
PCCC2[b)]	47.2	0.223	0.5
Human serum 1	52.3	0.252	0.5
Human serum 2	16.0	0.245	1.5
Human serum 3	32.7	0.280	0.9
Human serum 4	45.6	0.253	0.6
Human serum 5	49.5	0.258	0.5

Intermediate precision	*Mean* *g/L*	*SD* *g/L*	*CV* *%*
PCCC1[a)]	33.9	0.865	2.6
PCCC2[b)]	48.9	0.878	1.8
Human serum 1	52.3	0.656	1.3
Human serum 2	16.0	1.00	6.2
Human serum 3	32.7	0.878	2.7
Human serum 4	45.6	0.767	1.7
Human serum 5	51.4	0.696	1.4

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

Albumin 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) = 142

Passing/Bablok[13]	Linear regression
y = 0.987x + 1.75 g/L	y = 0.999x + 1.26 g/L
τ = 0.851	r = 0.992

The sample concentrations were between 2.60 and 57.7 g/L.

Albumin 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) = 72

Passing/Bablok[13]	Linear regression
y = 1.004x + 0.719 g/L	y = 1.001x + 0.852 g/L
τ = 0.922	r = 0.998

The sample concentrations were between 2.84 and 57.2 g/L.

Albumin 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) = 75

Passing/Bablok[13]	Linear regression
y = 1.005x - 0.450 g/L	y = 1.003x - 0.376 g/L
τ = 0.971	r = 0.999

The sample concentrations were between 3.97 and 58.8 g/L.

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.

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13 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit
→	Volume for reconstitution

ALB2

Albumin Gen.2

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

ALP2

cobas®

Alkaline phosphatase acc. to IFCC Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056757190*	08056757500	Alkaline Phosphatase acc. to IFCC Gen.2 (1100 tests)	System-ID 2011 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08056757214*	08056757500	Alkaline Phosphatase acc. to IFCC Gen.2 (1100 tests)	System-ID 2011 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
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	

* Some kits shown may not be available in all countries.

English

System information

ALP2: ACN 20110

Intended use

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

Summary

Measurement of alkaline phosphatase with this assay in human serum and plasma is used to aid in the diagnosis and monitoring of liver diseases and bone diseases.

Alkaline phosphatases (EC 3.1.3.1) are membrane-bound ectoenzymes that catalyze the hydrolysis of monophosphates from ester linkage under alkaline conditions (pH 8 to 10).[1] Alkaline phosphatase isoforms are encoded by 4 different genes: the liver-bone-kidney (tissue-nonspecific) variant, the intestinal variant, the placental variant and the variant from the germ cells (placental-like).[1,2] Alkaline phosphatase activity is present in various tissues, but its concentration varies, and the highest concentrations are typically found in the liver and bone. Although the exact metabolic function of the enzyme is not yet understood, it appears that it is associated with lipid transport in the intestine, with the calcification process in bone, and with host defense through endotoxin dephosphorylation. Minimal amounts of intestinal alkaline phosphatase may also be present and are subjected to increase after a meal.[2]

Total serum alkaline phosphatase measurement is used extensively as a clinical indicator of liver and bone health.[1,2,3,4,5,6,7,8,9] Any form of biliary tree obstruction induces the synthesis of alkaline phosphatase by hepatocytes, therefore a rise in the alkaline phosphatase activity in serum occurs with all forms of cholestasis and particularly with obstructive jaundice.[2,3,4,5] It is also elevated in diseases of the skeletal system associated with increased osteoblastic activity, such as Paget's disease, hyperparathyroidism, rickets and osteomalacia, as well as with fractures and malignant tumors.[1,6,7,8,9,10] A physiologic rise in the alkaline phosphatase activity is sometimes seen in children and juveniles. It is caused by increased osteoblast activity following accelerated bone growth.[1,2,10]

Decreased total alkaline phosphatase activity is rarely found in human serum but can occur in hypophosphatasia, in multiple myeloma with osteolytic lesions, secondary to growth hormone deficiency or in hypoparathyroidism.[1,10]

The assay method was first described by King and Armstrong, modified by Ohmori, Bessey, Lowry and Brock and later improved by Hausamen et al.[11,12,13,14] In 2011 the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) Scientific Division, Committee on Reference Systems of Enzymes (C-RSE) recommended a reference procedure for the determination of alkaline phosphatase using an optimized substrate concentration and 2-amino-2-methyl-1-propanol as buffer plus the cations magnesium and zinc at 37 °C.[15] This assay follows the recommendations of the IFCC, but was optimized for performance and stability.

Test principle[15]

Colorimetric assay in accordance with a standardized method.
In the presence of magnesium and zinc ions, p-nitrophenyl phosphate is cleaved by phosphatases into phosphate and p-nitrophenol.

$$\text{p-nitrophenyl phosphate} + H_2O \xrightarrow{\text{ALP}} \text{phosphate} + \text{p-nitrophenol}$$

The p-nitrophenol released is directly proportional to the catalytic ALP activity. It is determined by measuring the increase in absorbance.

Reagents - working solutions

R1 2-amino-2-methyl-1-propanol: 1.724 mol/L, pH 10.44 (30 °C); magnesium acetate: 3.83 mmol/L; zinc sulfate: 0.766 mmol/L; N-(2-hydroxyethyl)-ethylenediamine triacetic acid: 3.83 mmol/L

R3 p-nitrophenyl phosphate: 132.8 mmol/L, pH 8.50 (25 °C); preservatives

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

Precautions and warnings

For in vitro diagnostic use for laboratory 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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H315	Causes skin irritation.
H319	Causes serious eye irritation.

Prevention:

P264	Wash skin thoroughly after handling.
P280	Wear protective gloves/ eye protection/ face protection.

ALP2

Alkaline phosphatase acc. to IFCC Gen.2

Response:

P302 + P352 IF ON SKIN: Wash with plenty of water.

P332 + P313 If skin irritation occurs: Get medical advice/attention.

P337 + P313 If eye irritation persists: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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:	8 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 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:[16]	7 days at 20-25 °C
	7 days at 4-8 °C
	2 months at -20 °C (± 5 °C)

Freeze only once.

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)	480/450 nm		
Reagent pipetting		Diluent (H_2O)	
R1	56 µL	19 µL	
R3	13 µL	16 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.1 µL	–	–
Decreased	2.1 µL	20 µL	80 µL
Increased	2.1 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: C.f.a.s.
Calibration mode	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 IFCC procedure (2011).[15]

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 8 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 activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L x 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 10 U/L of initial values of samples ≤ 100 U/L and within ± 10 % for samples > 100 U/L.

Icterus:[17] 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:[17] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):[17] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[18,19]

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[20]

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.

ALP2

Alkaline phosphatase acc. to IFCC Gen.2

Limits and ranges

Measuring range

5-1200 U/L (0.084-20.0 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 5 U/L (0.084 µkat/L)
Limit of Detection	= 5 U/L (0.084 µkat/L)
Limit of Quantitation	= 5 U/L (0.084 µkat/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 activity 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 activity samples.

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

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

Expected values

U/L

Adults[21]

Males (n = 221)	40-129 U/L
Females (n = 229)	35-104 U/L

Children[22]

Males

Age	0-14 days	83-248 U/L
	15 days -< 1 year	122-469 U/L
	1-< 10 years	142-335 U/L
	10-< 13 years	129-417 U/L
	13-< 15 years	116-468 U/L
	15-< 17 years	82-331 U/L
	17-< 19 years	55-149 U/L

Females

Age	0-14 days	83-248 U/L
	15 days -< 1 year	122-469 U/L
	1-< 10 years	142-335 U/L
	10-< 13 years	129-417 U/L
	13-< 15 years	57-254 U/L
	15-< 17 years	50-117 U/L
	17-< 19 years	45-87 U/L

(measured at 37 °C)

µkat/L*

Adults[21]

Males (n = 221)	0.67-2.15 µkat/L
Females (n = 229)	0.58-1.74 µkat/L

Children[22]

Males

Age	0-14 days	1.39-4.14 µkat/L
	15 days -< 1 year	2.04-7.83 µkat/L
	1-< 10 years	2.37-5.59 µkat/L
	10-< 13 years	2.15-6.96 µkat/L
	13-< 15 years	1.94-7.82 µkat/L
	15-< 17 years	1.37-5.53 µkat/L
	17-< 19 years	0.92-2.49 µkat/L

Females

Age	0-14 days	1.39-4.14 µkat/L
	15 days -< 1 year	2.04-7.83 µkat/L
	1-< 10 years	2.37-5.59 µkat/L
	10-< 13 years	2.15-6.96 µkat/L
	13-< 15 years	0.95-4.24 µkat/L
	15-< 17 years	0.84-1.95 µkat/L
	17-< 19 years	0.75-1.45 µkat/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* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	98.9	0.408	0.4
PCCC2[b)]	223	0.673	0.3
Human serum 1	10.2	0.319	3.1
Human serum 2	36.2	0.293	0.8
Human serum 3	144	0.645	0.4
Human serum 4	606	1.27	0.2
Human serum 5	1094	2.66	0.2

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	98.4	1.42	1.4
PCCC2[b)]	223	2.83	1.3
Human serum 1	9.27	1.08	11.6
Human serum 2	35.3	1.21	3.4
Human serum 3	144	1.63	1.1
Human serum 4	607	3.30	0.5
Human serum 5	1095	5.21	0.5

a) PreciControl ClinChem Multi 1

b) PreciControl ClinChem Multi 2

ALP2

Alkaline phosphatase acc. to IFCC Gen.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

Alkaline phosphatase 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) = 88

Passing/Bablok[23]	Linear regression
y = 0.987x - 1.24 U/L	y = 1.013x - 4.31 U/L
τ = 0.985	r = 1.000

The sample activities were between 15.0 and 1171 U/L.

Alkaline phosphatase 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) = 75

Passing/Bablok[23]	Linear regression
y = 0.985x - 0.691 U/L	y = 0.996x - 3.04 U/L
τ = 0.994	r = 1.000

The sample activities were between 15.8 and 1177 U/L.

Alkaline phosphatase 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) = 75

Passing/Bablok[23]	Linear regression
y = 1.010x + 0.171 U/L	y = 1.019x - 1.18 U/L
τ = 0.999	r = 1.000

The sample concentrations were between 7.10 and 1129 U/L.

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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.

ALP2

Alkaline phosphatase acc. to IFCC Gen.2

CE 0123

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606

ALTP2

cobas®

Alanine Aminotransferase acc. to IFCC II

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08104697190*	08104697500	Alanine Aminotransferase acc. to IFCC II (800 tests)	System-ID 2014 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08104697214*	08104697500	Alanine Aminotransferase acc. to IFCC II (800 tests)	System-ID 2014 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
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	

* Some kits shown may not be available in all countries.

English

System information

ALTP2: ACN 20140

Intended use

In vitro test for the quantitative determination of alanine aminotransferase (ALT) with pyridoxal phosphate activation in human serum and plasma on **cobas c** systems.

Summary

Alanine aminotransferase (ALT) measurements, performed with this device, in human serum and plasma are used as an aid in diagnosis of hepatocellular injury and in monitoring chronic liver injury.

The enzyme alanine aminotransferase (ALT) is present in highest concentrations in the liver, in the cytosol of the hepatocytes, although it is also found in the kidney, and, in much smaller quantities, in heart and skeletal muscle cells.[1] ALT catalyzes the transfer of amino groups from L-alanine to α-ketoglutarate, resulting in L-glutamate and pyruvate. This is a critical process of the tricarboxylic acid cycle, in which the coenzyme pyridoxal phosphate (also known as pyridoxal-5-phosphate or active vitamin B6) is required. When liver injury occurs, ALT is released from injured liver cells and causes a significant serum elevation.[1]

Measurement of ALT activity is therefore used for the diagnosis of hepatic diseases such as acute and chronic viral hepatitis, nonalcoholic fatty liver disease (NAFLD), alcohol-related liver disease, ischemic hepatopathy, autoimmune hepatitis, biliary injury, suspected malignant infiltration, cholestasis.[1] Serum elevations of ALT activity are rarely observed in conditions other than parenchymal liver disease.[2] In addition, ALT testing is recommended for monitoring chronic hepatitis status and progression.[3]

Although both serum aspartate aminotransferase (AST) and ALT become elevated whenever disease processes affect liver cell integrity, evidence suggests that ALT is a more specific marker of hepatic injury than AST. Moreover, elevations of ALT activity persist longer than elevations of AST activity.[1,4]

In patients with vitamin B6 deficiency (insufficient endogenous pyridoxal phosphate), serum aminotransferase activity may be decreased. The addition of pyridoxal phosphate to this assay causes an increase in aminotransferase activity (activation higher for AST than for ALT) and prevents falsely low aminotransferase test results in these samples.[2]

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[5]

ALT catalyzes the transfer of an amino group between L-alanine and 2-oxoglutarate to form pyruvate and L-glutamate. The pyruvate then reacts with NADH in the presence of lactate dehydrogenase (LDH) to form L-lactate and NAD^+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

$$\text{L-Alanine} + \text{2-oxoglutarate} \xrightarrow{\text{ALT}} \text{pyruvate} + \text{L-glutamate}$$

$$\text{Pyruvate} + \text{NADH} + \text{H}^+ \xrightarrow{\text{LDH}} \text{L-lactate} + \text{NAD}^+$$

The rate of the NADH oxidation is directly proportional to the catalytic ALT activity. It is determined by measuring the decrease in absorbance.

Reagents - working solutions

R1 TRIS buffer: 230 mmol/L, pH 7.15 (37 °C); L-alanine: 1140 mmol/L; LDH (microorganisms): ≥ 94 µkat/L; pyridoxamine phosphate: 0.23 mmol/L; albumin (bovine): 0.25 %; stabilizers; preservative

R3 NADH: ≥ 0.71 mmol/L; 2-oxoglutarate: 96 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

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- and K_3-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.

ALTP2

Alanine Aminotransferase acc. to IFCC II

Stability:	4 days at 15-25 °C
	7 days 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	52 µL	48 µL	
R3	15 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	4.5 µL	–	–
Decreased	4.5 µL	10 µL	90 µL
Increased	4.5 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: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - 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 original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.[5]

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 12 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 activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L x 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 4.0 U/L of initial values of samples ≤ 40 U/L and ± 10 % for samples > 40 U/L.

Icterus:[6] 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:[6] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62.2 µmol/L or 100 mg/dL). Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[6] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.
Lipemic samples may cause > Abs flagging.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[7,8]

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[9]

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

5-700 U/L (0.08-11.7 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 5 U/L (0.08 µkat/L)
Limit of Detection	= 5 U/L (0.08 µkat/L)
Limit of Quantitation	= 5 U/L (0.08 µkat/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 activity 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 activity samples.

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

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

ALTP2

Alanine Aminotransferase acc. to IFCC II

cobas®

Expected values

U/L

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[10]

Males:	10-50 U/L
Females:	10-35 U/L

Consensus values with pyridoxal phosphate activation:[11]

Males:	up to 50 U/L
Females:	up to 35 U/L

µkat/L*

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[10]

Males:	0.17-0.84 µkat/L
Females:	0.17-0.58 µkat/L

Consensus values with pyridoxal phosphate activation:[11]

Males:	up to 0.84 µkat/L
Females:	up to 0.58 µkat/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* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	49.4	0.534	1.1
PCCC2[b)]	127	3.04	2.4
Human serum 1	12.8	0.474	3.7
Human serum 2	30.8	0.601	2.0
Human serum 3	54.7	0.965	1.8
Human serum 4	359	2.45	0.7
Human serum 5	630	2.81	0.4

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	49.2	1.80	3.7
PCCC2[b)]	127	4.96	3.9
Human serum 1	12.8	0.611	4.8
Human serum 2	30.8	0.818	2.7
Human serum 3	54.7	1.58	2.9
Human serum 4	359	3.28	0.9
Human serum 5	638	5.07	0.8

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

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

Sample size (n) = 100

Passing/Bablok[12]	Linear regression
y = 0.993x + 1.52 U/L	y = 0.988x + 1.71 U/L
τ = 0.988	r = 1.000

The sample activities were between 8.9 and 683 U/L.

ALT 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** 503 analyzer (x).

Sample size (n) = 50

Passing/Bablok[12]	Linear regression
y = 1.036x - 0.787 U/L	y = 1.039x - 1.61 U/L
τ = 0.997	r = 1.000

The sample activities were between 27.0 and 635 U/L.

ALT 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) = 65

Passing/Bablok[12]	Linear regression
y = 1.004x - 0.634 U/L	y = 1.003x - 0.635 U/L
τ = 0.974	r = 1.000

The sample concentrations were between 6.07 and 647 U/L.

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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 navifyportal.roche.com for definition of symbols used):

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

AMYL2

cobas®

α-Amylase EPS ver.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056811190*	08056811500	α-Amylase EPS ver.2 (750 tests)	System-ID 2017 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08056811214*	08056811500	α-Amylase EPS ver.2 (750 tests)	System-ID 2017 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
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	

* Some kits shown may not be available in all countries.

English

System information

AMYL2: ACN 20170 (Serum/plasma)

AMYL2U: ACN 20171 (Urine)

Intended use

In vitro test for the quantitative determination of α-amylase in human serum, plasma and urine on **cobas c** systems.

Summary

Measurements of α-amylase in human serum, plasma and urine with this assay are used in conjunction with other parameters to aid in the diagnosis and management of pancreatic diseases, such as acute pancreatitis, in suspected patients.

The α-amylases (1,4-α-D-glucanohydrolases, EC 3.2.1.1) are digestive enzymes that catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin and glycogen by cleaving 1,4-α-glucosidic bonds. Linear and branched polyglucans are hydrolyzed at different rates. End products for linear polyglucans (amylose) are maltose and some residual glucose; if branched-chain polyglucans are used as substrate, a residue of dextrins is formed in addition to maltose and glucose.[1]

Amylases are present in many organs and tissues. They are predominantly produced by salivary glands and pancreas and can be released into the digestive tract or transported to other organs via the bloodstream.[2] Due to its small size, amylase is able to pass through the glomeruli of the kidneys and is the only plasma enzyme normally found in the urine.[1] The two predominant types present in serum and urine are the pancreatic type (P-type) and the salivary type (S-type). The P-type is almost exclusively synthesized by the pancreas and the S-type is mainly secreted by the salivary glands.[1] Amylase activity is also found in tears, sweat, human milk, the lungs, thyroid, tonsils and the fallopian tube.[3]

Because of the sparsity of specific clinical symptoms of pancreatic diseases, α-amylase determinations are of considerable importance in pancreatic diagnostics. Elevated levels of amylase activities in serum or urine are characteristics of acute pancreatitis, and therefore they are mainly used in the diagnosis and monitoring of this disease.[4,5,6,7] Hyperamylasemia does not, however, only occur with acute pancreatitis, but also in renal failure (reduced glomerular filtration), tumors of the lungs or ovaries, pulmonary inflammation, diseases of the salivary gland, diabetic ketoacidosis, cerebral trauma, surgical interventions or in the case of macroamylasemia.[1,3,8,9,10,11,12]

Numerous methods have been described for the determination of α-amylase. These either determine the decrease in the amount of substrate viscometrically, turbidimetrically, nephelometrically and amyloclastically or measure the formation of degradation products saccharogenically or kinetically with the aid of enzyme-catalyzed subsequent reactions.[13,14] The kinetic method described here is based on the well-proven cleavage of 4,6-ethylidene-(G7)-1,4-nitrophenyl-(G1)-α,D-maltoheptaoside (Ethylidene Protected Substrate = EPS) by α-amylase and subsequent hydrolysis of all the degradation products to p-nitrophenol with the aid of α-glucosidase (100 % chromophore liberation).[15] The results of this method correlate with those obtained by HPLC. This assay follows the recommendation of the IFCC, but was optimized for performance and stability.[16]

Test principle[17,18]

Enzymatic colorimetric assay acc. to IFCC.

Defined oligosaccharides such as 4,6-ethylidene-(G_7) p-nitrophenyl-(G_1)-α-D-maltoheptaoside (ethylidene-G_7PNP) are cleaved under the catalytic action of α-amylases. The G_2PNP, G_3PNP and G_4PNP fragments so formed are completely hydrolyzed to p-nitrophenol and glucose by α-glucosidase.

Simplified reaction scheme:

$$5\ \text{ethylidene-G}_7\text{PNP}^{a)} + 5\ H_2O \xrightarrow{\alpha\text{-amylase}} 2\ \text{ethylidene-G}_5 + 2\ G_2\text{PNP} + 2\ \text{ethylidene-G}_4 + 2\ G_3\text{PNP} + \text{ethylidene-G}_3 + G_4\text{PNP}$$

a) PNP ≙ p-nitrophenol

$$2\ G_2\text{PNP} + 2\ G_3\text{PNP} + G_4\text{PNP} + 14\ H_2O \xrightarrow{\alpha\text{-glucosidase}} 5\ \text{PNP} + 14\ G^{b)}$$

b) G ≙ Glucose

The color intensity of the p-nitrophenol formed is directly proportional to the α-amylase activity. It is determined by measuring the increase in absorbance.

Reagents - working solutions

R1 HEPES: 52.4 mmol/L; sodium chloride: 87 mmol/L; calcium chloride: 0.08 mmol/L; magnesium chloride: 12.6 mmol/L; α-glucosidase (microbial): ≥ 66.8 µkat/L; pH 7.0 (37 °C); preservatives; stabilizers

R3 HEPES: 52.4 mmol/L; ethylidene-G_7-PNP: 22 mmol/L; pH 7.0 (37 °C); preservatives; stabilizers

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

Precautions and warnings

For in vitro diagnostic use for laboratory 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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

AMYL2

α-Amylase EPS ver.2

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Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Hazardous components:

- 2-methyl-2H-isothiazol-3-one hydrochloride

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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[16,19]

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 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.

Urine: Collect urine without additives. α-Amylase is unstable in acid urine. Assay promptly or adjust pH to alkaline range (just above pH 7) before storage.[20]
If stabilizers are added to the sample, the sample index feature must not be used.

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

Stability in *serum or plasma*:[20]	7 days at 15-25 °C 1 month at 2-8 °C
Stability in *urine*:[21]	2 days at 15-25 °C 10 days 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, plasma and urine

Test definition

Reporting time	10 min
Wavelength (sub/main)	700/415 nm

Reagent pipetting		Diluent (H_2O)	
R1	78 µL	–	
R3	16 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3.1 µL	–	–
Decreased	3.1 µL	20 µL	80 µL
Increased	3.1 µL	–	–

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

Calibration

Application for serum/plasma (ACN 20170)

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

Application for urine (ACN 20171)
Transfer of calibration from serum/plasma application (ACN 20170)

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

Traceability: This method has been standardized against Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and substrate-specific absorptivity, ε.

Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

Serum/plasma:	PreciControl ClinChem Multi 1 PreciControl ClinChem Multi 2
Urine:	Quantitative urine controls are recommended for routine quality control.

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 activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interference

A slight change in the yellow coloration of solution 2 does not interfere with the performance of the test.

Do not pipette by mouth, and ensure that the reagent does not come into contact with the skin. **Saliva and sweat** contain α-amylase!

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[22]

Serum/plasma

Criterion: Recovery within ± 10 U/L of initial values of samples ≤ 100 U/L and within ± 10 % for samples > 100 U/L.

Icterus:[23] 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:[23] No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L or 500 mg/dL).

Lipemia (Intralipid):[23] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

In rare cases, samples with a combination of elevated turbidity (L-index) and high Amylase activity may cause a >React or >Abs flag.

Highly turbid and grossly lipemic samples may cause Abs. flags.

Anticoagulants: Interference was found with citrate, fluoride, and EDTA.[19]

Glucose: No significant interference from glucose up to a concentration of 111 mmol/L (2000 mg/dL). Approximately 10 % higher recovery was found at glucose concentrations of 250 mmol/L (4500 mg/dL).

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 5.68 mmol/L (100 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[24,25]
Exception: Icodextrin-based drugs may lead to decreased amylase results.[26]

Urine

Criterion: Recovery within ± 46 U/L of initial values of samples ≤ 460 U/L and within ± 10 % for samples > 460 U/L.

Hemolysis: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L or 500 mg/dL).

Phosphate: No significant interference from phosphate up to a concentration of 70 mmol/L (217 mg/dL).

Urea: No significant interference from urea up to a concentration of 1500 mmol/L (9009 mg/dL).

Ascorbic acid: No significant interference from ascorbic acid up to a concentration of 2.27 mmol/L (40 mg/dL). Approximately 15 % lower recovery was found at ascorbic acid concentrations of 22.7 mmol/L (400 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[25]

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

Serum, plasma and urine

3-1500 U/L (0.05-25.0 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 3 U/L (0.05 µkat/L)
Limit of Detection	= 3 U/L (0.05 µkat/L)
Limit of Quantitation	= 3 U/L (0.05 µkat/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 activity 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 activity samples.

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

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

Expected values[16]

U/L

Serum/plasma	Men/Women	28-100 U/L
Spontaneously voided urine	Men	16-491 U/L
	Women	21-447 U/L
α-amylase/	Men	58-283 U/g
creatinine quotient	Women	75-390 U/g

µkat/L*

Serum/plasma	Men/Women	0.47-1.67 µkat/L
Spontaneously voided urine	Men	0.27-8.20 µkat/L
	Women	0.35-7.46 µkat/L
α-amylase/	Men	0.97-4.73 µkat/g
creatinine quotient	Women	1.25-6.51 µkat/g

*calculated by unit conversion factor

α-Amylase/creatinine quotient

To allow for fluctuations in the α-amylase activity in urine, it is advisable to determine the α-amylase/creatinine quotient. To do this, determine the α-amylase activity and creatinine concentration in spontaneously voided urine.

$$\text{Quotient [µkat/mmol or U/g]} = \frac{\text{α-amylase [µkat/L or U/L]}}{\text{creatinine [mmol/L or g/L]}}$$

Amylase/Creatinine Clearance Ratio (ACCR)[20]

The ACCR is calculated from amylase activity and creatinine concentration. Both the serum and urine samples should be collected at the same time.

$$\text{ACCR [\%]} = \frac{\text{urine amylase [U/L]} \times \text{serum creatinine [mg/L]}}{\text{serum amylase [U/L]} \times \text{urine creatinine [mg/L]}} \times 100$$

The ACCR is approximately equal to 2-5 %.

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

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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.

Serum/plasma

Repeatability	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[c)]	76.9	0.438	0.6
PCCC2[d)]	193	0.831	0.4
Human serum 1	7.38	0.231	3.1
Human serum 2	63.9	0.345	0.5
Human serum 3	509	1.63	0.3
Human serum 4	771	2.67	0.3
Human serum 5	1395	4.13	0.3

Intermediate precision	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[c)]	76.9	0.713	0.9
PCCC2[d)]	194	1.51	0.8
Human serum 1	7.38	0.263	3.6
Human serum 2	63.6	0.409	0.6
Human serum 3	509	2.51	0.5
Human serum 4	771	4.13	0.5
Human serum 5	1395	6.04	0.4

c) PreciControl ClinChem Multi 1

d) PreciControl ClinChem Multi 2

Urine

Repeatability	*Mean U/L*	*SD U/L*	*CV %*
Control 1[e)]	56.3	0.327	0.6
Control 2[e)]	180	0.707	0.4
Human urine 1	7.78	0.257	3.3
Human urine 2	263	0.913	0.3
Human urine 3	408	1.13	0.3
Human urine 4	766	1.96	0.3
Human urine 5	1385	3.62	0.3

Intermediate precision	*Mean U/L*	*SD U/L*	*CV %*
Control 1[e)]	56.3	0.370	0.7
Control 2[e)]	180	0.801	0.4
Human urine 1	7.74	0.403	5.2
Human urine 2	263	2.09	0.8
Human urine 3	409	10.6	2.6
Human urine 4	767	4.41	0.6
Human urine 5	1385	5.66	0.4

e) commercially available control material

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

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

Serum/plasma

Sample size (n) = 85

Passing/Bablok[27]	Linear regression
y = 1.006x - 0.00259 U/L	y = 1.008x - 0.399 U/L
τ = 0.993	r = 1.000

The sample activities were between 10.3 and 1439 U/L.

Urine

Sample size (n) = 67

Passing/Bablok[27]	Linear regression
y = 0.997x + 0.221 U/L	y = 0.996x + 0.571 U/L
τ = 0.985	r = 1.000

The sample activities were between 6.90 and 1467 U/L.

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

Serum/plasma

Sample size (n) = 73

Passing/Bablok[27]	Linear regression
y = 1.013x - 0.271 U/L	y = 1.012x - 0.182 U/L
τ = 0.993	r = 1.000

The sample activities were between 9.10 and 1460 U/L.

Urine

Sample size (n) = 71

Passing/Bablok[27]	Linear regression
y = 1.014x - 0.186 U/L	y = 1.019x - 0.515 U/L
τ = 0.991	r = 1.000

The sample activities were between 4.80 and 1444 U/L.

Amylase values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 73

Passing/Bablok[27]	Linear regression
y = 1.009x + 0.257 U/L	y = 1.006x + 0.976 U/L
τ = 0.995	r = 1.000

The sample concentrations were between 20.3 and 1412 U/L.

Urine

Sample size (n) = 75

Passing/Bablok[27]	Linear regression
y = 0.993x - 0.0323 U/L	y = 0.992x + 0.111 U/L
τ = 0.994	r = 1.000

The sample concentrations were between 5.20 and 1494 U/L.

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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

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606

Roche

AMY-P

cobas®

α-Amylase EPS pancreatic

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056820190	08056820500	α-Amylase EPS Pancreatic (450 tests)	System-ID 2018 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
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

AMYP2: ACN 20180 (Serum/plasma)

AMYP2U: ACN 20181 (Urine)

Intended use

In vitro test for the quantitative determination of pancreatic α-amylase in human serum, plasma and urine on **cobas c** systems.

Summary

Measurements of pancreatic α-amylase in human serum, plasma and urine with this assay are used in conjunction with other parameters to aid in the diagnosis and management of pancreatic diseases, such as acute pancreatitis, in suspected patients.

The α-amylases (1,4-α-D-glucanohydrolases, EC 3.2.1.1) are digestive enzymes that catalyze the hydrolytic degradation of polymeric carbohydrates such as amylose, amylopectin and glycogen by cleaving 1,4-α-glucosidic bonds. Linear and branched polyglucans are hydrolyzed at different rates. End products for linear polyglucans (amylose) are maltose and some residual glucose; if branched-chain polyglucans are used as substrate, a residue of dextrins is formed in addition to maltose and glucose.[1]

Amylases are present in many organs and tissues. They are predominantly produced by salivary glands and pancreas and can be released into the digestive tract or transported to other organs via the bloodstream.[2] Due to its small size, amylase is able to pass through the glomeruli of the kidneys and is the only plasma enzyme normally found in the urine.[1] The two predominant types present in serum and urine are the pancreatic type (P-type) and the salivary type (S-type). The P-type is almost exclusively synthesized by the pancreas and the S-type is mainly secreted by the salivary glands.[1] Amylase activity is also found in tears, sweat, human milk, the lungs, thyroid, tonsils and the fallopian tube.[3]

Because of the sparsity of specific clinical symptoms of pancreatic diseases, enzymatic determinations are of considerable importance in pancreas diagnostics. The determination of pancreatic α-amylase is suitable for the diagnosis and monitoring of acute pancreatitis and may also have diagnostic value in chronic pancreatitis.[1,4,5,6,7,8,9,10,11] In studies of acute pancreatitis, the clinical sensitivity of measuring circulating pancreatic α-amylase level for diagnosing acute pancreatitis is consistently high, while the clinical specificity varies according to population and clinical case mix. However, when the pancreatic α-amylase measurement was evaluated in individuals with acute abdominal pain and suspected pancreatitis using a specific decision limit (three times the upper reference limit), it was found to be highly specific (above 90 %) in diagnosing acute pancreatitis. Therefore, pancreatic α-amylase has been described as more sensitive and specific for pancreatic tissue damage than total α-amylase.[12]

A variety of methods have been described for determining pancreatic α-amylase: radio- and enzyme-immunoassays as well as the partial inhibition of salivary α-amylase by an inhibitor derived from wheatgerm and calculation of the pancreatic α-amylase from the remaining and total amylase activities.[13,14,15,16,17]

The kinetic method described here is based on inhibition of the activity of human salivary α-amylase by two different monoclonal antibodies and the well-proven cleavage of 4,6-ethylidene-(G7)-1,4-nitrophenyl-(G1)-α-D-maltoheptaoside (Ethylidene Protected Substrate = EPS) by pancreatic α-amylase followed by hydrolysis of all the degradation products to p-nitrophenol with the aid of α-glucosidase (100 % chromophore liberation). The results of this method correlate with those obtained by HPLC. This assay follows the recommendation of the IFCC, but was optimized for performance and stability.[18,19,20]

Test principle (simplified)[19,20]

Colorimetric assay
After immunoinhibition with antibodies against human salivary α-amylase the pancreatic α-amylase is selectively determined with an enzymatic colorimetric method using the substrate 4,6-ethylidene-p-nitrophenyl-α-D-maltoheptaoside (ethylidene-G_7PNP).[14]

Simplified reaction scheme:

$$5\ \text{ethylidene-G}_7\text{PNP}^{a)} + 5\ H_2O \xrightarrow{\text{pancreatic α-amylase}} 2\ \text{ethylidene-G}_5 + 2\ G_2PNP + 2\ \text{ethylidene-G}_4 + 2\ G_3PNP + \text{ethylidene-G}_3 + G_4PNP$$

a) PNP ≙ p-nitrophenol

$$2\ G_2PNP + 2\ G_3PNP + G_4PNP + 14\ H_2O \xrightarrow{\text{α-glucosidase}} 5\ PNP + 14\ G^{b)}$$

b) G ≙ Glucose

The rate of p-nitrophenol formation is directly proportional to the catalytic pancreatic α-amylase activity. It is determined by measuring the increase in absorbance photometrically.

Reagents - working solutions

R1 HEPES buffer: 52.4 mmol/L, pH 7.1 (37 °C); sodium chloride: 87 mmol/L; magnesium chloride: 12.6 mmol/L; calcium chloride: 0.075 mmol/L; α-glucosidase (microbial): ≥ 67 µkat/L; monoclonal antibodies (mouse): 97 mg/L; preservatives

R3 HEPES buffer: 52.4 mmol/L, pH 7.1 (37 °C); 4,6-ethylidene-G_7 PNP: 22 mmol/L; preservatives; stabilizers

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

AMY-P

α-Amylase EPS pancreatic

Warning

H317	May cause an allergic skin reaction.

Prevention:

P261	Avoid breathing mist or vapours.
P272	Contaminated work clothing should not be allowed out of the workplace.
P280	Wear protective gloves.

Response:

P333 + P313	If skin irritation or rash occurs: Get medical advice/attention.
P362 + P364	Take off contaminated clothing and wash it before reuse.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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[20,21]

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 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.

Urine: Collect without additives. Pancreatic α-amylase is unstable in acid urine. Assay promptly or adjust pH to alkaline range (about pH 7) before storage.[22]
If stabilizers are added to the sample, the sample index feature must not be used.

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

Stability in *serum or plasma*:[22]	7 days at 15-25 °C
	1 month at 2-8 °C
Stability in *urine*:[23]	2 days at 15-25 °C
	10 days 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, plasma and urine

Test definition

Reporting time	10 min		
Wavelength (sub/main)	700/415 nm		
Reagent pipetting		Diluent (H_2O)	
R1	78 µL	–	
R3	16 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3.1 µL	–	–
Decreased	3.1 µL	20 µL	80 µL
Increased	3.1 µL	–	–

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

Calibration

Application for serum/plasma (ACN 20180)

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

Application for urine (ACN 20181)
Transfer of calibration from serum/plasma application (ACN 20180)

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

Traceability: This method has been standardized against the Roche system reagent using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.

Quality control

For quality control, use control materials as listed in the "Order information" section. In addition, other suitable control material can be used.

Serum/plasma:	PreciControl ClinChem Multi 1 PreciControl ClinChem Multi 2
Urine:	Quantitative urine controls are recommended for routine quality control.

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.

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Calculation

cobas c systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L x 0.0167 = µkat/L

Limitations - interference[21,24]

The residual activity of salivary α-amylase is approx. 3 %. In rare cases, very high activities of salivary α-amylase can hence lead to elevated values being measured for pancreatic α-amylase.

A slight change in the yellow coloration of solution 2 does not interfere with the performance of the test.

Do not pipette by mouth, and ensure that the reagent does not come into contact with the skin. (**Saliva and sweat** contain α-amylase!)

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[25]

Patients with macroamylase may have elevated p-amylase results. The elevation is not due to an insufficient inhibition of salivary amylase in the serum immune complex. It is caused by a higher than normal level of p-amylase since the immune complex is not subject to glomerular filtration.

This elevated p-amylase is not diagnostic for pancreatitis. However, measurement of an elevated p-amylase in urine is confirmatory of pancreatitis, pancreatic trauma, or pancreatic carcinoma as the amylase released is not completely bound by the immune complex and thus subject to glomerular filtration.[26]

Serum/plasma

Criterion: Recovery within ± 5 U/L of initial values of samples ≤ 50 U/L and within ± 10 % for samples > 50 U/L.

Icterus:[27] 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:[27] No significant interference up to an H index of 200 (approximate hemoglobin concentration: 124 µmol/L or 200 mg/dL).

Lipemia (Intralipid):[27] No significant interference up to an L index of 1500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

In rare cases, samples with a combination of elevated turbidity (L-index) and high Amylase activity may cause a >React or >Abs flag.

Highly turbid and grossly lipemic samples may cause Abs. flags.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[28,29]

Exception: No interference from ascorbic acid up to 5.68 mmol/L (100 mg/dL). Icodextrin based drugs may lead to decreased amylase values.[26]

Urine

Criterion: Recovery within ± 35 U/L of initial values of samples ≤ 350 U/L and within ± 10 % for samples > 350 U/L.

Hemolysis: No significant interference up to an H index of 500 (approximate hemoglobin concentration: 311 µmol/L or 500 mg/dL).

Phosphate: No significant interference from phosphate up to a concentration of 60 mmol/L (186 mg/dL).

Urea: No significant interference from urea up to a concentration of 1500 mmol/L (9009 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[29]

Exception: Approximately 15 % lower recovery was found at ascorbic acid concentrations of 22.7 mmol/L (400 mg/dL).

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

Serum, plasma and urine

3-1500 U/L (0.05-25.0 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 3 U/L (0.05 µkat/L)
Limit of Detection	= 3 U/L (0.05 µkat/L)
Limit of Quantitation	= 3 U/L (0.05 µkat/L)

The Limit of Blank, the Limit of Detection and the 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 activity 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 activity samples.

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

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

Expected values[19]

U/L

Serum/plasma	Men/Women	13-53 U/L
Spontaneously voided urine	Men	7-356 U/L
	Women	13-319 U/L
Pancreatic α-amylase/creatinine quotient	Men	35-199 U/g
	Women	52-259 U/g

µkat/L*

Serum/plasma	Men/Women	0.22-0.88 µkat/L
Spontaneously voided urine	Men	0.12-5.95 µkat/L
	Women	0.22-5.33 µkat/L
Pancreatic α-amylase/creatinine quotient	Men	0.58-3.33 µkat/g
	Women	0.87-4.33 µkat/g

*calculated by unit conversion factor

Pancreatic α-amylase/creatinine quotient

To allow for fluctuations in the pancreatic α-amylase activity in urine, it is advisable to determine the pancreatic α-amylase/creatinine quotient. To do this, determine the pancreatic α-amylase activity and creatinine concentration in spontaneously voided urine.

$$\text{Quotient [µkat/mmol or U/g]} = \frac{\text{pancreatic α-amylase [µkat/L or U/L]}}{\text{creatinine [mmol/L or g/L]}}$$

Amylase/Creatinine Clearance Ratio (ACCR)[23]

The ACCR is calculated from amylase activity and creatinine concentration. Both the serum and urine samples should be collected at the same time.

$$\text{ACCR [\%]} = \frac{\text{Urine amylase [U/L]} \times \text{serum creatinine [mg/L]}}{\text{Serum amylase [U/L]} \times \text{urine creatinine [mg/L]}} \times 100$$

ACCR approximately equal to 2-5 %.

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

AMY-P

α-Amylase EPS pancreatic

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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.

Serum/plasma

Repeatability	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[c)]	38.3	0.314	0.8
PCCC2[d)]	94.7	0.556	0.6
Human serum 1	7.31	0.252	3.4
Human serum 2	31.7	0.248	0.8
Human serum 3	325	1.16	0.4
Human serum 4	737	2.44	0.3
Human serum 5	1254	3.80	0.3

Intermediate precision	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[c)]	38.3	0.358	0.9
PCCC2[d)]	93.5	0.695	0.7
Human serum 1	7.31	0.274	3.7
Human serum 2	31.7	0.293	0.9
Human serum 3	328	1.44	0.4
Human serum 4	737	2.85	0.4
Human serum 5	1254	5.05	0.4

c) PreciControl ClinChem Multi 1

d) PreciControl ClinChem Multi 2

Urine

Repeatability	*Mean U/L*	*SD U/L*	*CV %*
Control 1	39.2	0.308	0.8
Control 2	94.7	0.559	0.6
Human urine 1	7.05	0.261	3.7
Human urine 2	178	0.673	0.4
Human urine 3	325	0.988	0.3
Human urine 4	722	3.40	0.5
Human urine 5	1311	6.66	0.5

Intermediate precision	*Mean U/L*	*SD U/L*	*CV %*
Control 1	39.2	0.354	0.9
Control 2	94.5	0.727	0.8
Human urine 1	7.36	0.269	3.7
Human urine 2	178	0.979	0.5
Human urine 3	325	1.48	0.5
Human urine 4	722	4.85	0.7
Human urine 5	1311	7.41	0.6

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

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

Serum/plasma

Sample size (n) = 88

Passing/Bablok[30]	Linear regression
y = 1.005x + 0.0265 U/L	y = 1.002x + 0.381 U/L
τ = 0.983	r = 1.000

The sample activities were between 3.80 and 1456 U/L.

Urine

Sample size (n) = 69

Passing/Bablok[30]	Linear regression
y = 1.002x - 0.0394 U/L	y = 0.998x + 0.567 U/L
τ = 0.992	r = 1.000

The sample activities were between 5.40 and 1440 U/L.

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

Serum/plasma

Sample size (n) = 73

Passing/Bablok[30]	Linear regression
y = 1.015x - 0.148 U/L	y = 1.014x - 0.174 U/L
τ = 0.988	r = 1.000

The sample activities were between 7.30 and 1420 U/L.

Urine

Sample size (n) = 70

Passing/Bablok[30]	Linear regression
y = 1.005x + 0.00463 U/L	y = 1.014x - 0.829 U/L
τ = 0.997	r = 1.000

The sample activities were between 3.60 and 1441 U/L.

Pancreatic amylase values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 75

Passing/Bablok[30]	Linear regression
y = 1.008x - 0.00402 U/L	y = 1.006x + 0.528 U/L
τ = 0.993	r = 1.000

The sample concentrations were between 9.18 and 1467 U/L.

Urine

Sample size (n) = 73

Passing/Bablok[30]	Linear regression
y = 0.997x - 0.312 U/L	y = 0.997x - 0.265 U/L
τ = 0.996	r = 1.000

The sample concentrations were between 4.08 and 1395 U/L.

AMY-P

α-Amylase EPS pancreatic

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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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

ASTP2

cobas®

Aspartate Aminotransferase acc. to IFCC II

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08104719190*	08104719500	Aspartate Aminotransferase acc. to IFCC II (800 tests)	System-ID 2023 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08104719214*	08104719500	Aspartate Aminotransferase acc. to IFCC II (800 tests)	System-ID 2023 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
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	

* Some kits shown may not be available in all countries.

English

System information

ASTP2: ACN 20230

Intended use

In vitro test for the quantitative determination of aspartate aminotransferase (AST) with pyridoxal phosphate activation in human serum and plasma on **cobas c** systems.

Summary

Aspartate aminotransferase (AST) measurements, performed with this device, in human serum and plasma are used as an aid in diagnosis of hepatocellular injury and in monitoring chronic liver injury.

The enzyme aspartate aminotransferase (AST) is widely distributed in tissue, primarily in the liver, cardiac muscle, skeletal muscle, kidney, brain and erythrocytes.[1] AST catalyzes the transfer of amino groups from L-aspartate to α-ketoglutarate, resulting in L-glutamate and oxaloacetate. This is a critical process of the tricarboxylic acid cycle, in which the coenzyme pyridoxal phosphate (also known as pyridoxal-5-phosphate or active vitamin B6) is required. In particular, AST is vital for aerobic glycolysis. AST exists in human tissues as 2 distinct isoenzymes, 1 located in the cytoplasm (c-AST), and the other in mitochondria (m-AST), which differ in amino acid composition and immunochemical and kinetic properties. In healthy individuals, the circulating AST levels consist mainly of cytoplasmic AST, originating from cytoplasmic leakage, on the other side, mitochondrial AST activity in serum shows a marked increase in patients with extensive liver cell degeneration and necrosis. Although AST activity is important in all cells with high metabolic activity, it is more relevant for liver and muscle cells.[2]

Primarily, AST is a marker of hepatocellular injury. Measurement of AST activity is therefore used for the diagnosis of hepatic diseases such as acute and chronic viral hepatitis, nonalcoholic fatty liver disease (NAFLD), alcohol-related liver disease, ischemic hepatopathy, suspected malignant infiltration, cholestasis.[3] Although alanine aminotransferase (ALT) is considered a more specific indicator of liver disease, the concentration of AST may be a more sensitive indicator of liver injury in conditions such as alcohol-related liver disease and in some cases of autoimmune hepatitis.[4] Several international guidelines recommend AST testing for monitoring chronic hepatitis status and progression.[4,5]

Non-liver causes for increases in AST include damage to cardiac or skeletal muscle cells and haemolysis. Serum elevation of AST without elevation in ALT is suggestive of cardiac or muscle disease.[3] In patients undergoing renal dialysis or those with vitamin B6 deficiency, serum AST may be decreased.[6] AST serum levels can be affected by age, gender, alcohol consumption, body mass index, dietary and living habits, nutrition, metabolic status, and drug treatment, among other factors.[7]

In patients with vitamin B6 deficiency (insufficient endogenous pyridoxal phosphate), serum aminotransferase activity may be decreased. The addition of pyridoxal phosphate to this assay causes an increase in aminotransferase activity (activation higher for AST than for ALT) and prevents falsely low aminotransferase test results in these samples.[1]

Test principle

This assay follows the recommendations of the IFCC, but was optimized for performance and stability.[8]

AST catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to form oxaloacetate and L-glutamate. The oxaloacetate then reacts with NADH, in the presence of malate dehydrogenase (MDH), to form L-malate and NAD^+. Pyridoxal phosphate serves as a coenzyme in the amino transfer reaction. It ensures full enzyme activation.

$$\text{L-Aspartate} + \text{2-oxoglutarate} \xrightarrow{\text{AST}} \text{oxaloacetate} + \text{L-glutamate}$$

$$\text{Oxaloacetate} + \text{NADH} + H^+ \xrightarrow{\text{MDH}} \text{L-malate} + NAD^+$$

The rate of the NADH oxidation is directly proportional to the catalytic AST activity. It is determined by measuring the decrease in absorbance.

Reagents - working solutions

R1 TRIS buffer: 180 mmol/L, pH 7.65 (37 °C); L-aspartate: 550 mmol/L; MDH (microorganisms): ≥ 11 µkat/L; LDH (microorganisms): ≥ 80 µkat/L; pyridoxamine phosphate: 0.23 mmol/L; albumin (bovine): 0.25 %; stabilizers; preservative

R3 NADH: ≥ 0.71 mmol/L; 2-oxoglutarate: 96 mmol/L; preservative

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

Precautions and warnings

For in vitro diagnostic use for laboratory 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

Specimen collection and preparation

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

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Only the specimens listed below were tested and found acceptable.
Serum
Plasma: Li-heparin and K_2- and K_3-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:	4 days at 15-25 °C
	7 days at 2-8 °C
	3 months at -20 °C (± 5 °C)

Freeze only once.

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

Reaction time	10 min		
Wavelength (sub/main)	700/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	52 µL	48 µL	
R3	15 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	4.5 µL	–	–
Decreased	4.5 µL	10 µL	90 µL
Increased	4.5 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: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - 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 original IFCC formulation using calibrated pipettes together with a manual photometer providing absolute values and the substrate-specific absorptivity, ε.[8]

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 12 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 activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L x 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 4.0 U/L of initial values of samples ≤ 40 U/L and ± 10 % for samples > 40 U/L.

Icterus:[9] 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:[9] No significant interference up to an H index of 25 (approximate hemoglobin concentration: 15.6 µmol/L or 25 mg/dL).
Contamination with erythrocytes will elevate results, because the analyte level in erythrocytes is higher than in normal sera. The level of interference may be variable depending on the content of analyte in the lysed erythrocytes.

Lipemia (Intralipid):[9] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Lipemic specimens may cause > Abs flagging.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[10,11]

Drug interferences are measured based on recommendations given in CLSI guidelines EP07 and EP37 and other published literature. Effects of concentrations exceeding these recommendations have not been characterized.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[12]

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

5-700 U/L (0.08-11.7 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 5 U/L (0.08 µkat/L)
Limit of Detection	= 5 U/L (0.08 µkat/L)
Limit of Quantitation	= 5 U/L (0.08 µkat/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.

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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 activity 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 activity samples.

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

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

Expected values

U/L

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[13]

Males:	10-50 U/L
Females:	10-35 U/L

Consensus values with pyridoxal phosphate activation:[14]

Males:	up to 50 U/L
Females:	up to 35 U/L

µkat/L*

Acc. to IFCC/Standard Method 94 with pyridoxal phosphate activation measured at 37 °C:[13]

Males:	0.17-0.84 µkat/L
Females:	0.17-0.58 µkat/L

Consensus values with pyridoxal phosphate activation:[14]

Males:	up to 0.84 µkat/L
Females:	up to 0.58 µkat/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* U/L	*SD* U/L	*CV* %
PCCC1[a)]	46.4	0.551	1.2
PCCC2[b)]	149	1.49	1.0
Human serum 1	11.3	0.242	2.1
Human serum 2	33.0	0.497	1.5
Human serum 3	50.4	0.314	0.6
Human serum 4	345	1.31	0.4
Human serum 5	651	2.19	0.3

Intermediate precision	*Mean* U/L	*SD* U/L	*CV* %
PCCC1[a)]	46.5	1.33	2.9
PCCC2[b)]	148	3.30	2.2
Human serum 1	11.4	0.281	2.5
Human serum 2	33.0	0.552	1.7
Human serum 3	50.4	0.371	0.7
Human serum 4	345	1.79	0.5
Human serum 5	651	3.63	0.6

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

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

Sample size (n) = 111

Passing/Bablok[15]	Linear regression
y = 0.960x + 2.35 U/L	y = 0.931x + 6.22 U/L
τ = 0.978	r = 0.998

The sample activities were between 7.50 and 694 U/L.

AST 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** 503 analyzer (x).

Sample size (n) = 50

Passing/Bablok[15]	Linear regression
y = 0.984x + 0.903 U/L	y = 0.980x + 1.37 U/L
τ = 0.989	r = 1.000

The sample activities were between 7.79 and 667 U/L.

AST 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) = 65

Passing/Bablok[15]	Linear regression
y = 0.991x + 0.793 U/L	y = 0.989x + 1.15 U/L
τ = 0.981	r = 1.000

The sample concentrations were between 6.29 and 667 U/L.

References

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9 Glick MR, Ryder KW, Jackson SA. Graphical Comparisons of Interferences in Clinical Chemistry Instrumentation. Clin Chem 1986;32:470-475.

10 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

11 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.

12 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

13 Klauke R, Schmidt E, Lorentz K. Recommendations for carrying out standard ECCLS procedures (1988) for the catalytic concentrations of creatine kinase, aspartate aminotransferase, alanine aminotransferase and γ-glutamyltransferase at 37 °C. Eur J Clin Chem Clin Biochem 1993;31:907-909.

14 Thomas L, Müller M, Schumann G, et al. Consensus of DGKL and VDGH for interim reference intervals on enzymes in serum. J Lab Med 2005;29(5):301-308.

15 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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

Roche Diagnostics GmbH
Sandhofer Strasse 116
68305 Mannheim, Germany
www.roche.com
+800 5505 6606

Roche

BILD2

cobas®

Bilirubin Direct Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056951190	08056951500	Bilirubin Direct Gen.2 (1000 tests)	System-ID 2030 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
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	
10158046122	Precibil (4 x 2 mL)	Code 20306	

English

System information

Jendrassik-Grof method

BILD2-J: ACN 20301

Doumas method

BILD2-D: ACN 20300

Intended use

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

Summary

Measurements of direct bilirubin, performed with this assay in human serum and plasma of adults and neonates, are used for the diagnosis of hyperbilirubinemia (such as observed with abnormal destruction of red blood cells, liver diseases, and metabolic disorders, including hepatitis and gallbladder block), and in newborn screening for severe hyperbilirubinemia.

Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract. Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.[1,2,3,4]

In newborns, several mechanisms lead to an increased bilirubin load, such as increased turnover in fetal red blood cells, reduced bilirubin clearance, and increased enterohepatic circulation of bilirubin. Screening neonates for severe hyperbilirubinemia, especially in newborns with infant jaundice, has been proposed to help preventing chronic bilirubin encephalopathy.[5,6]

Test principle

Diazo method.[7]

Conjugated bilirubin and δ-bilirubin (direct bilirubin) react directly with 3,5 Dichlorophenyl diazonium salt in acid buffer to form the red-colored azobilirubin.

bilirubin + 3,5 DPD $\longrightarrow$ azobilirubin

The color intensity of the red azo dye formed is directly proportional to the direct (conjugated) bilirubin concentration and can be determined photometrically.

Remark: Under the influence of blue light, e.g. during phototherapy of newborn children, unconjugated bilirubin is partly transformed into a water-soluble isomer called photobilirubin, a substrate for direct bilirubin tests. This fraction is detected by BILD2 and may lead to above-normal results in healthy children.

Reagents - working solutions

R1 Phosphoric acid: 85 mmol/L; HEDTA: 4.0 mmol/L; NaCl: 50 mmol/L; detergent; pH 1.9

R2 3,5-Dichlorophenyl diazonium: 1.5 mmol/L; pH 1.3

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.

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: Collect serum using standard sample tubes.
Plasma: Li-heparin, K_2-, K_3-EDTA.

Protect specimens from exposure to light.

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:[a),8,9]	2 days at 15-25 °C
	7 days at 2-8 °C
	6 months at (-15)-(-25) °C

a) If care is taken to prevent exposure to light

Freeze only once.

Materials provided

See “Reagents – working solutions” section for reagents.

BILD2

Bilirubin Direct Gen.2

cobas®

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)	800/546 nm	
Reagent pipetting		Diluent (NaCl)
R1	79 µL	–
R2	16 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	Diluent (H_2O)
Normal	4.4 µL	–	–
Decreased	2.2 µL	–	–
Increased	4.4 µL	–	–

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

Calibration

Calibrator	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear regression
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - 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 manual test performance using the Jendrassik-Grof or Doumas method.[10,11]

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 µmol/L (mg/dL, mg/L).

Conversion factors:	µmol/L x 0.0585 = mg/dL
	µmol/L x 0.585 = mg/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial values at a direct bilirubin concentration of 34.2 µmol/L (2.0 mg/dL).

Hemolysis:[12] No significant interference up to an H index of 25 (approximate hemoglobin concentration: 15.5 µmol/L or 25 mg/dL).

Lipemia (Intralipid):[12] No significant interference up to an L index of 750. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

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

Exception: Phenylbutazone causes artificially low bilirubin results.

Samples containing indocyanine green must not be measured.

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.

In certain cases specimens may give a direct bilirubin result slightly greater than the total bilirubin result. This is observed in patient samples when nearly all the reacting bilirubin is in the direct form. In such cases the result for the total bilirubin should be reported for both direct bilirubin and total bilirubin values.

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

Jendrassik-Grof method

1.5-291 µmol/L (0.09-17 mg/dL)

Doumas method

1.4-236 µmol/L (0.08-14 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

Jendrassik-Grof method

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 1.0 µmol/L (0.06 mg/dL)
Limit of Detection	= 1.5 µmol/L (0.09 mg/dL)
Limit of Quantitation	= 3.0 µmol/L (0.18 mg/dL)

The Limit of Blank, the Limit of Detection and the 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 30 %. It has been determined using low concentration bilirubin samples.

Lower limits of measurement

Doumas method

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.8 µmol/L (0.05 mg/dL)
Limit of Detection	= 1.2 µmol/L (0.07 mg/dL)
Limit of Quantitation	= 1.4 µmol/L (0.08 mg/dL)

The Limit of Blank, the Limit of Detection and the 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 30 %. It has been determined using low concentration bilirubin samples.

Expected values

Jendrassik-Grof method[1]

µmol/L

Direct bilirubin ≤ 5 µmol/L

mg/dL

Direct bilirubin ≤ 0.30 mg/dL

An upper limit of 10 µmol/L direct bilirubin for neonates has been cited in the literature, although this has not been confirmed by internal data.[16]

Doumas method[17]

µmol/L

Direct bilirubin ≤ 3.4 µmol/L

mg/dL

Direct bilirubin ≤ 0.20 mg/dL

An upper limit of 10 µmol/L direct bilirubin for neonates has been cited in the literature, although this has not been confirmed by internal data.[16]

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

Jendrassik-Grof method

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* µmol/L	*SD* µmol/L	*CV* %
PCCC1[b)]	16.5	0.116	0.7
PCCC2[c)]	44.1	0.216	0.5
Human serum 1	4.32	0.0810	1.9
Human serum 2	9.76	0.141	1.4
Human serum 3	89.8	0.203	0.2
Human serum 4	139	0.488	0.4
Human serum 5	254	0.756	0.3

Intermediate precision	*Mean* µmol/L	*SD* µmol/L	*CV* %
PCCC1[b)]	16.5	0.212	1.3
PCCC2[c)]	44.1	0.573	1.3
Human serum 1	4.31	0.107	2.5
Human serum 2	9.76	0.241	2.5
Human serum 3	89.8	0.615	0.7
Human serum 4	139	2.23	1.6
Human serum 5	254	2.42	1.0

b) PreciControl ClinChem Multi 1

c) PreciControl ClinChem Multi 2

Doumas method

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* µmol/L	*SD* µmol/L	*CV* %
PCCC1[b)]	13.2	0.0838	0.6
PCCC2[c)]	35.6	0.146	0.4
Human serum 1	3.23	0.0673	2.1
Human serum 2	8.64	0.0899	1.0
Human serum 3	57.2	0.179	0.3
Human serum 4	109	0.393	0.4
Human serum 5	195	0.512	0.3

Intermediate precision	*Mean* µmol/L	*SD* µmol/L	*CV* %
PCCC1[b)]	13.2	0.175	1.3
PCCC2[c)]	35.9	0.429	1.2
Human serum 1	3.32	0.0945	2.8
Human serum 2	8.64	0.176	2.0
Human serum 3	57.8	0.421	0.7
Human serum 4	110	1.89	1.7
Human serum 5	195	1.98	1.0

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

Method comparison

Jendrassik-Grof method

Bilirubin values for human serum and plasma samples obtained with the Roche BILD2 reagent 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) = 582

Passing/Bablok[18]	Linear regression
y = 1.001x + 0.646 µmol/L	y = 0.987x + 1.28 µmol/L
τ = 0.965	r = 1.000

The sample concentrations were between 1.50 and 288 µmol/L.

Bilirubin values for human serum and plasma samples obtained with the Roche BILD2 reagent 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) = 64

Passing/Bablok[18]	Linear regression

y = 0.988x + 1.02 µmol/L	y = 0.938x + 2.53 µmol/L
τ = 0.952	r = 0.999

The sample concentrations were between 1.50 and 276 µmol/L.

Doumas method

Bilirubin values for human serum and plasma samples obtained with the Roche BILD2 reagent 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) = 66

Passing/Bablok[18]	Linear regression
y = 1.001x + 0.481 µmol/L	y = 0.985x + 1.22 µmol/L
τ = 0.966	r = 0.999

The sample concentrations were between 1.49 and 231 µmol/L.

Bilirubin values for human serum and plasma samples obtained with the Roche BILD2 reagent 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) = 62

Passing/Bablok[18]	Linear regression
y = 0.985x + 0.716 µmol/L	y = 0.941x + 1.81 µmol/L
τ = 0.928	r = 0.999

The sample concentrations were between 1.40 and 222 µmol/L.

References

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8 Quality of Diagnostic Samples, Recommendations of the Working Group on Preanalytical Quality of the German Society for Clinical Chemistry and Laboratory Medicine, 3rd completely revised ed. 2010.

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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 navifyportal.roche.com for definition of symbols used):

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

BILT3

Bilirubin Total Gen.3

cobas®

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08056960190	08056960500	Bilirubin Total Gen.3 (1050 tests)	System-ID 2031 001	**cobas c** 303, **cobas c** 503

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
10158046122	Precibil (4 x 2 mL)	Code 20306	
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

BILT3: ACN 20310

Intended use

In vitro test for the quantitative determination of total bilirubin in serum and plasma of adults and neonates on **cobas c** systems.

Summary

Measurements of total bilirubin, performed with this assay in human serum and plasma of adults and neonates, are used for the diagnosis of hyperbilirubinemia (such as observed with abnormal destruction of red blood cells, liver diseases, and metabolic disorders, including hepatitis and gallbladder block), and in newborn screening for severe hyperbilirubinemia. Total bilirubin is a combination of direct and indirect bilirubin.

Bilirubin is formed in the reticuloendothelial system during the degradation of aged erythrocytes. The heme portion from hemoglobin and from other heme-containing proteins is removed, metabolized to bilirubin, and transported as a complex with serum albumin to the liver. In the liver, bilirubin is conjugated with glucuronic acid for solubilization and subsequent transport through the bile duct and elimination via the digestive tract.

Diseases or conditions which, through hemolytic processes, produce bilirubin faster than the liver can metabolize it, cause the levels of unconjugated (indirect) bilirubin to increase in the circulation. Liver immaturity and several other diseases in which the bilirubin conjugation mechanism is impaired cause similar elevations of circulating unconjugated bilirubin. Bile duct obstruction or damage to hepatocellular structure causes increases in the levels of both conjugated (direct) and unconjugated (indirect) bilirubin in the circulation.[1,2,3,4]

Numerous guidelines, including those from the World Health Organization, the American College of Gastroenterology, and National Institute for Health and Care Excellence, recommend bilirubin testing as part of the diagnostic workup for liver injury.[3,4,5,6,7]

In newborns, several mechanisms lead to an increased bilirubin load, such as increased turnover in fetal red blood cells, reduced bilirubin clearance, and increased enterohepatic circulation of bilirubin. Screening neonates for severe hyperbilirubinemia, especially in newborns with infant jaundice, has been proposed to help preventing chronic bilirubin encephalopathy.[8,9] For infants born at >= 35 weeks of gestation, the American Academy of Pediatrics Subcommittee on Hyperbilirubinemia recommends to measure total serum bilirubin in case of jaundice in the first 24 hours after birth or if jaundice appears excessive for infants' age (all bilirubin levels should be interpreted according to the infant's age in hours).[8]

Test principle[10]

Colorimetric diazo method

Total bilirubin, in the presence of a suitable solubilizing agent, is coupled with 3,5-dichlorophenyl diazonium in a strongly acidic medium.

$$\text{Bilirubin} + \text{3,5-DPD} \xrightarrow{\text{acid}} \text{azobilirubin}$$

The color intensity of the red azo dye formed is directly proportional to the total bilirubin and can be determined photometrically.

Reagents - working solutions

R1 Phosphate: 50 mmol/L; detergents; stabilizers; pH 1.0

R3 3,5-dichlorophenyl diazonium salt: ≥ 1.35 mmol/L

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H290	May be corrosive to metals.
H319	Causes serious eye irritation.
H360FD	May damage fertility. May damage the unborn child.

Prevention:

P201	Obtain special instructions before use.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Response:

P308 + P313	IF exposed or concerned: Get medical advice/attention.
P337 + P313	If eye irritation persists: Get medical advice/attention.
P390	Absorb spillage to prevent material damage.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.
Contact phone: all countries: +49-621-7590

Reagent handling

Ready for use

BILT3

Bilirubin Total Gen.3

cobas®

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:	6 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-, K_3-EDTA plasma

(The use of EDTA-plasma with elevated hematocrit may lead to slightly lower values.)

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:[a),11]	1 day at 15-25 °C
	7 days at 2-8 °C
	6 months at (-15)-(-25) °C

a) If care is taken to prevent exposure to light

Freeze only once.

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)	600/546 nm		
Reagent pipetting		Diluent (H_2O)	
R1	78 µL	–	
R3	16 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.3 µL	–	–
Decreased	2.6 µL	20 µL	60 µL
Increased	1.3 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: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - as required following quality control procedures

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

Traceability: The method was standardized against the Doumas method.[12]

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 6 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 µmol/L (mg/dL, mg/L).

Conversion factors:	µmol/L x 0.0585 = mg/dL
	µmol/L x 0.585 = mg/L

Limitations - interference

Criterion: Recovery within ± 3.4 µmol/L (0.199 mg/dL) of initial values of samples ≤ 34 µmol/L (1.99 mg/dL) and within ± 10 % for samples > 34 µmol/L.

Hemolysis:[13] No significant interference up to an H index of 800 (approximate hemoglobin concentration: 497 µmol/L or 800 mg/dL).

Immunoglobulins: No significant interference from immunoglobulins up to a concentration of 28 g/L (187 µmol/L) (simulated by human immunoglobulin G).

Criterion: Recovery within ± 1.7 µmol/L (0.099 mg/dL) of initial values of samples ≤ 17 µmol/L (0.995 mg/dL) and within ± 10 % for samples > 17 µmol/L.

Hemolysis in neonates:[13] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[13] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[14,15]

Indican: No significant interference from indican up to a concentration of 0.12 mmol/L (3 mg/dL).

Cyanokit (Hydroxocobalamin) may cause falsely low results.

Samples containing indocyanine green must not be measured.

Results from certain multiple myeloma patients may show a positive bias in recovery. Not all multiple myeloma patients show the bias and the severity of the bias may vary between patients.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[16]

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

In certain cases specimens may give a direct bilirubin result slightly greater than the total bilirubin result. This is observed in patient samples when nearly all the reacting bilirubin is in the direct form. In such cases the result

for the total bilirubin should be reported for both D-bilirubin and total bilirubin values.

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

2.5-650 µmol/L (0.146-38.0 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

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 1.7 µmol/L (0.099 mg/dL)
Limit of Detection	= 2.5 µmol/L (0.146 mg/dL)
Limit of Quantitation	= 2.5 µmol/L (0.146 mg/dL)

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 30 %. It has been determined using low concentration bilirubin samples.

Expected values

µmol/L

Adults[17]	up to 21 µmol/L
Children with age ≥ 1 month[17]	up to 17 µmol/L

Reference range study with 500 well-characterized human serum samples:[18]

Males	up to 24 µmol/L
Females	up to 15 µmol/L

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term[19]

Age of newborn:

24 hours	≥ 137 µmol/L[b)]
48 hours	≥ 222 µmol/L[b)]
84 hours	≥ 290 µmol/L[b)]

b) 95th percentile

Levels > 95th percentile: Such levels of hyperbilirubinemia have been deemed significant and are generally considered to require close supervision, possible further evaluation, and sometimes intervention.

mg/dL

Adults[17]	up to 1.2 mg/dL
Children with age ≥ 1 month[17]	up to 1.0 mg/dL

Reference range study with 500 well-characterized human serum samples:[18]

Males	up to 1.4 mg/dL
Females	up to 0.9 mg/dL

High risk for developing clinically significant hyperbilirubinemia:

Newborns: Term and near-term[19]

Age of newborn:

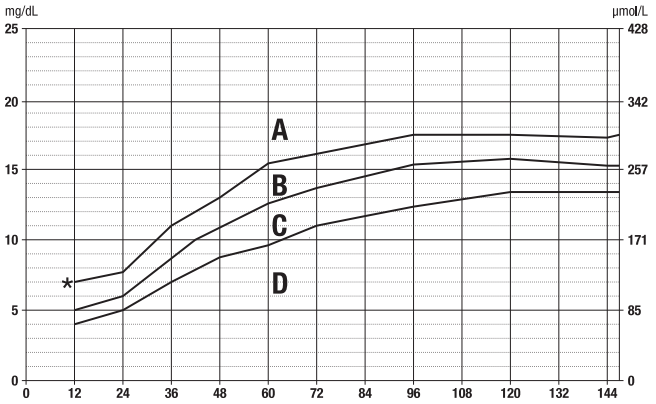
24 hours	≥ 8.0 mg/dL[b)]
48 hours	≥ 13.0 mg/dL[b)]
84 hours	≥ 17.0 mg/dL[b)]

b) 95th percentile

Levels > 95th percentile: Such levels of hyperbilirubinemia have been deemed significant and are generally considered to require close supervision, possible further evaluation, and sometimes intervention.

Nomogram for designation of risk in 2840 well newborns[19]

Serum bilirubin



Postnatal age (hours)

* 95th percentile

A High risk zone

B High intermediate risk zone

C Low intermediate risk zone

D Low risk zone

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*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
PCCC1[c)]	16.2	0.256	1.6
PCCC2[d)]	61.4	0.315	0.5
Human serum 1	5.43	0.211	3.9
Human serum 2	21.5	0.228	1.1

Human serum 3	91.6	0.507	0.6
Human serum 4	295	1.24	0.4
Human serum 5	519	1.97	0.4
Intermediate precision	*Mean*	*SD*	*CV*
	µmol/L	*µmol/L*	*%*
PCCC1[c)]	16.2	0.372	2.3
PCCC2[d)]	60.9	0.630	1.0
Human serum 1	5.43	0.222	4.1
Human serum 2	21.4	0.269	1.3
Human serum 3	91.6	0.706	0.8
Human serum 4	295	1.57	0.5
Human serum 5	516	3.26	0.6

c) PreciControl ClinChem Multi 1

d) PreciControl ClinChem Multi 2

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

Method comparison

Total bilirubin values for human serum and plasma samples obtained with the Roche Bilirubin Total Gen.3 reagent 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) = 649

Passing/Bablok[20]	Linear regression
y = 1.000x - 0.0394 µmol/L	y = 1.002x - 0.339 µmol/L
τ = 0.979	r = 1.000

The sample concentrations were between 2.51 and 622 µmol/L.

Total bilirubin values for human serum and plasma samples obtained with the Roche Bilirubin Total Gen.3 reagent 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) = 67

Passing/Bablok[20]	Linear regression
y = 1.010x - 0.247 µmol/L	y = 1.008x - 0.264 µmol/L
τ = 0.966	r = 1.000

The sample concentrations were between 2.90 and 615 µmol/L.

References

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14 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

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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 navifyportal.roche.com for definition of symbols used):

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.

BILT3

Bilirubin Total Gen.3

cobas®

CE 0123

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

Roche

CA2

Calcium Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057427190*	08057427500	Calcium Gen.2 (1500 tests)	System-ID 2034 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057427214*	08057427500	Calcium Gen.2 (1500 tests)	System-ID 2034 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
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	

* Some kits shown may not be available in all countries.

English

System information

CA2: ACN 20340 (Serum/plasma)

CA2U: ACN 20341 (Urine)

Intended use

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

Summary

Measurements of calcium, measured with this device, in human serum, plasma and urine, are used in the diagnosis of hypercalcemia/hypercalciuria (such as observed in hyperparathyroidism and cancer, endocrine disorders, inherited hypercalcemia, excessive vitamin D intake, chronic kidney disease) and of hypocalcemia/hypocalciuria (such as observed in hypoparathyroidism, vitamin D or magnesium deficiency, calcium homeostasis bone disease).[1]

Calcium is the most abundant mineral element in the body with about 99 % in the bones primarily as hydroxyapatite. The remaining calcium is distributed between the various tissues and the extracellular fluids where it performs a vital role for many life sustaining processes. Among the extra skeletal functions of calcium are involvement in blood coagulation, neuromuscular conduction, excitability of skeletal and cardiac muscle, enzyme activation, and the preservation of cell membrane integrity and permeability. Urinary calcium results from glomerular filtration of albumin-free plasma calcium and intense calcium reabsorption along the different tubular segments.[2]

Serum calcium levels and hence the body content are controlled by parathyroid hormone (PTH), calcitonin, and vitamin D. An imbalance in any of these modulators leads to alterations of the body and serum calcium levels. Increases in serum PTH or vitamin D are usually associated with hypercalcemia. Increased serum and urine calcium levels may also be observed in multiple myeloma and other neoplastic diseases. Hypocalcemia may be observed e.g. in hypoparathyroidism, nephrosis, and pancreatitis.[1]

Test principle

Calcium ions react with 5-nitro-5'-methyl-BAPTA (NM-BAPTA) under alkaline conditions to form a complex. This complex reacts in the second step with EDTA.

$$Ca^{2+} + \text{NM-BAPTA} \xrightarrow{\text{alkaline pH}} \text{calcium-NM-BAPTA complex}$$

$$\text{calcium-NM-BAPTA complex} + \text{EDTA} \longrightarrow \text{NM-BAPTA} + \text{calcium EDTA complex}$$

The change in absorbance is directly proportional to the calcium concentration and is measured photometrically.

Reagents - working solutions

R1 CAPSO:[a)] 557 mmol/L; NM-BAPTA: 2 mmol/L; pH 10.0; non-reactive surfactant; preservative

R3 EDTA: 7.5 mmol/L; pH 7.3; non-reactive surfactant, preservative

a) 3-[cyclohexylamino]-2-hydroxy-1-propanesulfonic acid

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H318 Causes serious eye damage.

Prevention:

P280 Wear eye protection/ face protection.

Response:

P305 + P351 + P338 + P310 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER/ doctor.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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

CA2

Calcium Gen.2

cobas®

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: Fresh serum collected in the fasting state is the preferred specimen.
Plasma: Li-heparin plasma.

Serum or plasma should be separated from blood cells as soon as possible, because prolonged contact with the clot may cause lower calcium values.[3] Sera from patients receiving EDTA (treatment of hypercalcemia) are unsuitable for analysis, since EDTA will chelate the calcium and render it unavailable for reaction with NM-BAPTA. Co-precipitation of calcium with fibrin (i.e. heparin plasma), lipids, or denatured protein has been reported with storage or freezing.[1,4]

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.

Urine

Urine specimens should be collected in acid-washed bottles. 24-hour specimens should be collected in containers containing 20-30 mL of 6 mol/L HCl to prevent calcium salt precipitation. Precipitated calcium salts may not be completely dissolved by the addition of HCl following urine collection.[5]

If stabilizers are added to the sample, the sample index feature must not be used.

Stability in *serum/plasma*:[6]	7 days at 15-25 °C
	3 weeks at 2-8 °C
	8 months at -20 °C (± 5 °C)

Freeze only once.

Stability in *urine*:[6]	2 days at 15-25 °C
	4 days at 2-8 °C
	3 weeks at -20 °C (± 5 °C)

Freeze only once.

Stored serum or urine specimens must be mixed well prior to analysis.

Centrifuge samples containing precipitates before performing the assay.

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

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)	376/340 nm	
Reagent pipetting		Diluent (H_2O)
R1	15 µL	120 µL
R3	15 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.3 µL	–	–
Decreased	2.3 µL	–	–
Increased	2.3 µL	–	–

Application for urine

Test definition

Reporting time	10 min	
Wavelength (sub/main)	376/340 nm	
Reagent pipetting		Diluent (H_2O)
R1	15 µL	120 µL
R3	15 µL	–

Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.5 µL	–	–
Decreased	1.5 µL	15 µL	60 µL
Increased	1.5 µL	–	–

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

Calibration

Application for serum/plasma (ACN 20340)

Calibrators	S1: H_2O
	S2: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - every 8 weeks - as required following quality control procedures

Application for urine (ACN 20341)
Transfer of calibration from serum/plasma application (ACN 20340)

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

Traceability: This method has been standardized against the SRM 956 c Level 2 reference material.

Quality control

For quality control, use control materials as listed in the “Order information” section. In addition, other suitable control material can be used.

Serum/plasma:	PreciControl ClinChem Multi 1, PreciControl ClinChem Multi 2
Urine:	Quantitative urine controls are recommended for routine quality control.

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 mmol/L (mg/dL, mg/L).

Conversion factors: mmol/L x 4.01 = mg/dL
mmol/L x 40.1 = mg/L

In studies with 24-hour urine, multiply the value obtained by the 24-hour volume in order to obtain a measurement in mg/24 h or mmol/24 h.

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a calcium concentration of 2.2 mmol/L.

Serum/plasma

Icterus:[7] 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:[7] No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Lipemia (Intralipid):[7] No significant interference up to an L index of 1000. There is a poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Magnesium: No significant interference from magnesium up to a concentration of 15 mmol/L (36.5 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[8,9]

The interference of intravenously administered gadolinium containing MRI (magnetic resonance imaging) contrast media was tested (Omniscan®, Optimark®) but no interference was found at the therapeutic concentration. Interferences at higher concentrations were observed.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[10]

Urine

Icterus: No significant interference up to a conjugated bilirubin concentration of 1026 µmol/L or 60 mg/dL.

Hemolysis: No significant interference up to an H index of 1000 (approximate hemoglobin concentration: 621 µmol/L or 1000 mg/dL).

Magnesium: No significant interference from magnesium up to a concentration of 60 mmol/L (145.8 mg/dL).

Urea: No significant interference from urea up to a concentration of 1600 mmol/L (9610 mg/dL).

Drugs: No interference was found at therapeutic concentrations using common drug panels.[9]

The interference of intravenously administered gadolinium containing MRI (magnetic resonance imaging) contrast media was tested (Omniscan®, Optimark®). For Omniscan® no interference was observed at the therapeutic concentration, but there was interference at higher concentrations. For Optimark® interference was observed at therapeutic and higher concentrations.

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

Serum/plasma

0.20-5.0 mmol/L (0.8-20.1 mg/dL)

Urine

0.20-7.5 mmol/L (0.8-30.1 mg/dL)

Determine urine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:5 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 5.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Serum/plasma and urine

Limit of Blank	= 0.10 mmol/L (0.4 mg/dL)
Limit of Detection	= 0.20 mmol/L (0.8 mg/dL)
Limit of Quantitation	= 0.20 mmol/L (0.8 mg/dL)

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 30 %. It has been determined using low concentration calcium samples.

Expected values[11]

mmol/L

Serum/plasma

Children (0-10 days):	1.90-2.60 mmol/L
Children (10 days-2 years):	2.25-2.75 mmol/L
Children (2-12 years):	2.20-2.70 mmol/L
Children (12-18 years):	2.10-2.55 mmol/L
Adults (18-60 years):	2.15-2.50 mmol/L
Adults (60-90 years):	2.20-2.55 mmol/L
Adults (> 90 years):	2.05-2.40 mmol/L

Urine

2.5-7.5 mmol/24 h with normal food intake.

mg/dL

Serum/plasma

Children (0-10 days):	7.6-10.4 mg/dL
Children (10 days-2 years):	9.0-11.0 mg/dL
Children (2-12 years):	8.8-10.8 mg/dL
Children (12-18 years):	8.4-10.2 mg/dL
Adults (18-60 years):	8.6-10.0 mg/dL
Adults (60-90 years):	8.8-10.2 mg/dL
Adults (> 90 years):	8.2-9.6 mg/dL

Urine

100-300 mg/24 h with normal food intake.

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.

Serum/plasma

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[b)]	2.22	0.0103	0.5
PCCC2[c)]	3.41	0.0136	0.4
Human serum 1	0.398	0.00739	1.9
Human serum 2	1.43	0.00982	0.7
Human serum 3	2.12	0.0112	0.5
Human serum 4	2.42	0.0152	0.6
Human serum 5	4.13	0.0155	0.4

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[b)]	2.21	0.0140	0.6
PCCC2[c)]	3.41	0.0272	0.8
Human serum 1	0.398	0.00924	2.3
Human serum 2	1.43	0.0109	0.8
Human serum 3	2.13	0.0134	0.6
Human serum 4	2.42	0.0212	0.9
Human serum 5	4.13	0.0185	0.4

b) PreciControl ClinChem Multi 1

c) PreciControl ClinChem Multi 2

Urine

Repeatability	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
Control 1[d)]	1.73	0.0138	0.8
Control 2[d)]	2.39	0.0150	0.6
Human urine 1	0.374	0.0104	2.8
Human urine 2	1.44	0.0127	0.9
Human urine 3	2.25	0.0161	0.7
Human urine 4	3.56	0.0217	0.6
Human urine 5	6.25	0.0294	0.5

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
Control 1[d)]	1.73	0.0150	0.9
Control 2[d)]	2.39	0.0180	0.8
Human urine 1	0.374	0.0170	4.5
Human urine 2	1.43	0.0159	1.1
Human urine 3	2.25	0.0236	1.0
Human urine 4	3.56	0.0282	0.8
Human urine 5	6.22	0.0425	0.7

d) commercially available control material

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

Calcium values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 71

Passing/Bablok[12]	Linear regression
y = 0.988x + 0.0216 mmol/L	y = 0.985x + 0.0295 mmol/L
τ = 0.964	r = 0.999

The sample concentrations were between 0.260 and 4.84 mmol/L.

Urine

Sample size (n) = 68

Passing/Bablok[12]	Linear regression
y = 0.964x + 0.0148 mmol/L	y = 0.967x + 0.0055 mmol/L
τ = 0.987	r = 1.000

The sample concentrations were between 0.270 and 7.12 mmol/L.

Calcium values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 73

Passing/Bablok[12]	Linear regression
y = 1.024x - 0.0379 mmol/L	y = 1.024x - 0.0304 mmol/L
τ = 0.977	r = 0.999

The sample concentrations were between 0.250 and 4.63 mmol/L.

Urine

Sample size (n) = 71

Passing/Bablok[12]	Linear regression
y = 0.996x - 0.00631 mmol/L	y = 0.990x + 0.00625 mmol/L
τ = 0.989	r = 0.999

The sample concentrations were between 0.300 and 7.42 mmol/L.

Calcium values for human serum, plasma and urine 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).

Serum/plasma

Sample size (n) = 73

Passing/Bablok[12]	Linear regression
y = 1.021x - 0.0334 mmol/L	y = 1.011x - 0.0110 mmol/L
τ = 0.955	r = 0.999

The sample concentrations were between 0.464 and 4.85 mmol/L.

Urine

Sample size (n) = 72

Passing/Bablok[12]	Linear regression
y = 1.017x + 0.00977 mmol/L	y = 1.016x + 0.0110 mmol/L
τ = 0.992	r = 1.000

The sample concentrations were between 0.250 and 7.13 mmol/L.

References

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8 Breuer J. Report on the Symposium "Drug effects in Clinical Chemistry Methods". Eur J Clin Chem Clin Biochem 1996;34:385-386.

9 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.

10 Bakker AJ, Mücke M. Gammopathy interference in clinical chemistry assays: mechanisms, detection and prevention. Clin Chem Lab Med 2007;45(9):1240-1243.

11 Wu AHB, ed. Tietz Clinical Guide to Laboratory Tests, 4th ed. St. Louis (MO): Saunders Elsevier 2006:202-207.

12 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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 navifyportal.roche.com for definition of symbols used):

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

CHOL2

cobas®

Cholesterol Gen.2

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057443190*	08057443500	Cholesterol Gen.2 (2600 tests)	System-ID 2041 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057443214*	08057443500	Cholesterol Gen.2 (2600 tests)	System-ID 2041 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
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	

* Some kits shown may not be available in all countries.

English

System information

CHOL2-A: ACN 20410: Abell/Kendall Standardization

CHOL2-I: ACN 20411: ID/MS Standardization

Intended use

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

Summary

Measurements of cholesterol, performed with this assay, in human serum and plasma, are used in screening an individual's risk of developing atherosclerotic disease and as an aid in diagnosis, therapy guidance and monitoring of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.

Cholesterol is a steroid with a secondary hydroxyl group in the C3 position. It is synthesized in many types of tissue, but particularly in the liver and intestinal wall. Approximately three quarters of cholesterol is newly synthesized and a quarter originates from dietary intake. Cholesterol assays are used for screening for atherosclerotic risk and in the diagnosis and treatment of disorders involving elevated cholesterol levels as well as lipid and lipoprotein metabolic disorders.[1,2,3]

Cholesterol analysis was first reported by Liebermann in 1885 followed by Burchard in 1889.[4,5] In the Liebermann-Burchard reaction, cholesterol forms a blue-green dye from polymeric unsaturated carbohydrates in an acetic acid/acetic anhydride/concentrated sulfuric acid medium. The Abell and Kendall method is specific for cholesterol, but is technically complex and requires the use of corrosive reagents.[6] In 1974, Roeschlau and Allain described the first fully enzymatic method.[7,8] This method is based on the determination of Δ4-cholestenone after enzymatic cleavage of the cholesterol ester by cholesterol esterase, conversion of cholesterol by cholesterol oxidase, and subsequent measurement by the Trinder reaction of the hydrogen peroxide formed.[9] Optimization of ester cleavage (> 99.5 %) allows standardization using primary and secondary standards and a direct comparison with the CDC and NIST reference methods.[10,11]

Nonfasting sample results may be slightly lower than fasting results.[12,13,14]

The Roche cholesterol assay meets the 1992 National Institutes of Health (NIH) goal of less than or equal to 3 % for both precision and bias.[14]

The assay is optionally standardized against Abell/Kendall and isotope dilution/mass spectrometry.

Test principle

Enzymatic, colorimetric method.

Cholesterol esters are cleaved by the action of cholesterol esterase to yield free cholesterol and fatty acids. Cholesterol oxidase then catalyzes the oxidation of cholesterol to cholest-4-en-3-one and hydrogen peroxide. In the presence of peroxidase, the hydrogen peroxide formed effects the oxidative coupling of phenol and 4-aminoantipyrine (4-AAP) to form a red quinone-imine dye.

CE

$$\text{Cholesterol esters} + H_2O \longrightarrow \text{cholesterol} + \text{RCOOH}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{cholest-4-en-3-one} + H_2O_2$$

$$2\,H_2O_2 + \text{4-AAP} + \text{phenol} \xrightarrow{\text{POD}} \text{quinone-imine dye} + 4\,H_2O$$

The color intensity of the dye formed is directly proportional to the cholesterol concentration. It is determined by measuring the increase in absorbance.

Reagents – working solutions

R1 PIPES buffer: 225 mmol/L, pH 6.8; Mg^{2+}: 10 mmol/L; sodium cholate: 0.6 mmol/L; 4-aminoantipyrine: ≥ 0.45 mmol/L; phenol: ≥ 12.6 mmol/L; fatty alcohol polyglycol ether: 3 %; cholesterol esterase (Pseudomonas spec.): ≥ 25 µkat/L (≥ 1.5 U/mL); cholesterol oxidase (E. coli): ≥ 7.5 µkat/L (≥ 0.45 U/mL); peroxidase (horseradish): ≥ 12.5 µkat/L (≥ 0.75 U/mL); stabilizers; preservative

R1 is in position B.

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H319 Causes serious eye irritation.

Prevention:

P264 Wash skin thoroughly after handling.

P280 Wear eye protection/ face protection.

Response:

CHOL2

Cholesterol Gen.2

cobas®

P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337 + P313	If eye irritation persists: Get medical advice/attention.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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

Do not use citrate, oxalate or fluoride.[15]

Fasting and nonfasting samples can be used.[13]

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:[1,16]	7 days at 15-25 °C
	7 days at 2-8 °C
	3 months at -20 °C (± 5 °C)

Freeze only once.

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)	700/505 nm		
Reagent pipetting		Diluent (H_2O)	
R1	26 µL	51 µL	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	1.1 µL	–	–
Decreased	1.1 µL	10.0 µL	90 µL
Increased	1.1 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: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Blank calibration - every 7 days on-board - every 7 days during shelf life
	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 according to Abell/Kendall[14] and also by isotope dilution/mass spectrometry.[17]

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 mmol/L (mg/dL, g/L).

Conversion factors:	mmol/L x 38.66 = mg/dL
	mmol/L x 0.3866 = g/L

Limitations – interference

Criterion: Recovery within ± 10 % of initial value at a cholesterol concentration of 5.2 mmol/L.

Icterus:[18] No significant interference up to an I index of 16 for conjugated bilirubin and 14 for unconjugated bilirubin (approximate conjugated bilirubin concentration 274 µmol/L or 16 mg/dL; approximate unconjugated bilirubin concentration 239 µmol/L or 14 mg/dL).

Hemolysis:[18] No significant interference up to an H index of 700 (approximate hemoglobin concentration: 435 µmol/L or 700 mg/dL).

Lipemia (Intralipid):[18] No significant interference up to an L index of 2000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[19,20]

Acetaminophen intoxications are frequently treated with N-acetylcysteine. N-Acetylcysteine at the therapeutic concentration when used as an antidote and the acetaminophen metabolite N-acetyl-p-benzoquinone imine (NAPQI) independently may cause falsely low results.

Venipuncture should be performed prior to the administration of metamizole. Venipuncture immediately after or during the administration of metamizole may lead to falsely low results.

In very rare cases, gammopathy, in particular type IgM (Waldenström’s macroglobulinemia), may cause unreliable results.[21]

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

CHOL2

Cholesterol Gen.2

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

0.1-20.7 mmol/L (3.86-800 mg/dL)

Determine samples having higher concentrations via the rerun function. Dilution of samples via the rerun function is a 1:10 dilution. Results from samples diluted using the rerun function are automatically multiplied by a factor of 10.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 0.1 mmol/L (3.86 mg/dL)
Limit of Detection	= 0.1 mmol/L (3.86 mg/dL)
Limit of Quantitation	= 0.1 mmol/L (3.86 mg/dL)

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 cholesterol samples.

Expected values

mmol/L

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[2]

	mmol/L	*Lipid metabolic disorder*
Cholesterol	< 5.2	No
Triglycerides	< 2.3	No
Cholesterol	5.2-7.8	Yes, if HDL-cholesterol < 0.9 mmol/L
Cholesterol	> 7.8	Yes
Triglycerides	> 2.3	Yes

Recommendations of the NCEP Adult Treatment Panel for the following risk-cutoff thresholds for the US American population:[3]

Desirable cholesterol level	< 5.17 mmol/L
Borderline high cholesterol	5.17-6.18 mmol/L
High cholesterol	≥ 6.21 mmol/L

mg/dL

Clinical interpretation according to the recommendations of the European Atherosclerosis Society:[2]

	mg/dL	*Lipid metabolic disorder*
Cholesterol	< 200	No
Triglycerides	< 200	No
Cholesterol	200-300	Yes, if HDL-cholesterol < 35 mg/dL
Cholesterol	> 300	Yes
Triglycerides	> 200	Yes

Recommendations of the NCEP Adult Treatment Panel for the following risk-cutoff thresholds for the US American population:[3]

Desirable cholesterol level	< 200 mg/dL
Borderline high cholesterol	200-239 mg/dL
High cholesterol	≥ 240 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. 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*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[a)]	2.36	0.00970	0.4
PCCC2[b)]	5.15	0.0184	0.4
Human serum 1	0.226	0.00478	2.1
Human serum 2	5.02	0.0167	0.3
Human serum 3	6.02	0.0214	0.4
Human serum 4	9.55	0.0314	0.3
Human serum 5	17.9	0.0845	0.5

Intermediate precision	*Mean*	*SD*	*CV*
	mmol/L	*mmol/L*	*%*
PCCC1[a)]	2.39	0.0257	1.1
PCCC2[b)]	5.11	0.0363	0.7
Human serum 1	0.249	0.0185	7.4
Human serum 2	5.02	0.0355	0.7
Human serum 3	6.01	0.0369	0.6
Human serum 4	9.55	0.0432	0.5
Human serum 5	17.9	0.0959	0.5

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

Cholesterol 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) = 75

Passing/Bablok[22]	Linear regression
y = 1.019x + 0.00509 mmol/L	y = 1.020x - 0.0158 mmol/L
τ = 0.985	r = 1.000

The sample concentrations were between 0.344 and 18.8 mmol/L.

CHOL2

Cholesterol Gen.2

Cholesterol 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) = 66

Passing/Bablok[22]	Linear regression
y = 1.024x + 0.00124 mmol/L	y = 1.022x + 0.00775 mmol/L
τ = 0.993	r = 1.000

The sample concentrations were between 0.330 and 18.2 mmol/L.

Cholesterol 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) = 91

Passing/Bablok[22]	Linear regression
y = 1.014x - 0.0144 mmol/L	y = 1.018x - 0.0486 mmol/L
τ = 0.987	r = 0.999

The sample concentrations were between 0.141 and 19.3 mmol/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 (for USA: see navifyportal.roche.com for definition of symbols used):

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

CK

Creatine Kinase

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057460190*	08057460500	Creatine Kinase (500 tests)	System-ID 2042 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057460214*	08057460500	Creatine Kinase (500 tests)	System-ID 2042 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

10759350190	Calibrator f.a.s. (12 x 3 mL)	Code 20401	
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	

* Some kits shown may not be available in all countries.

English

System information

CK2: ACN 20420

Intended use

In vitro test for the quantitative determination of creatine kinase (CK) in human serum and plasma on **cobas c** systems.

Summary

Measurements of creatine kinase (CK), performed with this assay in human serum and plasma, are used as an aid in diagnosis of muscular injuries and diseases.

CK is a dimeric enzyme occurring in 4 different forms: a mitochondrial isoenzyme and the cytosolic isoenzymes CK MM (skeletal muscle type), CK BB (brain type) and CK MB (myocardial type).[1] Elevated total CK is observed in patients with skeletal and heart muscle injuries and diseases.[2]

The determination of CK and CK isoenzyme activities is utilized in the diagnosis and monitoring of muscular injuries and diseases in the acute (e.g. rhabdomyolysis or acute myocardial injury) and chronic settings (e.g. myopathies such as the progressive Duchenne muscular dystrophy). In acute rhabdomyolysis, for example, serum CK activities above 200 times the upper reference limit may be found. Serum CK activity is elevated in all types of muscular dystrophy.[3] In progressive muscular dystrophy, enzyme activity in serum may be increased long before the disease is clinically apparent.[3]

Following injury to the myocardium, such as occurs with acute myocardial infarction,[1] CK is released from the damaged myocardial cells. In early cases, a rise in the CK activity can be found just 4 hours after an infarction.[1,4] The CK activity reaches a maximum after 12-24 hours and then falls back to the normal range after 3-4 days.[1,4] According to the 4th Universal Definition of Myocardial Infarction, cardiac troponins are the preferred biomarkers for the evaluation of myocardial injury, since other biomarkers are less specific and less sensitive.[5]

The determination of CK levels can also be used for evaluation of drug toxicity. The European Society of Cardiology and the European Atherosclerosis Society recommend measuring CK in patients before initiation of lipid-lowering drug therapy, and in patients on lipid-lowering drugs, presenting with muscle pain and weakness, in order to identify the limited number of patients where treatment is contraindicated.[6]

The assay method using creatine phosphate and ADP was first described by Oliver,[7] modified by Rosalki[8] and further improved for optimal test conditions by Szasz et al.[9] CK is rapidly inactivated by oxidation of the sulfhydryl groups in the active center. The enzyme can be reactivated by the addition of acetylcysteine (NAC).[9] Interference by adenylate kinase is prevented by the addition of diadenosine pentaphosphate[10] and AMP.[9,10]

Standardized methods for the determination of CK with activation by NAC were recommended by the German Society for Clinical Chemistry (DGKC)[10] in 1977 and the International Federation of Clinical Chemistry (IFCC)[11] in 1991. In 2002 the IFCC confirmed their recommendation and extended it to 37 °C.[12,13] The method described here is derived from the formulation recommended by the IFCC and was optimized for performance and stability.

Test principle

UV-test

$$\text{Creatine phosphate} + \text{ADP} \xrightarrow{\text{CK}} \text{creatine} + \text{ATP}$$

$$\text{ATP} + \text{D-glucose} \xrightarrow{\text{HK}} \text{ADP} + \text{G6P}$$

$$\text{G6P} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{D-6-phosphogluconate} + \text{NADPH} + \text{H}^+$$

Equimolar quantities of NADPH and ATP are formed at the same rate. The photometrically measured rate of formation of NADPH is directly proportional to the CK activity.

Reagents - working solutions

R1 Imidazole buffer: 123 mmol/L, pH 6.5 (37 °C); EDTA: 2.46 mmol/L; Mg^{2+}: 12.3 mmol/L; ADP: 2.46 mmol/L; AMP: 6.14 mmol/L; diadenosine pentaphosphate: 19 µmol/L; $NADP^+$ (yeast): 2.46 mmol/L; N-acetylcysteine: 24.6 mmol/L; HK (yeast): ≥ 36.7 µkat/L; G6PDH (E. coli): ≥ 23.4 µkat/L; preservative; stabilizers; additives.

R3 CAPSO* buffer: 20 mmol/L, pH 8.8 (37 °C); glucose: 120 mmol/L; EDTA: 2.46 mmol/L; creatine phosphate: 184 mmol/L; preservative; stabilizers.

*CAPSO: 3-(cyclohexylamine)-2-hydroxy-1-propanesulfonic acid

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

CK

Creatine Kinase

Danger

H360D	May damage the unborn child.

Prevention:

P201	Obtain special instructions before use.
P202	Do not handle until all safety precautions have been read and understood.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Response:

P308 + P313	IF exposed or concerned: Get medical advice/attention.

Storage:

P405	Store locked up.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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:	8 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: Nonhemolyzed serum is the specimen of choice and also recommended by IFCC.
Plasma: Li-heparin, K_2-, K_3-EDTA plasma.

Please note: Differences in the degree of hemolysis resulting from the blood sampling procedure used can lead to deviating results in serum and 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 in serum:[14]	2 days at 20-25 °C
	7 days at 4-8 °C
	4 weeks at -20 °C (± 5 °C)

Freeze only once.

Stability in EDTA/heparin plasma:	2 days at 15-25 °C
	7 days at 2-8 °C
	4 weeks at -20 °C (± 5 °C)

Freeze only once.

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)	546/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	79 µL	–	
R3	16 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	2.2 µL	–	–
Decreased	2.2 µL	10 µL	100 µL
Increased	2.2 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: C.f.a.s.
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - 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 IFCC Method for Creatine Kinase.[12]

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 8 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.

CK

Creatine Kinase

cobas®

Calculation

cobas c systems automatically calculate the analyte activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L x 0.0167 = µkat/L

Limitations - interference

Criterion: Recovery within ± 10 % of initial value at a creatine kinase activity of 140 U/L.

Icterus:[15] 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:[15] No significant interference up to an H index of 100 (approximate hemoglobin concentration: 62.1 µmol/L or 100 mg/dL). The level of interference may be variable depending on the exact content of erythrocytes.

Lipemia (Intralipid):[15] No significant interference up to an L index of 1000. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration. Highly lipemic specimens (L index > 1000) may cause high absorbance flagging.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[16,17] Cyanokit (hydroxocobalamin) at therapeutic concentrations interferes with the test.

In very rare cases, gammopathy, in particular type IgM (Waldenström's macroglobulinemia), may cause unreliable results.[18]

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

7-2000 U/L (0.12-33.4 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:11 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 11.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 7 U/L (0.12 µkat/L)
Limit of Detection	= 7 U/L (0.12 µkat/L)
Limit of Quantitation	= 7 U/L (0.12 µkat/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 activity 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 activity samples.

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

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

Expected values

Reference intervals strongly depend on the patient group and the specific clinical situation.

U/L

For healthy people, according to Klein et al.:[19]

CK	Men	39-308 U/L
	Women	26-192 U/L

Consensus values:[20]

CK	Men	< 190 U/L
	Women	< 170 U/L
CK-MB	Men/women	< 25 U/L

Myocardial infarction: There is a high probability of myocardial damage when the following 3 conditions are fulfilled:[21]

1	CK_{men}	> 190 U/L
	CK_{women}	> 167 U/L
2	CK-MB	> 24 U/L
3	The CK-MB activity accounts for 6-25 % of the total CK-activity.	

According to Tietz:[22]

CK	Adult males > 19 years	20-200 U/L
	Adult females > 19 years	20-180 U/L

µkat/L

For healthy people, according to Klein et al.:[19]*

CK	Men	0.65-5.14 µkat/L
	Women	0.43-3.21 µkat/L

*calculated by unit conversion factor

Consensus values:[20]

CK	Men	< 3.20 µkat/L
	Women	< 2.85 µkat/L
CK-MB	Men/women	< 0.42 µkat/L

Myocardial infarction: There is a high probability of myocardial damage when the following 3 conditions are fulfilled:[21]

1	CK_{men}	> 3.17 µkat/L
	CK_{women}	> 2.79 µkat/L
2	CK-MB	> 0.40 µkat/L
3	The CK-MB activity accounts for 6-25 % of the total CK-activity.	

According to Tietz:[22]*

CK	Adult males > 19 years	0.33-3.34 µkat/L
	Adult females > 19 years	0.33-3.01 µkat/L

*calculated by unit conversion factor

The reference values according to Klein et al. are based on the 95th percentile of a group of healthy persons (202 men and 217 women) not involved in high-intensity athletic activities.

In order to ensure high sensitivity in the diagnosis of heart diseases the values given by Tietz are recommended. The loss of diagnostic specificity thereby incurred can be compensated for by additionally determining CK-MB and/or troponin T. When myocardial infarction is suspected the diagnostic strategy proposals in the consensus document of European and American cardiologists should in general be followed.[23]

If despite the suspicion of myocardial infarction the values found remain below the stated limits, a fresh infarction may be involved. In such cases, the determinations should be repeated after 4 hours.

CK varies with physical activity level and race in healthy individuals.[22,24]

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

CK

Creatine Kinase

cobas®

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* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	155	0.764	0.5
PCCC2[b)]	287	0.988	0.3
Human serum 1	19.5	0.524	2.7
Human serum 2	85.7	0.510	0.6
Human serum 3	176	1.12	0.6
Human serum 4	900	3.28	0.4
Human serum 5	1588	4.52	0.3

Intermediate precision	*Mean* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	155	1.04	0.7
PCCC2[b)]	287	2.02	0.7
Human serum 1	19.4	0.582	3.0
Human serum 2	85.7	1.01	1.2
Human serum 3	176	1.96	1.1
Human serum 4	895	10.7	1.2
Human serum 5	1588	18.7	1.2

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

Creatine kinase 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) = 80

Passing/Bablok[25]	Linear regression
y = 0.988x + 1.20 U/L	y = 0.993x - 0.788 U/L
τ = 0.996	r = 1.000

The sample activities were between 8.20 and 1938 U/L.

Creatine kinase 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) = 110

Passing/Bablok[25]	Linear regression
y = 1.006x + 0.553 U/L	y = 1.013x - 1.03 U/L
τ = 0.990	r = 1.000

The sample activities were between 11.0 and 1959 U/L.

Creatine kinase 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) = 75

Passing/Bablok[25]	Linear regression
y = 1.004x + 0.512 U/L	y = 1.006x - 0.256 U/L
τ = 0.995	r = 1.000

The sample concentrations were between 51.2 and 1939 U/L.

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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.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

CKMB

Creatine Kinase-MB

cobas®

Order information

REF		CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
08057486190*	08057486500	Creatine Kinase-MB (150 tests)	System-ID 2043 001	**cobas c** 303, **cobas c** 503, **cobas c** 703
08057486214*	08057486500	Creatine Kinase-MB (150 tests)	System-ID 2043 001	**cobas c** 303, **cobas c** 503, **cobas c** 703

Materials required (but not provided):

11447394216	Calibrator f.a.s. CK-MB (3 x 1 mL)	Code 20402	
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	

* Some kits shown may not be available in all countries.

English

System information

CKMB2: ACN 20430

Intended use

In vitro test for the quantitative determination of the catalytic activity of creatine kinase MB subunit (CK-MB) in human serum and plasma on **cobas** c systems.

Summary

Measurements of CK-MB, performed with this assay in human serum and plasma, are used as an aid in diagnosis of myocardial infarction.

Creatine kinase (CK) appears as 3 isoenzymes which are dimers composed of 2 types of monomer subunits. The isoenzymes comprise all 3 combinations of monomers, M (for skeletal muscle derived) and B (for brain derived), as represented by the notations MM, MB, and BB.[1,2]

Many organs contain CK, but the distribution of isoenzymes is different in each one. Skeletal muscle is very rich in the MM isoenzyme, while brain, stomach, intestine, bladder, and lung contain primarily the BB isoenzyme. The MB isoenzyme has been found in appreciable amounts only in myocardial tissue (15 to 20 percent of the total myocardial CK).[3] Therefore, total serum CK activity is elevated in a number of diseases. This lack of specificity limits its diagnostic value. However, the striking difference in the CK isoenzyme patterns from different organs has made CK one of the most useful enzymes for diagnostic purposes in acute myocardial infarction. CK-MB appears in serum reflecting its unique presence in myocardial tissue. It is in supporting the diagnosis of suspected myocardial infarction that serial determinations of CK isoenzymes find their most frequent application in the clinical laboratory.[1,2]

Because of their higher sensitivity and specificity, cardiac troponins, measured by high-sensitivity assays, are the preferred biomarkers to define myocardial infarction,[4] and if a troponin assay is not available, the best alternative is CK-MB measured by a mass assay.[4]

After immunoinhibition with antibodies to the CK-M subunit,[5] the CK-B activity is determined with a standardized method for the determination of CK with activation by NAC as recommended by the German Society for Clinical Chemistry (DGKC)[6] and the International Federation of Clinical Chemistry (IFCC)[7,8] in 1977 and 2002 respectively. This assay meets the recommendations of the IFCC and DGKC, but was optimized for performance and stability.

Test principle

Immunological UV assay

- Sample and addition of R1 (buffer/enzymes/coenzyme)
- Addition of R2 (buffer/substrate/antibody) and start of reaction.

Human CK-MB is composed of 2 subunits, CK-M and CK-B which both have an active site. With the aid of specific antibodies to CK-M, the catalytic activity of CK-M subunits in the sample is inhibited to 99.6 % without affecting the CK-B subunits. The remaining CK-B activity, corresponding to half the CK-MB activity, is determined by the total CK method. As the CK-BB isoenzyme only rarely appears in serum and the catalytic activity of the CK-M and CK-B subunits hardly differ, the catalytic activity of the CK-MB isoenzyme can be calculated from the measured CK-B activity by multiplying the result by 2.

Reagents - working solutions

R1 Imidazole buffer: 123 mmol/L, pH 6.5 (37 °C); EDTA: 2.46 mmol/L; Mg^{2+}: 12.3 mmol/L; ADP: 2.46 mmol/L; AMP: 6.14 mmol/L; diadenosine pentaphosphate: 19 µmol/L; NADP (yeast): 2.46 mmol/L; N-acetylcysteine: 24.6 mmol/L; HK (yeast): ≥ 36.7 µkat/L; G6P-DH (E. coli): ≥ 23.4 µkat/L; preservative; stabilizers; additives.

R2 CAPSO* buffer: 20 mmol/L, pH 8.8 (37 °C); glucose: 120 mmol/L; EDTA: 2.46 mmol/L; creatine phosphate: 184 mmol/L; 4 monoclonal anti-CK-M antibodies (mouse), inhibiting capacity: > 99.6 % up to 66.8 µkat/L (4000 U/L) (37 °C) CK-M subunit; preservative; stabilizers; additive.

*CAPSO: 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid

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.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Danger

H360D May damage the unborn child.

Prevention:

P201 Obtain special instructions before use.

P202 Do not handle until all safety precautions have been read and understood.

P280	Wear protective gloves/ protective clothing/ eye protection/ face protection/ hearing protection.

Response:

P308 + P313	IF exposed or concerned: Get medical advice/attention.

Storage:

P405	Store locked up.

Disposal:

P501	Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

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:	8 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: Nonhemolyzed serum is the specimen of choice and also recommended by IFCC.
Plasma: Li-heparin, K_2-, K_3-EDTA plasma.

Li-heparin in the usual concentration does not interfere with the test, but IFCC warns against its use.[7]

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 in serum:[9]	8 hours at 20-24 °C
	8 days at 2-8 °C
	4 weeks at -20 °C (± 5 °C)

Freeze only once.

Stability in heparin plasma:[9]	8 hours at 20-24 °C
	5 days at 2-8 °C
	8 days at -20 °C (± 5 °C)

Freeze only once.

Stability in EDTA plasma:[10]	2 days at 20-25 °C
	7 days at 4-8 °C
	1 year at -20 °C (± 5 °C)

Freeze only once.

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)	546/340 nm		
Reagent pipetting		Diluent (H_2O)	
R1	79 µL	–	
R2	16 µL	–	
Sample volumes	*Sample*	*Sample dilution*	
		Sample	*Diluent (NaCl)*
Normal	3.9 µL	–	–
Decreased	11.7 µL	10 µL	80 µL
Increased	3.9 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: C.f.a.s. CK-MB
Calibration mode	Linear
Calibration frequency	Automatic full calibration - after reagent lot change
	Full calibration - 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 IFCC Method for Creatine Kinase[8] with addition of antibodies.

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 8 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 activity of each sample in the unit U/L (µkat/L).

Conversion factor: U/L × 0.0167 = µkat/L

Limitations - interference

The total CK activity of the specimen should be determined prior to performing the CK-MB assay. The amount of anti-human CK-M subunit antibody in the CK-MB reagent is sufficient for the complete inhibition of up to 4000 U/L CK-M activity. If the total CK activity exceeds 4000 U/L, the specimen requires dilution because complete inhibition of the CK-M subunit is no longer assured. In patients with a disposition to macro-CK formation, implausibly high CK-MB values may be measured in relation to the total CK, since the macroforms mainly consist of CK-B subunits. As these patients

have generally not suffered a myocardial infarction, additional diagnostic measures are necessary.[11]

Criterion: Recovery within ± 10 % of initial value at a CK-MB activity of ≥ 25 U/L.

Icterus:[12] No significant interference up to an I index of 60 for conjugated and 20 for unconjugated bilirubin (approximate conjugated bilirubin concentration: 1026 µmol/L or 60 mg/dL and approximate unconjugated bilirubin concentration: 342 µmol/L or 20 mg/dL).

Hemolysis:[12] No significant interference up to an H index of 20 (approximate hemoglobin concentration: 12.4 µmol/L or 20 mg/dL).

Lipemia (Intralipid):[12] No significant interference up to an L index of 500. There is poor correlation between the L index (corresponds to turbidity) and triglycerides concentration.

Adenylate kinase: Adenylate kinase (AK) may cause positive interference. Sources of AK in the blood are erythrocytes, muscle, and liver. In order to reduce AK interference to a minimum, AMP and Ap_5A are included in the reagent. The AMP/Ap_5A mixture causes 97 % inhibition of the AK from erythrocytes and muscle, and 95 % inhibition of the AK from liver.[6] The slight residual AK activity does not influence the assay of total CK, but may affect the low CK-MB activities.

Drugs: No interference was found at therapeutic concentrations using common drug panels.[13,14] Exceptions: Cyanokit (hydroxocobalamin) and cefoxitin at therapeutic concentrations interfere with the test.

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

3-2000 U/L (0.05-33.4 µkat/L)

Determine samples having higher activities via the rerun function. Dilution of samples via the rerun function is a 1:3 dilution. Results from samples diluted by the rerun function are automatically multiplied by a factor of 3.

Lower limits of measurement

Limit of Blank, Limit of Detection and Limit of Quantitation

Limit of Blank	= 3 U/L (0.05 µkat/L)
Limit of Detection	= 3 U/L (0.05 µkat/L)
Limit of Quantitation	= 5 U/L (0.08 µkat/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 activity 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 activity samples.

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

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

Expected values

Reference intervals strongly depend on the patient group regarded and the specific clinical situation.

U/L

For healthy people: Reference range (37 °C) according to Klein et al.[16] and consensus values:[17]

< 25 U/L

For myocardial infarction diagnosis using the combination CK and CK-MB (activity), and representing a CK consensus value based on long-term experience:[17,18]

1. CK_{men} > 190 U/L
   CK_{women} > 167 U/L
2. CK-MB > 24 U/L
3. The CK-MB activity accounts for 6-25 % of the total CK activity.

µkat/L

For healthy people: Reference range (37 °C) according to Klein et al.[16] and consensus values:[17]*

< 0.418 µkat/L

*calculated by unit conversion factor

For myocardial infarction diagnosis using the combination CK and CK-MB (activity), and representing a CK consensus value based on long-term experience:[17,18]

1. CK_{men} > 3.17 µkat/L
   CK_{women} > 2.79 µkat/L
2. CK-MB > 0.40 µkat/L
3. The CK-MB activity accounts for 6-25 % of the total CK activity.

When myocardial infarction is suspected the diagnostic strategy proposals in the consensus document of European and American cardiologists should in general be followed.[19]

If despite the suspicion of myocardial infarction the values found remain below the stated limits, a fresh infarction may be involved. In such cases the determinations should be repeated after 4 hours.

Maximum diagnostic efficiency of the CK-MB determination will be obtained when a sequential sampling protocol is used and consideration is given to the time pattern of activity over a 6 to 48 hour period. When only CK-MB activity is used, the diagnostic efficiency will be lower and will vary with the sampling time.[1,11]

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* *U/L*	*SD* *U/L*	*CV* *%*
PCCC1[a)]	42.9	0.380	0.9
PCCC2[b)]	96.9	0.365	0.4
Human serum 1	16.0	0.358	2.2
Human serum 2	22.6	0.365	1.6
Human serum 3	190	0.779	0.4
Human serum 4	997	2.61	0.3

Human serum 5	1782	4.80	0.3
Intermediate precision	*Mean U/L*	*SD U/L*	*CV %*
PCCC1[a)]	42.9	0.557	1.3
PCCC2[b)]	96.6	0.712	0.7
Human serum 1	15.5	0.507	3.3
Human serum 2	22.3	0.560	2.5
Human serum 3	190	3.24	1.7
Human serum 4	997	11.1	1.1
Human serum 5	1784	28.1	1.6

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

CK-MB 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) = 69

Passing/Bablok[20]	Linear regression
y = 1.014x - 1.73 U/L	y = 1.013x - 1.24 U/L
τ = 0.964	r = 1.000

The sample activities were between 4.90 and 1876 U/L.

CK-MB 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) = 69

Passing/Bablok[20]	Linear regression
y = 1.015x + 0.202 U/L	y = 1.023x + 0.108 U/L
τ = 0.932	r = 1.000

The sample activities were between 3.50 and 1970 U/L.

CK-MB 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) = 66

Passing/Bablok[20]	Linear regression
y = 1.003x + 1.52 U/L	y = 1.005x + 1.43 U/L
τ = 0.975	r = 1.000

The sample concentrations were between 3.81 and 1857 U/L.

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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.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see navifyportal.roche.com for definition of symbols used):

CONTENT	Contents of kit

CKMB

Creatine Kinase-MB

→	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

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606